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# Differences between the Fecal Microbiota of Coeliac Infants and Healthy Controls

**María Carmen Collado<sup>1</sup>, Miguel Calabuig<sup>2</sup>, and Yolanda Sanz<sup>1\*</sup>**

<sup>1</sup>Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Apartado 73, 46100 Burjassot, Valencia, Spain

<sup>2</sup>Hospital General Universitario, Avenida Tres Cruces s/n 46014 Valencia, Spain

## Abstract

Coeliac disease (CD) is an immune-mediated enteropathy with a multifactorial aetiology, characterized by chronic inflammation of the small intestinal mucosa. Although evidence suggests that the gut microbiota contributes to other chronic inflammatory disorders, its possible role in CD has not been determined. In this study, the composition of the fecal microbiota of coeliac children and age-matched controls was investigated by culture-dependent and -independent methodologies, using fluorescent *in situ* hybridization (FISH). The levels of *Bacteroides*, *Clostridium* and *Staphylococcus* were significantly higher ( $p < 0.05$ ) in fecal samples from coeliac patients than in healthy subjects when analysed by culture methods. The numbers of *Bacteroides-Prevotella*, *Clostridium histolyticum*, *Eubacterium rectale-C. coccoides*, *Atopobium*, and sulfate reducing bacterial groups were also significantly higher ( $p < 0.05$ ) in fecal samples from coeliac infants when analysed by FISH. The counts of *Bifidobacterium* tended to be higher in healthy controls by the two type of analysis but the differences were not significant. This is the first report on the identification of the specific bacterial groups responsible for alterations in the intestinal microecology of children with active CD. The bacterial pattern detected in coeliac patients, correlates with the epidemiological data and metabolic deviations associated with CD, and involve bacterial groups link to other chronic inflammatory disorders.

## Introduction

Coeliac disease (CD) is an autoimmune enteropathy characterized by a permanent intolerance to wheat-gluten proteins and related prolamines of rye and barley. In coeliac patients, the ingestion of gluten proteins triggers an abnormal T cell-mediated immune response that leads to inflammation of the small intestinal mucosa. CD can present at any age with a varied of clinical features but typical cases often manifest in early childhood with small intestinal mucosal injury and signs of malabsorption (Fasano and Catassi, 2005). The aetiology of CD involves a combination of genetic and environmental factors that lead to alterations in the gut-barrier function with loss of gluten tolerance (Kagnoff, 2005). The human major histocompatibility complex (MHC) molecules DQ2 and DQ8 are important genetic factors for CD, but only

account for 40% of the genetic risk (Louka and Sollid, 2003). The ingestion of gluten is regarded as the key environmental factor involved in the presentation of CD. Moreover, epidemiological and clinical evidence suggest that other environmental factors, which are involved in shaping the composition of the gut microbiota, could be additional trigger elements or predispose to CD. These include the milk-feeding practices, the incidence of early microbial infections, and the use of antibiotics (Ivarsson *et al.*, 2002; 2003).

The commensal microbiota provides a natural defence against pathogenic microorganisms and plays a pivotal role in numerous physiological functions, including regulation of epithelial permeability and immune tolerance (Majamaa and Isolauri, 1996; Isolauri *et al.* 2001; Sanz and Collado, 2005). It has been demonstrated that the intestinal microbiota is implicated in the pathogenesis of chronic inflammatory bowel diseases, and other immune-related disorders (Cummings *et al.*, 2003; Bullock *et al.*, 2004; Sartor, 2004; Swidsinski *et al.*, 2005). In contrast, specific strains of the gut microbiota and the use of probiotics have been found to reduce intestinal inflammation and normalize gut mucosal dysfunction (Sartor, 2004). At present, there are not data on the composition of the intestinal microbiota of coeliac patients and only alterations in the composition of fecal short-chain fatty acids (SCFA) have been reported (Tjellström *et al.*, 2005). In addition, rod-shaped bacteria were often found to be associated with the mucosa of CD patients (Forsberg *et al.*, 2004).

The aim of this study was to compare the composition of the fecal microbiota of coeliac children and healthy controls in order to define the specific microbial changes associated with CD and, thereby, contribute to the understanding of the possible role of the microbiota in this disorder.

## Results

The results of the analysis of the fecal microbiota of coeliac and control children by conventional culture techniques are shown in Table 1. Significantly higher counts ( $p < 0.05$ ) of strict anaerobic bacteria of the genera *Bacteroides* and *Clostridium* were detected in fecal samples of coeliac children than in those of controls. The aerobic genus *Staphylococcus* was also present in significantly higher levels ( $p < 0.05$ ) in fecal samples of coeliac patients. *Bacteroides*, *Clostridium*, and *Enterobacteriaceae* were the dominant bacterial groups in coeliac samples while *Bifidobacterium*, and *Enterobacteriaceae* were dominant in controls. The levels of *Bifidobacterium* were slightly higher in controls than in coeliac infants but the differences were not significant.

The results obtained by FISH using 16S rRNA probes targeting the main intestinal bacterial groups are shown in Table 2. Overall, these results confirmed the trends and

\*For correspondence: yolsanz@iata.csic.es

Microbial group	Coeliac children (n=26)		Control children (n=23)	
	Median	Range	Mean	SD
Total anaerobs	10.0	10.9–9.2	10.1	11.2–8.0
<i>Bacteroides</i>	8.4*	8.6–6.0	6.2	7.7–4.0
<i>Clostridium</i>	8.5*	9.0–5.3	5.9	8.3–5.0
<i>Bifidobacterium</i>	8.3	8.6–5.3	8.7	9.4–7.9
<i>Lactobacillus</i>	6.2	7.4–5.2	6.3	8.4–5.0
<i>Enterococcus</i>	7.7	9.4–5.8	7.2	8.7–5.3
<i>Enterobacteriaceae</i>	7.9	9.2–6.8	7.8	8.7–6.8
<i>Staphylococcus</i>	5.7*	7.7–3.7	4.3	5.8–2.1
Yeast	3.5	5.9–1.7	3.2	4.9–2.0

\* Significant differences between coeliac and control subjects ( $p < 0.05$ ).

major differences detected between the two population groups by culture techniques. The counts of *Bacteroides-Prevotella* group were significantly higher ( $p < 0.05$ ) in coeliac samples than in controls. The numbers of *Clostridium histolyticum* and *Eubacterium rectale-C. coccoides* groups were also significantly higher ( $p < 0.05$ ) in coeliac samples, indicating that both clostridial groups contributed to the differences detected in total clostridial levels by culture techniques. The *C. histolyticum* probe allows the detection of *Clostridium* clusters I and II (Collins *et al.*, 1994), including a representative number of the known clostridial species commonly found in the infant gut such as *C. paraputrificum*, *C. butyricum*, and *C. perfringens* as well as *Eubacterium multiforme* (Stark *et al.*, 1982; Franks *et al.*, 1998). The *E. rectale-C. coccoides* probe allows the detection of *Clostridium* clusters XIVa and b (Collins *et al.*, 1994), which also includes other species of the genera *Eubacteria*, *Clostridium* and *Ruminococcus* (Franks *et al.*, 1998). Therefore, other bacterial groups besides clostridia could contribute to the differences found by FISH analysis using these two probes. The numbers of sulphate-reducing bacteria (SRB) were significantly higher ( $p < 0.05$ ) in coeliac infants than in controls. The counts of the *Atopobium* group (which includes the *Coriobacterium*

group and comprises the family *Coriobacteriaceae* without the genera *Slakia* and *Denitrobacterium*; Harmsen *et al.*, 2000b) were also significantly higher ( $p < 0.05$ ) in coeliac infants than in healthy controls while significant differences were not observed for the *Coriobacterium* group.

## Discussion

This study has demonstrated for the first time the specific bacterial groups that define the alterations of the intestinal microecology of coeliac children at the presentation of the disease. Increased levels of *Bacteroides*, *Clostridium*, and SRB were detected in fecal samples of coeliac children as reported for inflammatory bowel diseases, which are also characterized by T-helper 1 polarized responses (Stator, 2004). In these cases, mucosal cytokine profiles are associated with up-regulation of inflammatory cytokines (mainly IFN gamma), with concurrent induction of nitric oxide synthase, which are known to alter regulation of cell junction proteins and thus barrier integrity (De Stefano *et al.*, 2006; Tagkalidis *et al.*, 2006). This inflammatory milieu could lead to modifications of the intestinal microbiota in coeliac infants. Otherwise, the alterations detected in the intestinal microbiota could contribute to the pathological process by increasing the permeability of the mucosa to

Microbial group	Coeliac children (n=26)		Control children (n=23)	
	Median	Range	Median	Range
Total microbial counts <sup>a</sup>	11.1	11.7–10.3	11.2	11.2–10.2
<i>Bacteroides/Prevotella</i>	8.7*	10.4–7.4	6.7	9.1–5.1
<i>Clostridium histolyticum</i>	6.9*	10.1–5.6	6.2	9.3–3.1
<i>Clostridium lituseburense</i>	6.4	9.1–3.5	6.1	11.1–3.7
<i>E. rectale-C. coccoides</i>	9.1*	10.5–7.1	8.1	9.7–4.8
<i>Bifidobacterium</i>	7.6	10.3–5.0	8.3	11.2–5.2
<i>Lactobacillus-Enterococcus</i>	7.2	10.5–3.0	6.5	10.4–3.2
<i>E. coli</i>	7.3	10.8–4.8	6.6	9.9–4.2
<i>Atopobium</i>	9.3*	10.4–8.3	7.3	10.4–5.3
<i>Coriobacterium</i>	8.6	9.7–6.2	8.3	8.9–3.0
Sulphate-reducing bacteria	8.4*	11.3–6.4	7.4	10.9–4.9

<sup>a</sup>Assessed by 4', 6-diamidino-2-phenylindole (DAPI) staining.

\*Significant differences between coeliac and control children ( $p < 0.05$ ).

gluten antigens and promoting gut inflammation through the activation of macrophages and dendritic cells with production of pro-inflammatory cytokines (Kagnoff, 2005). In patients suffering from ulcerative colitis and Crohn's disease the concentration of *Bacteroides* associated with the mucosa has been shown to be higher and increased with the severity of the disease (Swidsinski *et al.*, 2002). The species *Bacteroides vulgatus* and *B. fragilis* are thought to be implicated in the pathogenesis of these diseases by triggering inflammatory responses (Setoyama *et al.*, 2003; Medina *et al.*, 2005). In addition, induction of intestinal inflammation has been detected in rodent models of colitis mono-associated with *Bacteroides vulgatus* (Setoyama *et al.*, 2003). Although the present study was only focused on the fecal microbiota, a similar role of species of the *Bacteroides* genus in CD cannot be disregarded. In contrast to what we detected in coeliac infants, a higher presence of *Clostridium* groups has not been reported in patients with inflammatory bowel diseases (Sokol *et al.*, 2006). Nevertheless, the clostridium toxins were found to exacerbate inflammation in patients with chronic colitis (Stator *et al.*, 2004). The presence of higher levels of SRB in coeliac patients could be of clinical significance for their recognized contribution to the generation of hydrogen sulphide as end metabolic product, which is a highly cytotoxic compound. Hydrogen sulphide can inhibit butyrate oxidation, which is the primary energy source of colonocytes leading to cell death, epithelia atrophy, and chronic inflammation (Babidge *et al.*, 1998). As a consequence, SRB have been proposed to play a role in inflammatory bowel diseases and cancer (Pitcher and Cummings, 1996). The high prevalence of other bacterial groups such as *Clostridium* in coeliac infants could also favour the growth of SRB by supplying the hydrogen necessary for their particular mode of energy generation. In addition, a higher cell turnover of the small intestine is expected to occur in coeliac patients as a reflection of the crypt hyperplasia that develops with villous atrophy. This could lead to an increase in the availability of endogenous substrates such as chondroitin sulfate, mucins, and endogenous amino acids derived from the expelled cells, which could promote the growth of SRB. The abundance of the integrants of the *Coriobacterium* group and the *Atopobium* clusters has been associated with the type of feeding and infant age, but only some species of the *Coriobacterium* group have been isolated from patients with colon cancer and inflammatory bowel diseases (Harmsen *et al.*, 2000b). The relevance of the higher prevalence of the *Atopobium* group in coeliac patients remains to be investigated. The aerobic genus *Staphylococcus* was also present in higher levels in fecal samples of coeliac patients. A specific higher prevalence of *Saphylococcus* has only been found in fecal samples of allergic infants (Bjorksten *et al.*, 2001).

The information about the relationships between coeliac disease and the gut microbiota is scarce. Moreover, epidemiological and clinical evidence have suggested a role for the microbiota in CD. In the present study, the bacterial pattern of coeliac children at the presentation of the disease was also in agreement with the differences found between the fecal microbiota of Swedish and Estonian infants, two population groups

with high and low incidence of CD, respectively. In that study, the former population group also showed increased levels of *Clostridium* and *Bacteroides* (Sepp *et al.*, 1997). Recent studies also indicated that levels of total SCFA, as well as acetic, valeric and butyric acids were higher in fecal samples of coeliac patients than in those of healthy controls (Tjellström *et al.*, 2005). In this study, the higher prevalence of bacterial groups (*Bacteroides*, and *Clostridium*) that are important producers of SCFA and particularly of butyric acid (*E. rectale-C. coccoides* group; MacFarlane and Gibson, 1997; Hold *et al.*, 2003) found in coeliac children could account for the alteration of SCFA previously reported by other authors (Tjellström *et al.*, 2005).

In summary, this study has provided the first evidence of the bacterial groups that are responsible for microecological changes in the intestinal tract of coeliac infants. The bacterial groups overrepresented in these patients could be either a consequence of the pro-inflammatory status of individuals with symptomatic coeliac disease or involved in its presentation and evolution. Further studies should be carried out to determine whether the microbial unbalance persists after remission under a gluten-free diet in order to progress in the understanding of the role of the microbiota in this pathology.

## Experimental procedures

### Subjects and sampling

The studied population comprised a total of 49 children, 26 coeliac patients (medium age 26.3 months, range 12–48 months) and 23 age-matched controls (medium age 23.0 months, range 11–45 months). The diagnosis of CD was based on clinical symptoms, positive detection of coeliac serology markers (antigliadin, antiendomysial and antitransglutaminase antibodies), and small duodenal biopsy showing severe enteropathy.

Fecal samples from both population groups were immediately kept at 4°C, under anaerobiosis using AnaeroGen sachets (Oxoid, Hampshire, UK), and analysed in less than 12 h. Samples from coeliac children were collected at the presentation of the disease and still following a normal-gluten diet. The children included in the study were not treated with antibiotics for at least one month before the sampling time.

The study protocol was approved by the Committee on Ethical Practice of General University Hospital and CSIC, and infants were enrolled in the study after written informed consent obtained from their parents.

### Analysis of cultivable fecal bacteria using plate culture media

Fecal samples (2 g wet weight) were 10-fold diluted in phosphate-buffered saline (PBS, 130 mM sodium chloride, 10 mM sodium phosphate, [pH 7.2]), and homogenized in a Lab Blender 400 stomacher (Seward Medical London, UK). Appropriate serial decimal dilutions were made in PBS and aliquots were plated on each agar media in duplicated. Total anaerobes were enumerated on Wilkins-Chalgren agar (Oxoid, Hampshire, England), *Bifidobacterium* on BFM agar (Nebra and Blanch, 1999),

*Clostridium* on Reinforced Clostridial Medium (Oxoid, Hampshire, England) supplemented with novobiocin (8 mg/L) and colistin (8 mg/L.), *Bacteroides* on Schaedler agar (Scharlau, Barcelona, Spain) supplemented with kanamycin (100 mg/L), vancomycin (7.5 mg/L) and vitamin K (0.5 mg/L). In all these cases, plates were incubated in anaerobic conditions at 37°C for 72 h. *Enterobacteriaceae* were enumerated on VRBD agar (Scharlau, Barcelona, Spain) and *Staphylococcus* on Baird Parker agar (BP, Scharlau, Barcelona, Spain) after incubation in anaerobic conditions at 37°C for 48 h. *Lactobacillus* were enumerated on Rogosa Agar (Scharlau, Barcelona, Spain) after incubation in anaerobiosis at 30°C for 72 h. *Enterococcus* were enumerated on kanamycin-esculine agar (KAA, Scharlau, Barcelona, Spain) and yeast in Bengale Pink agar (Scharlau, Barcelona, Spain), after incubation under aerobic conditions at 30°C for 72 h. The identity of representative colonies recovered from each selective medium was confirmed by conventional microbiological methods including colony and cellular morphology, Gram staining, biochemical test using the API systems (BioMerieux, Lyon, France) and antibiotic susceptibility assays (Simpson *et al.*, 2004). Bacterial counts were expressed as the log of the number of colony forming units (CFU) per gram of wet-weight feces.

#### Analysis of fecal bacteria by fluorescent *in situ* hybridization (FISH)

One volume of the first decimal dilution of the fecal samples was added to three volumes of 4% paraformaldehyde (PFA) in PBS and fixed at 4°C overnight. After fixation, bacteria were washed twice in PBS by centrifugation (12000 rpm for 5 min). The bacterial pellets were suspended and stored in 50% ethanol-PBS at -80°C until use. The enumeration of cells present in fecal samples was carried out by fluorescent *in situ* hybridization (FISH) using fluorescein isothiocyanate (FITC)-labelled oligonucleotide probes as described elsewhere (MOLBIOL, Berlin, Germany; Collado *et al.*, 2006). The group- and genus-specific probes and hybridization conditions used in this study are summarized in Table 3. Total cell numbers were enumerated using the nucleic acid stain 4', 6-diamidino-2-phenylindole (DAPI). Briefly, fixed cell suspensions were hybridized with the corresponding probe in hybridization

buffer (10 mM Tris-HCl, 0.9 M NaCl and 10% SDS) overnight. Then, cells were washed with the same buffer but without SDS, applied to a 0.2 µm polycarbonate filter (Millipore Corporation, Bedford, USA) and mounted on a glass slide. Slides were visualized in a Nikon Eclipse E800 microscope (Nikon corporation, Tokyo, Japan). Results were the average of 20 fields counted per sample and probe, and were expressed as the log of cells per gram of wet-weight feces.

#### Statistical analysis

The differences in bacterial counts between control and coeliac children were determined by applying the Mann-Whitney U-test using StatGraphics software (Manugistics, Rockville, MD, USA). Significant differences were established at a confidence level of 95% (*p* value less than 0.05).

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#### References

- Babidge W., Millard, S. and Roediger, W. (1998). Sulfides impair short chain fatty acid beta-oxidation at acyl-CoA dehydrogenase level in colonocytes: implications for ulcerative colitis. *Mol. Cell. Biochem.* **181**, 117–24.
- Bjorksten, B, Sepp, E., Julge, K., Voor, T and Mikelsaar, M. (2001). Allergy development and the intestinal microflora during the first year of life. *J. Allergy Clin. Immunol.* **108**, 516–20.
- Bullock, N.R., Booth, J.C. and Gibson, G.R. (2004). Comparative composition of bacteria in the human intestinal microflora during remission and active ulcerative colitis. *Curr. Issues Intestinal Microbiol.* **5**, 59–64.
- Collado, M.C., Moreno, Y., Cobo, J.N., Mateos, J.A. and Hernández, M. (2006). Molecular detection of *Bifidobacterium animalis* DN-173010 in human faeces

Table 3. Oligonucleotide probes and hybridization conditions used in FISH analysis of fecal bacteria.

Probe	Target bacterial group	Sequence (5'–3')	Hybridization conditions	References
Bif164	<i>Bifidobacterium</i>	CATCCGGCATTACCACCC	50	Langendijk <i>et al.</i> (1995)
Bac303	<i>Bacteroides/Prevotella</i>	CCAATGTGGGGGACCTT	45	Manz <i>et al.</i> (1996)
Erec0482	<i>Eubacterium rectale/Clostridium coccoïdes</i>	GCT TCT TAG TCA GGTACCG	50	Franks <i>et al.</i> (1998)
CLis135	<i>Clostridium lituseburense</i>	GTT ATC CGT GTG TAC AGG G	50	Hold <i>et al.</i> (2003)
CHis150	<i>Clostridium histoyiticum</i>	TTA TGC GGT ATT AAT CT(C/T) CCT TT	50	Harmsen <i>et al.</i> (2000a)
Lac168	<i>Lactobacillus/Enterococcus</i>	GGT ATT AGC A(C/T)C TGT TTC CA	45	Harmsen <i>et al.</i> (2000a)
Ecol1513	<i>Escherichia coli</i>	CAC CGT AGT GCC TCG TCA TCA	37	Poulsen <i>et al.</i> (1994)
Ato291	<i>Atopobium</i>	GGTCGGTCTCTCAACCC	50	Harmsen <i>et al.</i> (2000b)
Cor653	<i>Coriobacterium</i>	CCC TCC C(A/C)A CCG GAC CC	50	Harmsen <i>et al.</i> (2000b)
SBR	Sulphate-reducing bacteria	TAC GGA TTT CAC TCC T	48–50	Bullock <i>et al.</i> , (2004)

- during fermented milk administration. *Food Res. Int.* 39, 530–535.
- Collins, M.D., Lawson, P.A., Willems, A., Cordoba, J.J., Fernandez-Garayzabal, J., Garcia, P., Cai, H., Hippe, J. and Farrow, J.A. (1994). The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int. J. Syst. Bacteriol.* 44, 812–826.
- Cummings, J.H., Macfarlane, G.T. and Macfarlane, S. (2003). Intestinal bacteria and ulcerative colitis. *Curr. Issues Intestinal Microbiol.* 4, 9–20.
- De Stefano, D., Maiuri, M.C., Iovine, B., Ialenti, A., Bevilacqua, M.A. and Carnuccio, R. (2006). The role of NF-kappaB, IRF-1, and STAT-1alpha transcription factors in the iNOS gene induction by gliadin and IFN-gamma in RAW 264.7 macrophages. *J. Mol. Med.* 84, 65–74.
- Fasano, A. and Catassi, C. (2005). Coeliac disease in children. *Best Pract. Res. Clin. Gastroenterol.* 19, 467–78.
- Forsberg, G., Fahlgren, A., Horstedt, P., Hammarstrom, S., Hernell, O. and Hammarstrom, M.L. (2004). Presence of bacteria and innate immunity of intestinal epithelium in childhood coeliac disease. *Am. J. Gastroenterol.* 99, 894–904.
- Franks, A.H., Harmsen, H.J., Raangs, G.C., Jansen, G.J., Schut, F. and Welling, G.W. (1998). Variations of bacterial populations in human feces measured by fluorescent *in situ* hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Appl. Environ. Microbiol.* 64, 3336–45.
- Harmsen, H.J.M., Gibson, G.R., Efferich, P., Degener, J.E. and Welling, G.W. (2000a). Comparison of viable cell counts and fluorescence *in situ* hybridization using specific rRNA-based probes for the quantification of human faecal bacteria. *FEMS Microbiol. Lett.* 183, 125–129.
- Harmsen H.J.M., Wildeboer-Veloo, A.C., Grijpstra, J., Knol, J., Degener, J.E. and Welling, G.W. (2000b). Development of 16S rRNA-based probes for the *Coriobacterium* group and the *Atopobium* cluster and their application for enumeration of Coriobacteriaceae in human feces from volunteers of different age groups. *Appl. Environ. Microbiol.* 66, 4523–4527.
- Hold, G.L., Schwiertz, A., Aminov, R.I., Blaut, M. and Flint, H.J. (2003). Oligonucleotide probes that detect quantitatively significant groups of butyrate-producing bacteria in human feces. *Appl. Environ. Microbiol.* 69, 4320–4324.
- Ivansson, A., Hernall, O., Stenlund, H. and Persson, L.A. (2002). Breast-feeding protects against coeliac disease. *Am. J. Clin. Nutr.* 75, 914–21.
- Ivansson, A., Hernall, O., Nyström, L. and Persson, L.A. (2003). Children born in the summer have increased risk for coeliac disease. *J. Epidemiol. Community Health* 57, 36–9.
- Isolauri, E., Sütas, Y., Kankaanpää, P., Arvilommi, H. and Salminen, S. (2001). Probiotics: effects on immunity. *Am. J. Clin. Nutr.* 73, 444S–450S.
- Kagnoff, M.F. (2005). Overview and pathogenesis of coeliac disease. *Gastroenterology* 128, S10–S18.
- Langendijk, P.S., Schut, F., Jansen, G.J., Raangs, G.C., Kamphuis, G.R., Wilkinson, M.H.F. and Welling, G.W. (1995). Quantitative fluorescence *in situ* hybridization of *Bifidobacterium* spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples. *Appl. Environ. Microbiol.* 61, 3069–3075.
- Louka, A.S. and Sollid, L.M. (2003). HLA in coeliac disease: unraveling the complex genetics of a complex disorder. *Tissue Antigens* 61, 105–117.
- MacFarlane, G.T. and Gibson, G.R. (1997). Carbohydrate fermentation, energy transduction and gas metabolism in the human large intestine. In *Human Health. The contribution of microorganisms*, R.I. Mackie and B.A. White. London: UK: Springer-Verlag. pp. 17–52.
- Majamaa, H. and Isolauri, E. (1996). Evaluation of the gut mucosal barrier: evidence for increased antigen transfer in children with atopic eczema. *J. Allergy Clin. Immunol.* 97, 985–990.
- Manz, W., Amann R., Ludwig, W., Vancanneyt, M. and Schleifer, K.H. (1996). Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum Cytophaga-Flavobacter-Bacteroides in the natural environment. *Microbiology* 142, 1097–1106.
- Medina, C., Santana, A., Llopis, M., Paz-Cabrera, M.C., Antolin, M., Mourelle, M., Guarnier, F., Vilaseca, J., Gonzalez, C., Salas, A., Quintero, E. and Malagelada, J.R. (2005). Induction of colonic transmural inflammation by *Bacteroides fragilis*: implication of matrix metalloproteinases. *Inflamm. Bowel Dis.* 11, 99–105.
- Nebra, Y. and Blanch, A.R. (1999). A new selective medium for *Bifidobacterium* spp. *Appl. Environ. Microbiol.* 65, 5173–6.
- Pitcher, M.C. and Cummings, J.H. (1996). Hydrogen sulphide: a bacterial toxin in ulcerative colitis? *Gut.* 39, 1–4.
- Poulsen, L.K., Lan, F., Kristensen, C.S., Hobolth, P., Molin, S. and Krogfelt, K.A. (1994). Spatial distribution of *Escherichia coli* in the mouse large intestine inferred from rRNA *in situ* hybridization. *Infect. Immun.* 62, 5191–4.
- Sanz, Y. and Collado, M.C. (2005). The role of *Bifidobacterium* in the host defense against pathogenic microorganisms. In *Recent Research Developments in Microbiology*, S.G. Pandalai. Trivandrum, India: Research Signpost, Vol 9 pp. 271–287
- Sartor, B.R. (2004). Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. *Gastroenterology* 126, 1620–1633.
- Sepp, E., Julge, K. and Vasar, M. (1997). Intestinal microflora of Estonian and Swedish infants. *Acta Paediatr.* 86, 956–961.
- Setoyama, H., Imaoka, A., Ishikawa, H. and Umesaki, Y. (2003). Prevention of gut inflammation by *Bifidobacterium* in dextran sulfate-treated gnotobiotic mice associated with *Bacteroides* strains isolated from ulcerative colitis patients. *Microbes Infect.* 5, 115–22.
- Simpson, P.J., Fitzgerald, G.F., Stanton C. and Ross, R.P. (2004). The evaluation of a mupirocin-based selective

- medium for the enumeration of bifidobacteria from probiotic animal feed. *J. Microbiol. Methods*, 57, 9–16.
- Sokol, H., Seksik, P., Rigottier-Gois, L., Lay, C., Lepage, P., Podglajen, L., Marteau, P. and Dore, J. (2006). Specificities of the fecal microbiota in inflammatory bowel disease. *Inflamm. Bowel Dis.* 12,106–11.
- Stark, P.L. and Lee A. (1982). Clostridia isolated from the faeces of infants during the first year of life. *J. Pediatr.* 100, 362–365.
- Swidsinski, A., Ladhoff, A., Pernthaler, A., Hale, L.P. and Lochs, H. (2002). Mucosal flora in inflammatory bowel disease. *Gastroenterology* 122, 44–54.
- Tagkalidis, P.P., Gibson, P., Bhathal, P.S. (2006). Microscopic colitis demonstrates a TH1 mucosal cytokine profile. *J. Clin. Pathol.* Jun 14; [Epub ahead of print]
- Tjellstrom B., Stenhammar, L., Hogberg, L., Falth-Magnusson, K., Magnusson, K.E., Midtvedt, T., Sundqvist, T. and Norin, E. (2005). Gut microflora associated characteristics in children with coeliac disease. *Am. J. Gastroenterol.* 100, 2784–8.