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REVIEW ARTICLE

# Celiac disease microbiota and its applications

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**Abstract** Intestinal microbiota plays an important role in maintaining the overall health of an individual. It can be affected by diet but also inflammation of the intestine due to various causes. In the last decade, particular attention has been paid to the study of the interaction between mucosal cells and intestinal microbiota, and to the host immune response to change in community structure. Here, we review the most significant studies on human microbiota in patients with celiac disease, and also the potential biotechnological use of microorganisms for the production of gluten-free products.

**Keywords** Adolescence · Gluten-free · Microbiology · Sorghum

## Introduction

Most microorganisms in the human microbiota are in the intestine. The intestinal microbiota plays an important role in metabolic and nutritional processes, but also has a defensive role in assuring intestinal health. In recent years, special attention has been paid to studying the intestinal microbiota in intestinal disease, as well as changes in the microbiata with dietary variations and with age.

An important therapeutic strategy for many mucosal and non-mucosal immune-related conditions is to harness the skill of microbiota to disturb host immunity (Ivanov and Honda 2012). Alterations in microbial flora accompany various medical conditions (Serban 2011), such as inflammatory bowel diseases and celiac disease (CD). The intestinal microbiota also changes substantially during aging (Cheng et al. 2011). This review discusses important recent discoveries regarding microbiota in relation to gluten-free diet (GFD) in patients with CD.

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## Celiac disease

### Immunopathological state of CD

Under immunopathological conditions, e.g., during infection, inflammatory bowel disease and CD, both epithelial cells and intraepithelial lymphocytes participate substantially in inflammatory reactions (Tlaskalová-Hogenová et al. 1995). Intestinal microbiota influence the Th1 pro-inflammatory milieu characteristic of CD (Medina et al. 2008). Serological responses to different microbial antigens have been demonstrated (Ashorn et al. 2009). Persons with selective IgA deficiency carry an increased risk of CD. The microbiota of IgA-deficient people is characterized by an increased frequency of *Escherichia coli* with inflammatogenic potential, and can thus contribute to the development of inflammatory bowel diseases (Friman et al. 2002). A reduction in IgA-coated bacteria is associated with intestinal dysbiosis, providing new insights into the possible relationships between gut microbiota and host defences in CD patients (De Palma et al. 2010).

### Celiac disease studies

CD patients with intestinal enzymopathy have, in general, diminished protective flora and increased hemolytic and lactose-negative enterobacteria (Kamilova et al. 2001). The levels of *Bacteroides*, *Clostridium* and *Staphylococcus* were much higher ($P<0.05$) in fecal samples from CD patients than in those of healthy subjects when analyzed by culture methods (Collado et al. 2007). The species *Lactobacillus curvatus*, *Leuconostoc mesenteroides* and *Leuconostoc carnosum* were characteristic of CD patients, while those of the *Lactobacillus casei* group were characteristic of healthy controls (Sanz et al. 2007).

Analysis of fecal samples and duodenal biopsies using denaturing gradient gel electrophoresis specific for total bacteria, lactobacilli and bifidobacteria the microbial population showed that bacteria adhering to duodenal biopsies were not dominating in stool samples (Kopecný et al. 2008). A peculiar microbial temporal temperature gradient gel electrophoresis profile and a significant higher biodiversity were identified in the duodenal mucosa of CD pediatric patients. *Bacteroides vulgatus* and *E. coli* were detected more often in CD patients than in controls ($P<0.0001$) (Schippa et al. 2010). Thus, virulence features of the enteric microbiota are linked to CD (Sánchez et al. 2008).

Examination of differences in *Bacteroides* spp. and their pathogenic features between CD patients and controls revealed an increased abundance of *Bacteroides fragilis* strains with metalloprotease activities, which could play a role in CD pathogenesis (Sánchez et al. 2012a). Two novel, obligately anaerobic, Gram-stain-positive, saccharolytic and non-proteolytic spore-forming bacilli [strains CD3:22(T) and N1 (T)] were described. One of them, strain CD3:22(T) was isolated from a biopsy of the small intestine of a child with CD (Hedberg et al. 2012).

### Active and non-active celiac disease

The proportions of total bacteria and Gram-negative bacteria were much higher in CD patients with active disease than in symptom-free CD patients and controls (Nadal et al. 2007). Reductions in total *Bifidobacterium* and *Bacteroides longum* populations were associated with both active and non-active CD when compared to controls (Collado et al. 2008). *Bacteroides*, *Bifidobacterium* and lactic acid bacteria (LAB) populations in the duodenum of children with typical CD (active and treated) and controls differ in diversity and species composition; this could help illustrate features of the disease (Sánchez et al. 2010). Increased abundance of *Staphylococcus epidermidis* carrying the *mecA* gene in active and non-active CD patients most likely reflects increased exposure of these subjects to opportunistic pathogens and antimicrobials (Sánchez et al. 2012b).

### Clinical implications

Cesarean delivery can affect the early biodiversity of intestinal bacteria (Biasucci et al. 2008). An association was described between cesarean delivery and CD. Alterations of the mucosal host-microbial interaction during the neonatal period might contribute to the pathogenesis of inflammatory diseases such as CD (Decker et al. 2011).

Breast milk is the gold-standard feeding method during infancy for optimal nutrition (Salvatore et al. 2008). But milk-feeding type can also play a role in CD (Sanz et al. 2011). The effects of milk-feeding practices on intestinal colonization of *Bacteroides* species in infants at risk of CD development was investigated. Breast-fed infants showed a higher prevalence of *Bacteroides uniformis* at 1 and 4 months of age, while formula-fed infants had a higher prevalence of *Bacteroides intestinalis* at all sampling times, of *Bacteroides caccae* at 7 days and 4 months, and of *Bacteroides plebeius* at 4 months. The conclusion was that both the type of milk feeding influences the colonization process of *Bacteroides* species, and possibly disease risk (Sánchez et al. 2011). Another study came to a similar conclusion, i.e., that milk-feeding type plays a role in establishing infants' gut microbiota (De Palma et al. 2012).

## Gluten-free diet

### Gluten-free diet and CD

The accepted treatment for CD is a gluten-free diet (GFD) (Samaşca et al. 2013). However, GFD influences the structure of the gut bacterial population and its metabolism markedly (Kopecný et al. 2006). GFD has been shown to lead to reductions in beneficial gut bacteria populations and stimulate the host's immunity (De Palma et al. 2009). The analysis of fecal microbiota and dietary intake after GFD indicated that the numbers of healthy gut bacteria decreased, while numbers of unhealthy bacteria increased parallel to reductions in the intake of polysaccharides in CD adults (Sanz 2010). In conclusion, there are bacterial differences in the upper small bacteria microbiota between treated and untreated CD adults due to treatment with a GFD (Nistal et al. 2012). Duodenal and fecal microbiotas are unbalanced in children with untreated CD and only partially restored after long-term treatment with GFD (Collado et al. 2009). A lower ratio of cultivable LAB and *Bifidobacterium* to *Bacteroides* and enterobacteria in CD children after GFD compared to healthy children was observed—even lower in CD children before GFD(Di Cagno et al. 2009). Therefore, GFD lasting at least 2 years did not completely restore the microbiota and, thus, the metabolome of CD children (Di Cagno et al. 2011). So what are the solutions?

### Plant extract solutions

The crude water-soluble extract from amaranth seeds has an inhibitory effect towards many fungal species isolated from bakeries and is used as an ingredient in the manufacture of gluten-free (GF) and wheat bread (Rizzello et al. 2009), extending the shelf-life of gluten-free and wheat flour breads. The alcohol-soluble fraction from some cereals (tef, millet, amaranth, and quinoa) has been shown to be safe for diets of CD patients, with the absence of any immune cross-reactivity of them toward wheat gliadin being of note (Bergamo et al. 2011). The aqueous extract of kiwifruit puree, which has good stability and contains polyphenols and antioxidants, was also considered as a functional ingredient for GF bread (Sun-Waterhouse et al. 2009). But the replacement of gluten still presents a significant technological challenge, as it is an essential structure-building protein necessary for formulating high-quality baked goods (Zannini et al. 2012a).

### Sourdough biotechnologies

Sourdough has been used since ancient times for the production of rye and wheat bread; its universal usage has been attributed to the improved quality, nutritional properties and shelf life of sourdough-based breads (Arendt et al. 2011). Producing sourdough bread that is tolerated by CD patients has been an important focus for many researchers (Di Cagno et al. 2004)with biotechnologies that use selected sourdough LAB and probiotics and their potential to decrease the risk of gluten contamination in gluten-free products being investigated (Gobbetti et al. 2007).

*Lactobacillus plantarum* CRL 759 and CRL 778 have an active proteolytic system that is responsible for high amino acid release during sourdough fermentation and the hydrolysis of 31–43 alpha-gliadin-like fragments (Rollán et al. 2005). The kinetics of the hydrolysis of this 33-mer by lactobacilli were highly efficient (Rizzello et al. 2007). The ability of LAB strains to degrade alpha-gliadin fragments is not correlated to individual peptidase activities. Results have shown that several strains separately degraded 31–43 and 62–75 alpha-gliadin fragments, while a mixture of peptidase profiles from pooled LAB strains was associated with 57–89 peptide degradation (Gerez et al. 2008). Other studies supported the same conclusion. The use of sourdough in GF baking was suggested as a new frontier for increasing the quality, safety and acceptability of GF bread (Moroni et al. 2009).

The use of a pool of selected enterococci and fungal proteases (*Rhizopus oryzae*) to hydrolyze wheat gluten during long-duration fermentation was investigated. This microbial and protein cocktail showed a decrease in the gluten concentration of more than 98 % during longer fermentation (M'hir et al. 2009). The biodiversity of spontaneous environmental biota from buckwheat and teff sourdoughs showed a broad spectrum of yeast species and LAB. The selection of dominant yeast and *Lactobacillus* species as starters for the production of sourdough destined for GF bread production was suggested (Moroni et al. 2011). Another promising approach to improve bread-making for GFD can be to manipulate microbial exopolysaccharide amounts to reach effectual levels through optimization, as corroborated by the application of microbial exopolysaccharide-forming starter cultures (Rühmkorf et al. 2012; Galle et al. 2012). The use of two lactic bacteria (*Lactobacillus plantarum* CRL 775 and *Pediococcus pentosaceus* CRL 792) as cell free extract and pooled cells showed a higher efficiency of cell extracts in the reduction of gliadin concentration, thus use of cell-free extract may be considered a practical alternative technology for the manufacture of GF products (Gerez et al. 2012). Microbial fermentation by LAB and yeast is considered one of the most ecological/economical methods of producing and preserving food (Zannini et al. 2012b).

## Conclusions

The role of intestinal microbial flora in certain pathologies such as CD currently remains unknown. Most studies in this area have revealed changes in gut microbiota but not their role in the evolution of CD pathology. Bacterial species other than those present in healthy subjects prevail in the intestinal microbiota of patients with CD. The most effective treatment in CD, i.e., GFD, does not contribute to restoring of the intestinal microbiota, with the observed imbalance being maintained even after treatment. Meanwhile, microbial biotechnology can provide solutions for making gluten-free products.

**Conflict of interest** The authors report no conflicts of interest.

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