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SUMMARY

Celiac disease (CD) is a common chronic autoimmune enteropathy caused by gluten intake. To date, the only therapy for CD is the complete exclusion of dietary sources of grains and any food containing gluten. It has been hypothesized that the intestinal microbiota is somehow involved in CD. For this reason, probiotics are appearing as an interesting adjuvant in the dietetic management of CD. This review aims to discuss the characteristics of the microbiota in CD subjects and the use of probiotics as a novel therapy for CD. Comparisons between children with CD and controls show that their microbiota profiles differ; the former have fewer lactobacilli and bifidobacteria. Specific probiotics have been found to digest or alter gluten polypeptides. It has also been demonstrated that some bacterial species belonging to the genera *Lactobacillus* and *Bifidobacterium* exert protective properties on epithelial cells from damage caused by gliadin.

INTRODUCTION

Celiac disease (CD) is a common chronic lifelong autoimmune enteropathy triggered by the consumption of specific proteins by genetically predisposed individuals (1, 2). Such proteins are present specifically in cereals and receive specific names according to the food source, such as gliadin (present in wheat), hordein (present in barley), and secalin (present in rye) (Fig. 1). As these proteins share structural similarities, they are collectively known as gluten (3, 4). Among gluten proteins, two main fractions can be distinguished: the soluble gliadins and the insoluble glutenins. Both groups are characterized by high glutamine and proline contents (5).

Genetic predisposition is an important aspect of CD. It is associated mostly with the human leukocyte antigen (HLA-DQ) system, which participates in the recognition of self and nonself molecules by the immune system. The variants HLA-DQ2 and/or -DQ8 as well as HLA-DP and HLA-DR are commonly observed in CD patients (6, 7). These gene variants produce receptors that bind to gliadin peptides more tightly than other forms of the antigen-presenting receptor. This may increase the likelihood for immune cell activation and autoimmunity. Additionally, proteases from the intestine of CD patients may inefficiently break down gluten peptides, therefore enhancing the availability of entire peptides. These may thus translocate through the intestinal

epithelial mucosa via either epithelial transcytosis or increased epithelial tight junction (TJ) permeability (2). In the lamina propria, HLA molecules present gluten peptides to CD4⁺ T immune cells (8), thus activating the secretion of Th1 cytokines, i.e., gamma interferon (IFN- γ) and tumor necrosis factor alpha (TNF- α), and matrix metalloproteinases. Together, this response promotes matrix degradation, mucosal remodeling, villous atrophy, crypt cell hyperplasia, and increases in intraepithelial cell numbers (9).

Therefore, an overload of peptides, such as gluten peptides, in the lamina propria may lead to a loss of tolerance to their epitopes in predisposed subjects. Peptide transport through intestinal mucosa, which is also regulated by TJ assembly, may be an important step in the development of CD (10). Thus, the disassembly of TJ and the consequent increased paracellular transport may favor this overload of peptides in the lamina propria and immune dysregulation. Emerging evidence strongly suggests that enhanced intestinal permeability is one of the factors involved in the development of various autoimmune disorders as well as CD (11–14). However, it is still not clear whether altered intestinal permeability is a primary cause or a consequence of CD and also if this alteration is induced by gluten itself, by alterations of the microbiota, or by a combination of both. Zonulin is a protein that exhibits the ability to reversibly modulate intercellular TJ (15). Gliadin activates zonulin signaling in CD patients, leading to increased intestinal permeability to macromolecules (16). On the contrary, some studies indicate that shifts in gut microbiota may also lead to increased intestinal permeability in diseases different from CD (17, 18).

In this context, it has been hypothesized that the microbiota is somehow involved in CD. In addition, probiotics appear to be an interesting adjuvant in the dietetic management of CD (Fig. 2). This review aims to discuss the characteristics of the microbiota of patients with CD and the application of probiotics as a novel therapy for CD.

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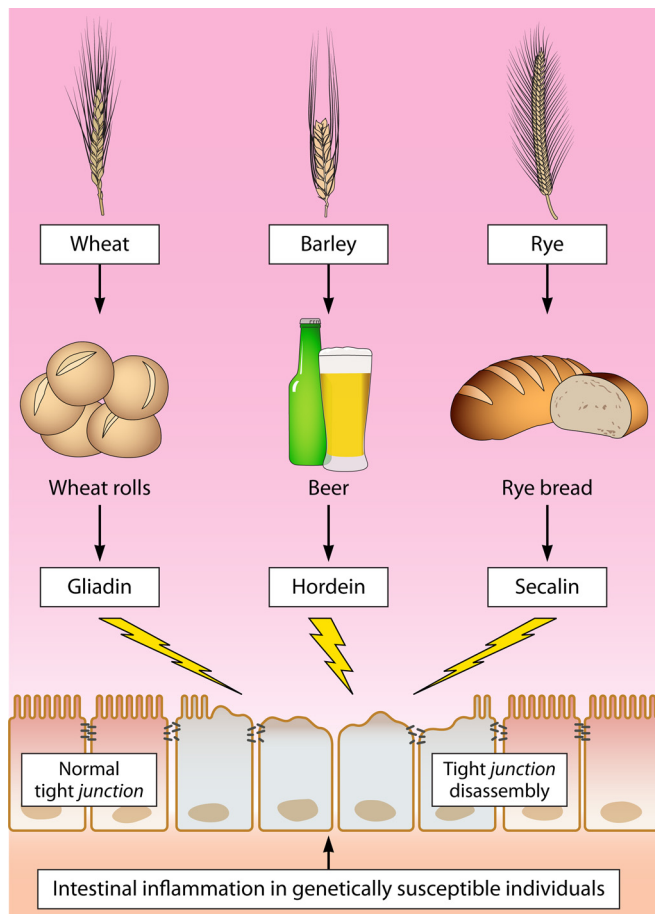


FIG 1 Different cereal-derived products and intestinal inflammation in CD subjects. Consumption of food-derived products containing wheat, barley, and rye by individuals genetically susceptible to CD leads to villous atrophy, intestinal inflammation, and disassembly of tight junctions.

MICROBIOTA AND CD

The human gastrointestinal tract is a complex and dynamic environment, sheltering a vast number and variety of commensal microorganisms (19). This balanced microecosystem provides the host a natural defense against invasion of potential pathogens. Recently, research has focused on the important role of the human intestinal microbiota in health and disease (20). Studies on the role of the gut microbiota in CD pathophysiology are still in their early stages. The main findings related to microbiota composition in CD subjects are summarized in Table 1.

CD is a common disorder in both children and adults (21). Nevertheless, our knowledge about the intestinal microbiota of adults with CD is still sparse. Indeed, studies characterizing the microbiota of adult CD patients only began in 2012 (12, 22). A year later, two studies concerning gut microbiota and CD were reported (23, 24). Studies before 2012 were conducted notably with children (13, 25–31). A single study of both children and adults reported a slight difference in the percentages of the main phyla between subjects and also a more diverse profile in duodenal biopsy specimens from adults (12). The *Firmicutes* are the most abundant bacteria in CD adults, while *Proteobacteria* are present mainly in CD children. Other phyla shared between CD adults and

CD children belong to the *Bacteroidetes* and *Actinobacteria*. Regarding bacterial genera, CD adults harbor larger numbers of *Mycobacterium* spp. and *Methylobacterium* spp., while *Neisseria* spp. and *Haemophilus* spp. are more abundant in CD children. Future studies should focus on the similarities between children and adults with CD compared with healthy controls. If the aim is to establish causality, a specific bacterial group might be expected to be pathogenic in both adults and children.

It is still not clear whether an altered microbiota in CD patients could be the cause or the consequence of this disease. It is hypothesized that Gram-negative bacteria in genetically susceptible individuals may contribute to the loss of tolerance to gluten. If a modified microbiota is a result of this disease, the disrupted mucosa overlaid by immature enterocytes could lead to conditions favoring Gram-negative instead of Gram-positive bacterial colonization. Duodenal biopsy specimens from untreated CD children showed higher total and Gram-negative populations than did treated CD and healthy control groups. Furthermore, the counts of Gram-positive bacteria were reduced in CD children (untreated and treated) compared to controls (26). Thus, the proportions of Gram-negative and Gram-positive bacteria seem to be of importance.

The possibility that unfavorable bacteria may colonize the intestinal mucosa indicates the need to evaluate the microbiota from this site. Sampling by biopsy is an invasive method in healthy individuals, while feces still remain the easiest and most noninvasive source of data collection. Even so, the numbers of studies of CD using biopsy specimens and those using fecal samples for microbiota characterization are almost the same so far. In general, clear differences between mucosa-associated microorganisms and fecal microbiota are expected (32, 33). Indeed, Ouwehand and collaborators (33) found 4-times-higher numbers of bifidobacteria in the feces of healthy infants than in the mucosa of a group with rectal bleeding. Corroborating this finding, Di Cagno and coworkers (31) did not find bifidobacteria in biopsy specimens of CD subjects but detected them in fecal samples. In addition, those authors showed that the level microbiota diversity was higher in fecal samples than in biopsy specimens (31). In contrast, Collado et al. (28) showed a high level of correlation between the fecal and biopsy specimen levels of *Bifidobacterium*, *Bacteroides*, *Staphylococcus*, *Clostridium coccoides*, *Clostridium leptum*, *Lactobacillus*, and *Escherichia coli* in untreated and treated CD patients and a control group. An *Akkermansia muciniphila* correlation was detected only in controls. Nevertheless, the presented data suggest that the unidentified part of the microbiota, especially in the mucosa, deserves more attention.

Comparison between CD children and controls shows that their microbiota profiles differ. Higher *Bacteroides* counts are detected in CD children (13, 26) than in controls. Particularly, *Bacteroides* bacteria are an important fraction of the human gut microbiota, and some species, such as *B. vulgatus* and *B. fragilis*, have been found to exhibit proinflammatory effects (34), indicating the importance of investigations of this group at the species level. Data on the levels of *Atopobium*, *Staphylococcus*, *E. coli*, *Eubacterium rectale-C. coccoides*, the *Clostridium histolyticum* group, *Clostridium lituseburense*, and sulfate-reducing bacteria are still contradictory, as there have been reports showing increased levels in CD patients (13) or no difference (26, 28, 30) in comparison to controls. Reports regarding the characterization of the main groups containing probiotic species, such as *Lactobacillus* and *Bifidobac-*

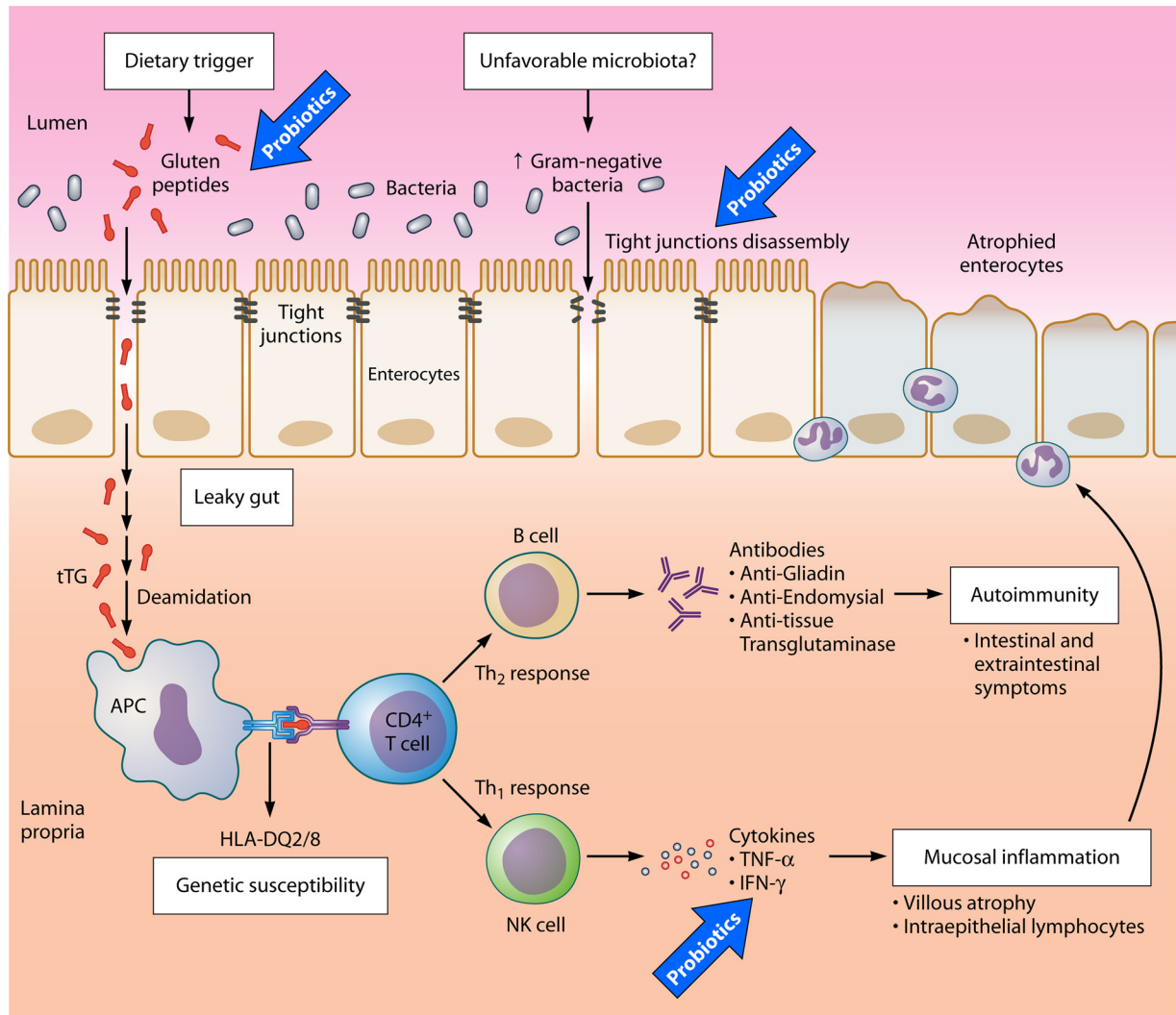


FIG 2 Inflammation process and possible routes of probiotic action in the maintenance of CD. In CD patients, increased epithelial tight junction permeability (“leaky gut”) favors the entrance of non-well-digested gluten peptides from the lumen to the lamina propria. Once there, they are deamidated by the tissue transglutaminase (tTG) enzyme and presented to $CD4^+$ T immune cells by the human leukocyte antigen (HLA) in antigen-presenting cells (APCs), which in CD patients is often of the haplotypes DQ2 and DQ8. Thereafter, Th1 and Th2 immune responses are triggered, resulting in autoimmunity, mucosal inflammation, and the growth of unfavorable microbiota, worsening the prognosis of disease. Three large arrows indicate where probiotics could act.

terium, are of great interest. Since these bacteria are associated with protective beneficial mechanisms for the host and anti-inflammatory effects, it is expected that CD subjects would present lower lactobacillus and bifidobacterial levels. Indeed, their levels tend to be lower in CD children than in healthy controls (13). The ratio of beneficial lactobacilli and bifidobacteria to possibly harmful Gram-negative bacteria, such as *Bacteroides-Prevotella* and *E. coli*, was found to be significantly higher in controls than in CD children (26). Regarding bifidobacterial diversity in CD patients, contradictory results have been reported: lower diversity in CD children (25) and higher diversity of lactobacilli and bifidobacteria in CD adults (22) than in controls. It has been shown that levels of specific species of lactobacilli and bifidobacteria may be higher, lower, or not detected in CD patients in comparison to controls (Table 1). However, the exact value of this information still remains unclear.

Since intake of gluten is a common characteristic of this disease

worldwide, it has been demonstrated that there are clear differences in microbiota composition associated with geographical location (35–39). To search for similarities of microbiota among CD patients from different global regions, it may be helpful to identify groups of microbes involved in the development of disease or in alleviating the symptoms of already-present CD.

It is also important to note that gluten is not an issue for CD patients only. Ideally, characterization and comparison of microbiota, genetic background, intestinal permeability, and immune function of subjects presenting CD and other gluten-related disorders, i.e., gluten ataxia, dermatitis herpetiformis, wheat allergy, and nonceliac gluten sensitivity, may advance the knowledge to elaborate treatment options adequate for each condition.

In summary, low levels of lactobacilli and bifidobacteria are the most consistent findings in CD children. Different techniques have been applied to study the mucosal and luminal microbiota of CD patients, providing quantitative (fluorescent *in situ* hybridiza-

TABLE 1 Main findings related to microbiota composition in CD subjects^a

Subjects	Specimen type(s)	Method	Main finding(s) for comparison between untreated and treated subjects vs controls	Reference
26 untreated CD children and 23 controls	Fecal samples	Culture	↑ <i>Bacteroides</i> , <i>Clostridium</i> , and <i>Staphylococcus</i> (untreated vs control) ↑ <i>Bacteroides-Prevotella</i> and <i>Clostridium histolyticum</i> (untreated vs controls); ↑ <i>Eubacterium rectale-Clostridium cocoides</i> , <i>Atopobium</i> , and sulfate-reducing bacteria (untreated vs control); ↓ <i>Bifidobacterium</i> (untreated vs control)	13
10 untreated CD children and 10 controls	Fecal samples	DGGE	↑ bacterial diversity (untreated vs control); ⇌ <i>Lactobacillus</i> diversity (untreated vs control); ↓ <i>Bifidobacterium</i> diversity (untreated vs controls); ↑ prevalence of <i>Lactobacillus armitus</i> , <i>Lactospor mesenteroides</i> , and <i>Lactospor carnosum</i> (untreated vs control); ↓ prevalence of <i>Lactobacillus casei</i> group bacteria, including <i>L. casei</i> , <i>L. parvasei</i> , <i>L. rhamnosus</i> , or <i>L. zeae</i> (untreated vs control); <i>Bifidobacterium adolescentis</i> not detected in CD children	25
20 untreated CD children, 10 treated CD children, and 8 controls	Duodenal biopsy specimens	FISH-FC	↑ total and Gram-negative bacteria (untreated vs treated and control); ↑ <i>Bacteroides-Prevotella</i> group and <i>Escherichia coli</i> (untreated vs control); ↓ <i>Lactobacillus-Bifidobacterium/Bacteroides-Prevotella</i> ratio (untreated and treated vs control); ↑ Gram positive (untreated and treated vs control); ⇌ <i>Atopobium</i> , <i>Eubacterium rectale-Clostridium cocoides</i> , <i>Clostridium histolyticum</i> , <i>Clostridium histohydrum</i> , <i>Clostridium histohydrum</i> , sulfate-reducing bacteria, and <i>Facalibacterium prausnitzii</i> groups (untreated vs control)	26
30 untreated CD children, 18 treated CD children, and 30 controls	Fecal samples	qPCR	↑ prevalence of <i>Bifidobacterium adolescentis</i> and <i>Bifidobacterium breve</i> in untreated CD children; ↓ prevalence of <i>Bifidobacterium dentium</i> (also found in controls); ↓ <i>Bifidobacterium catenulatum</i> (untreated and treated vs control)	27
25 untreated CD children, 8 treated CD children, and 8 controls	Duodenal biopsy specimens	qPCR	<i>Bifidobacterium longum</i> , <i>Bifidobacterium bifidum</i> , and <i>Bifidobacterium catenulatum</i> detected in all samples; <i>Bifidobacterium dentium</i> detected only in untreated and treated CD children; ↑ <i>Bifidobacterium breve</i> (untreated vs treated and control); ↓ <i>Bifidobacterium lactis</i> (untreated vs treated and control); ↓ <i>Bifidobacterium attenuatum</i> (untreated and treated vs control)	27
25 untreated CD children, 8 treated CD children, and 8 controls	Fecal samples	qPCR	↑ total bacteria (untreated and treated vs control); ↑ <i>Bifidobacterium dentium</i> (treated vs control); ↑ <i>Bifidobacterium breve</i> (untreated vs treated and control); ⇌ <i>Bacteroides</i> , <i>Clostridium leptum</i> , and <i>Escherichia coli</i> (untreated vs treated); ↓ <i>Staphylococcus</i> (untreated vs treated)	28
7 untreated CD children, 7 treated CD children, and 7 controls	Biopsy specimens	qPCR	↓ <i>Clostridium cocoides</i> (untreated and treated vs control); ↑ <i>Lactobacillus</i> group and <i>Akkermansia muciniphila</i> (untreated vs treated and control); ↑ <i>Staphylococcus</i> (untreated and treated vs control); ⇌ <i>Bacteroides</i> , <i>Clostridium leptum</i> , and <i>Escherichia coli</i> (untreated and treated vs control)	29
24 untreated CD children, 18 treated CD children, and 20 controls	Fecal samples	Culture	↓ lactic acid bacteria, <i>Bifidobacterium</i> , and <i>Staphylococcus-Micrococcus</i> (untreated and treated vs control); ↑ <i>Bacteroides</i> and <i>Clostridium</i> (untreated and treated vs control); <i>L. plantarum</i> , <i>L. parvasei</i> , <i>L. rhamnosus</i> , and <i>B. longum</i> detected in all groups; <i>L. brevis</i> , <i>L. rossiae</i> , <i>L. pentosus</i> , and <i>Bifidobacterium bifidum</i> not found in untreated children; <i>L. fermentum</i> , <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> , and <i>L. gasseri</i> detected only in controls	29
19 treated CD children and 15 controls	Fecal samples and biopsy specimens	IgA coated DGGE	↓ IgA-coated bacteria (untreated and treated vs control); ↓ total Gram-positive bacterial populations (untreated and treated vs control); ↓ <i>Bifidobacterium</i> proportions; <i>C. histolyticum</i> , <i>C. lituseburense</i> and <i>Facalibacterium prausnitzii</i> groups (untreated vs control); ↑ <i>Bacteroides-Prevotella</i> group (untreated vs control); ⇌ <i>Escherichia coli</i> , <i>Staphylococcus</i> , <i>Lactobacillus-Enterococcus</i> , and sulfate-reducing bacteria (untreated and treated vs control)	30
8 untreated CD children and 5 controls, and 5 untreated CD adults, 5 treated CD adults, and 5 controls	Fecal samples and biopsy specimens	DGGE	DGGE profiles of fecal samples were richer than those of biopsy specimens; bifidobacteria not found in CD biopsy specimens	31
10 untreated CD adults, 11 treated CD adults, 11 controls, and 10 controls on GFD	Fecal samples	Culture	↓ <i>Lactobacillus</i> , <i>Enterococcus</i> and <i>Bifidobacterium</i> (treated vs control) in fecal samples; ↑ <i>Bacteroides-Prevotella</i> , <i>Porphyrromonas</i> , and <i>Staphylococcus</i> (treated vs control) in fecal samples; ⇌ <i>Salmonella</i> , <i>Shigella</i> , <i>Klebsiella</i> , and <i>Clostridium</i> (treated vs control) in fecal samples	31
10 untreated CD children and 5 controls, and 5 untreated CD adults, 5 treated CD adults, and 5 controls	Duodenal biopsy specimens	16S rRNA gene sequencing	↑ <i>Firmicutes</i> (38% vs 34% for CD adults vs CD children), <i>Proteobacteria</i> (29% vs 38%), <i>Bacteroidetes</i> (17% vs 13%), <i>Actinobacteria</i> (10% vs 4%), <i>Fusobacteria</i> (4% vs 2.9%), <i>Deinococcus-Thermus</i> (0 vs 2.7%); adults (6) different genera and children (36 different genera); ↑ <i>Prevotella</i> spp. and <i>Streptococcus</i> spp. (treated vs untreated) in adults; <i>Mycobacterium</i> spp. and <i>Methylobacterium</i> spp. (untreated and treated vs control) in adults; ↓ <i>Streptococcus</i> and <i>Prevotella</i> (untreated vs control) in children; ↑ <i>Neisseria</i> spp. and <i>Haemophilus</i> spp. (untreated vs control) in children	12
10 untreated CD adults, 11 treated CD adults, 11 controls, and 10 controls on GFD	Fecal samples	DGGE	↑ diversity of <i>Lactobacillus</i> and <i>Bifidobacterium</i> groups (untreated and control vs treated); ↑ <i>Lactobacillus sakei</i> (untreated and control vs treated); ↑ <i>Bifidobacterium bifidum</i> and <i>Bifidobacterium catenulatum</i> (untreated vs control)	22
10 untreated CD children, 6 treated CD adults, and 9 controls	Duodenal biopsy specimens	Microarray HITChip	⇌ in diversity (untreated vs control)	23
33 untreated CD adults and 18 controls	Duodenal biopsy specimens	DGGE	↑ microbial diversity and richness (untreated vs control); ↓ composition and structure dominated by <i>Proteobacteria</i> (untreated vs control)	24

^aThe content includes studies developed with distinct populations, samples, and methodology approaches. ↑, higher; ↓, lower; ⇌, no difference; qPCR, quantitative PCR.

tion coupled with flow cytometry [FISH-FC] and real-time PCR) and qualitative (denaturing gradient gel electrophoresis [DGGE] and HITChip [human intestinal tract chip]) results. This may contribute to the lack of a consensus about the exact bacterial content in patients with CD, together with the patients' age range, specimen type (biopsy specimen or fecal sample), small number of studies, and small sample size.

PROBIOTICS AND CD

To date, the only therapy for CD is the mandatory and complete exclusion of dietary sources of grains and any food containing gluten (40, 41). However, many patients face difficulties in following a gluten-free diet (GFD). The compliance to therapy varies widely, from around 80% in patients diagnosed before 4 years of age to <40% in those diagnosed after 4 years of age (42).

Recent advances in CD pathophysiology have contributed to the development of novel and promising therapeutic solutions. Thus, many other treatments have been identified, such as genetically modified gluten, zonulin inhibitors, therapeutic vaccines, tissue transglutaminase inhibitors, and, recently, probiotics (43).

According to the FAO/WHO (44), a probiotic is defined as a "live microorganism, which when administered in adequate amounts confers a health benefit on the host." Abnormalities in the gut microbiome in CD patients have led to the use of probiotics as a promising alternative.

The beneficial effects of probiotics on the gut health of the host can be manifested through (i) production of inhibitory substances against pathogens (hydrogen peroxide, bacteriocins, and organic acids), (ii) blockage of adhesion sites, (iii) competition for nutrients, (iv) degradation of toxin receptors, and (v) regulation of immunity (45). The molecular mechanisms of probiotic action still need to be characterized. More studies are required to assess the actions of particular probiotics against specific pathogens and disorders and to define which of these actions may benefit CD patients.

Some probiotics have been found to digest or alter gluten polypeptides. De Angelis and coworkers (37) analyzed the potential role of the specific probiotic preparation VSL#3 (a cocktail of eight strains belonging to the species *Bifidobacterium breve*, *B. longum*, *B. infantis*, *Lactobacillus plantarum*, *L. acidophilus*, *L. casei*, *L. delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus*) in decreasing the toxic properties of wheat flour during prolonged fermentation. That study found that the probiotic VSL#3 was highly effective in hydrolyzing gliadin polypeptides compared to other commercial probiotic products such as Oxadrop (*B. infantis*, *L. acidophilus*, *L. brevis*, and *S. thermophilus*), Florisia (*L. brevis*, *L. salivarius* subsp. *salicinius*, and *L. plantarum*), and Yovis (*B. breve*, *B. infantis*, *B. longum*, *L. acidophilus*, *L. plantarum*, *L. casei*, *L. delbrueckii* subsp. *bulgaricus*, *Streptococcus salivarius* subsp. *thermophilus*, and *Enterococcus faecium*). Furthermore, the activities of enzymes digesting proline-rich peptides and aminopeptidases, which regulate the hydrolysis of gliadin epitopes, were widely present in VSL#3. The other commercial probiotic products appear to lack the same ability to break down gliadin polypeptides. Interestingly, another study by De Angelis et al. (46) also reported that the capacity of VSL#3 to degrade gliadin was disabled when the probiotic strains were tested individually. The outcomes suggest that a single probiotic strain is not sufficient to degrade gliadin peptides and therefore must be used together with other strains to exert the beneficial effect against CD. The probiotic

preparation VSL#3 may thus provide better effectiveness in the treatment of CD, since following a gluten-free diet is often a great challenge for patients, for instance, due to cross-contamination.

Specific lactobacillus and bifidobacterial strains have been found to improve gut health. De Palma and collaborators (30) evaluated *in vitro* immunomodulatory properties of *B. bifidum* strain IATA-ES2 and *B. longum* strain ATCC 15707 versus *B. fragilis* strain DSM2451, *E. coli* strain CBL2, and *Shigella* sp. strain CBD8 on peripheral blood mononuclear cells (PBMCs), under effects of gliadin and IFN- γ . *B. bifidum* strain IATA-ES2 and *B. longum* strain ATCC 15707 were able to induce lower levels of interleukin-12 (IL-12) and IFN- γ secretion than *E. coli* CBL2 and *Shigella* sp. CBD8. The release of TNF- α was induced by all strains tested, but its level was lower with *B. bifidum* IATA-ES2 than with *B. fragilis* DSM2451 and *Shigella* strain CBD8. The highest level of IL-10 secretion was observed in the presence of *B. longum* ATCC 15707. It seems that Gram-negative bacteria, such as *E. coli* CBL2 and *Shigella* strain CBD8, usually trigger higher levels of production of proinflammatory cytokines, which in turn contribute to the development of disease. On the other hand, *B. bifidum* IATA-ES2 was able to improve intestinal epithelial permeability, since it stimulated the lowest levels of production of TNF- α and IFN- γ .

Lindfors and coworkers (47) found that *B. lactis* exerted a protective effect on epithelial cells against cellular damage induced by gliadin incubation. Furthermore, it was observed that the addition of 10^6 and 10^7 CFU/ml, but not 10^5 CFU/ml, of *B. lactis* was able to preserve TJ in comparison to epithelial cells maintained in the presence of gliadin alone. Administration of *L. fermentum* at the tested concentrations was unable to stimulate the recovery of transepithelial resistance.

Recently, a study using a gliadin-induced enteropathy animal model was developed to observe whether *B. longum* CECT 7347 could provide beneficial effects. The administration of *B. longum* CECT 7347 enhanced villus width and enterocyte height, which partially restored alterations in animals sensitized with IFN- γ and fed gliadin. In addition, it also reduced levels of TNF- α and increased levels of IL-10 synthesis, demonstrating its ability to favor an anti-inflammatory response in the gut mucosa. *B. longum* CECT 7347 administered to gliadin-fed animals sensitized with IFN- γ was able to moderately diminish some of the alterations in jejunal structure. This effect could apparently contribute to an improvement in the gut barrier function and prevent the translocation of gliadin to the lamina propria (48). Similar to previous work, *L. casei* ATCC 9595 administration was able to significantly reduce the levels of TNF- α and to repair the intestinal injury induced by gliadin in HLA-DQ8 transgenic mice under indomethacin treatment (49).

Studies regarding probiotics and CD in humans are very scarce. In a randomized, double-blind, placebo-controlled study, Smecuol and coworkers (50) evaluated the effect of the *B. infantis* Natren Life Start (NLS) superstrain on gut permeability, the occurrence of symptoms, and the presence of inflammatory cytokines in untreated adult CD patients. Results showed that probiotic administration was unable to modify gut barrier function, probably due to a short time of treatment or inadequate dose. After 3 weeks from the beginning of treatment with the *B. infantis* NLS superstrain, a marked improvement in digestion and reduction in constipation were noted. Abdominal pain and diarrheal symptom scores were also diminished although without significance. In addition, no differences in inflammatory markers were

observed in either of the groups. Although there was a slight improvement in digestive symptoms, this noteworthy study demonstrates that the *B. infantis* NLS superstrain could be a promising tool in CD therapy.

CONCLUSIONS

CD is an autoimmune enteropathy triggered by gluten proteins. Consequently, damage in the mucosa often occurs, accompanied by altered intestinal microbiota and increased epithelial permeability. The causality association is not yet defined. It has been demonstrated that levels of bifidobacteria and lactobacilli are reduced in CD patients, and thus, these bacteria have been seen as promising targets for probiotic therapy. However, there is still a lack of consensus regarding the shifts in bacterial composition, primarily at the species level. Thus, future studies should emphasize microbiota characterization with potential benefits to gut health. Strains capable of producing enzymes that degrade gliadin peptides and induce anti-inflammatory effects are believed to be better suited for the treatment of this disorder. Moreover, studies including a larger sample size and involving international health and research centers would contribute to the design of common directions and guidelines for the treatment of CD and advance the knowledge regarding the importance of microbiota in CD development.

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