Dietary Interventions for Complementing Celiac Disease and Beyond

Subjects: Gastroenterology & Hepatology | Nutrition & Dietetics | Biotechnology & Applied Microbiology

Contributor: Sandip K. Wagh , Karen M. Lammers , Manohar V. Padul , Alfonso Rodriguez-Herrera , Veronica I. Dodero

Celiac Disease (CeD) is a chronic small intestinal immune-mediated enteropathy caused by ingesting dietary gluten proteins in genetically susceptible individuals. CeD is one of the most common autoimmune diseases, affecting around 1.4% of the population globally. The only acceptable treatment for CeD is strict, lifelong adherence to a gluten-free diet (GFD). However, in some cases, GFD does not alter gluten-induced symptoms. In addition, strict adherence to a GFD reduces patients' quality of life and is often a socio-economic burden. Therefore, dietary and non-dietary interventions are investigated. This entry concentrates on the recent research on the degradation of gluten through enzymes, the modulation of the microbiome, and the different types of "biotics" strategies, from probiotics to the less explored "viromebiotics" as possible beneficial complementary interventions for CeD management and other less understood gluten-related disorders beyond the GFD.

celiac disease (CeD) | gut microbiota | dietary therapies | probiotics | Viromebiotics | Prebiotics | postbiotics | Gluten | gliadin

1. Enzymes as a Nutritional Supplement Therapy for CeD

Several proteases and peptidases have been proven to degrade gluten in vitro and/or in vivo [1][2]. As aforementioned, mammalian gastrointestinal proteases partially digested immunogenic gluten sequences [3][4]. Therefore, the detoxification of gluten can theoretically be achieved by proteolytic fragmentation by oral enzymatic therapy. The idea is to inactivate gluten peptides in the human gastrointestinal tract by peptidase supplementation, thereby minimizing the amount of gluten peptides reaching the small intestine. The gluten-hydrolyzing enzymes produced by the *Rothia mucilaginosa* were have been identified as two structurally closely related *subtilisins* [5]. Previously, some of us reported the significant hydrolysis of wheat gliadin by Peptidase S9, isolated from the *B. tequilensis* strain [6]. Several gluten-detoxifying peptidases have been isolated from probiotic preparations involving *lactobacilli* [7][8], other microorganisms [5][9], and germinating cereals [10].

The withdrawal or modification of celiac peptides during food processing using enzymes is already commercialized. For example, a dietary supplement based on *Aspergillus niger*-Prolyl endopeptidase (PEP) can degrade gluten at a particular stage. However, it is not currently a treatment for CeD because it does not entirely break down gluten, and the resulting accumulation of gluten peptides in the duodenum has not been determined [11]. Another commercialized product is based on caricain, a proteolytic enzyme obtained from the papaya plant and papain.

Previous studies have reported that caricain has the potential specificity to target gluten amino acid sequence and helps reduce gluten concentration during food processing [12][13]. However, to date, all the commercialized enzymatic cocktails are not prescribed for CeD patients.

Other prolyl endopeptidases (PEPs) isolated from *Myxococcus xanthus* and *Flavobacterium meningosepticum* showed the ability to hydrolyze toxic gliadin peptides significantly. However, the presence of immunopeptides has not been determined [14][15][16][17]. PEPs from *Sphingomonas capsulate,* showed complete hydrolysis of immunogenic gluten peptides after mixing with barley cysteine endoproteases [18][19]. Another interesting PEP is latiglutenase, in which experiments in subjects receiving 900 mg of latiglutenase led to improvements (*p*-values) in the severity of symptoms relative to placebo-dosed subjects for week 12. The reduction in symptoms trended higher for more symptomatic patients [20]. However, previous randomized phase 2 trials were conducted with latiglutenase (IMGX003, formerly ALV003) (ClinicalTrials.gov, NCT03585478), and they reported contradictory findings regarding its effect on villous atrophy and clinical symptoms, showing only 88% gluten hydrolysis efficiency [21][22]. Nowadays, a phase I clinical study is being conducted to evaluate the bacterial endopeptidase TAK-062 that simultaneously targets proline and glutamine peptide motifs in the stomach (ClinicalTrials.gov Identifier: NCT05353985). TAK-062 is a second generation of the engineered endopeptidase kuma030 [23]. When healthy individuals ingested TAK-062 before a complex meal containing 1–6 g gluten, it was observed that after 20–65 min post-TAK-062 ingestion, 97–99% of the gluten was degraded as a measure in aspirate samples from the stomach [24]. The calculated remaining gluten showed a median amount of up to 38 mg. This is the first glutenase that showed this high gliadin hydrolysis efficiency in vivo. This has a potential clinical relevance since amounts as low as 10 mg of gluten may be able to trigger the immunological cascade [25][26]. Yet, these data showed the high efficiency of TAK-062, and further studies in CeD patients are in progress to test the efficiency of Tak-062 in degrading inadvertent gluten exposure (ClinicalTrials.gov Identifier: NCT05353985).

2. Human Microbiota and Dysbiosis in CeD

During the co-evolution of humans and microbes, thousands of bacterial species have colonized the human body. The vast amount of microbial presence in the host's body is termed "normal flora," "microbiota," or "microflora" [27][28][29]. The microflora consists of bacteria accompanied by fungi, archaea, viruses, and protozoans [30][31][32]. This colonization occurs at birth, covering every human body surface, including the ear, oral cavity, respiratory tract, genitourinary tract, and gastrointestinal (GI) tract [29][33]. The GI tract is loaded with a plethora of molecules providing nutrition to microbes, facilitating heavy colonization of harmful and beneficial microbes.

The indigenous gut bacteria maintain themselves and protect the host against freshly ingested microbes, including pathogens. It is an essential immune mechanism in the host, referred to as the "barrier effect" or "colonization resistance" [34][35]. Indigenous microbes present in the gut microflora were also reported to regulate the development of the structure and morphology of the GI tract.

Each healthy individual has a unique gut microbiota [36]. The two major bacterial phyla are *Firmicute*s (*Bacillota*) and *Bacteroidetes (Bacteroidota)*, which are 90% of the whole gut microbiota [37]. The *Firmicutes* species is

composed of ≥ 200 different genera, and *Clostridium* genera are 95% of the *Firmicutes* phyla. Bacteroidetes consist of predominant genera such as *Bacteroides* and *Prevotella*. *Actinobacteria, Proteobacteria, Fusobacteria*, and *Verrucomicrobia* are the next most numerous phyla, which are reported in a "healthy gut microbiota composition" [38].

Recently, many findings have reported that gluten metabolism is closely related to the GI microbiota [39][40][41]. The detailed mechanisms of microorganisms that play a protective role in CeD pathogenesis are broad. They comprise the metabolism of the triggering antigen (e.g., gliadin), increased intestinal barrier permeability, and inflection of innate and adaptive immune responses [42]. In 2016, Caminero et al. reported that the bacteria in the human GI tract could hydrolyze gluten in vivo and efficiently reduce its immunogenicity [43]. *Faecalibacterium prausnitzii*, *Roseburia intestinalis*, and *Eubacterium hallii* demonstrated a capability to restore and improve intestinal permeability [44]. Furthermore, orally administered bacteria, *Lactococcus lactis*, has been reported to induce antigen-specific tolerance in an experimental animal model [45]. Moreover, gluten hydrolyzing actions by dental plaque bacteria were reported [46], showing that the host's indigenous bacteria could be able to degrade gluten.

Interestingly, microbial dysbiosis has been identified in patients with active CeD, which was exquisitely reviewed by Girvoban A. et al. in 2017 [47]. Their main conclusion was that both duodenal and colonic dysbiosis are associated with CeD. They reported that the most frequent Gram-negative bacterial species isolated from CeD patients were: *Bacteroides* spp., *Salmonella* spp., *Shighellaspp*, *Klebsiella* spp., *Neisseria* spp., and *Prevotella* spp. Although CeD is associated with a decrease in the number of Gram-positive bacteria, pathogenic Gram-positive species, such as *Clostridium* spp., *Staphylococcus* spp., and *Actinomyces* spp., were isolated from CeD patients. Of note is that bacterial virulence features are considered higher in CeD patients. Among them, it was reported that a peculiar *Neisseria flavescens* strain was identified in adults affected by CeD [48][49], using the 16S rRNA technique for duodenal and oropharyngeal samples from celiac patients and control subjects. This *Neisseria flavescens* strain, isolated from the CeD patients, induced an immune-inflammatory response in human and murine dendritic cells, both in CaCo-2 cells and in ex vivo duodenal mucosal explants of control subjects, thereby suggesting that it could play a role in CeD [48]. Leonard et al. reported that intestinal dysbiosis is associated with CeD onset in infants. They performed a prospective metagenomic analysis of the gut microbiota of infants at risk of CeD to track shifts in the microbiota before CeD development. The cross-sectional analysis at CeD onset identified an altered abundance of six microbial strains of *B. longum* and several metabolites between cases and controls but no change in microbial species or pathway abundance [26]. One of the main findings was the dysregulated interaction between the genus *Bifidobacteria* and butyrate-producing bacteria *Faecalibacteriumprausnitzii*, and *Clostridium clostridioforme* which could be critical in the development of CeD. Additionally, they reported new microbes (e.g., *Porphyromonas* sp.), pathways (e.g., high mannose–typeN-glycan biosynthesis), and metabolites (e.g., serine) that can be CeD-specific biomarkers. In another study, it was found that the stool microbiota of children with CeD active showed a significant abundance of *Bacteroides-Prevotella*, *Akkermansia*, and *Staphylococcaceae* compared with healthy controls. Interestingly, at the symptom level, the authors found a significantly increased mean relative abundance of *Bacillaceae* and *Enterobaeriaceae* in patients with abdominal pain. Meanwhile, those patients with diarrhea had a significantly reduced mean relative abundance, particularly

of *Akkermansia.* The main conclusion was that CeD active patients' microbiota differed from controls, where a pro-inflammatory microflora was found. Following the microbiota of such patients in GFD could shed light on the role of gluten in the observed disbalance [50].

3. Probiotics

Probiotics are live microorganisms that have demonstrated beneficial effects on human health after being administered in adequate amounts by restoring the composition of the gut microbiome to prevent gut microbiota dysbiosis and improve immunity [51][52][53][54]. In this regard, probiotic bacteria are constantly being studied, and their applications are also being considered in promising adjuvant treatments for various intestinal diseases, including CeD [55][56]. Most of the probiotic bacteria belong to the genus *Lactobacillus* and *Bifidobacterium*. They are considered "Generally Recognized As Safe'' (GRAS) by the United States Food and Drug Administration (USFDA) [57]. However, some researchers reported that several *Bacillus* sp. also fulfill the essential probiotic characteristics, such as resistance to antibiotics as well as acid, bile salt, and sodium chloride tolerance, and produce a group of antimicrobial peptides with a broader inhibition spectrum [58]. Probiotic *Lactobacillus* sp. and *Bacillus* sp. isolated from different sources are mainly used as probiotic candidates because they are generally safe and cost-effective [59]. Both of these species are usually found in abundance in the upper GI tracts of both humans and animals. De Angelis et al. reported the formulation of commercial enzymes with microbial consortia of *Lactobacillus* and *Bacillus*, named consortia I: *Lactobacillus (Lp.) plantarum*, *(Lc.) paracasei*, *Bacillus subtilis*, *Bacillus pumilus*, and consortia II: *Lp. plantarum*, *Lc. Paracasei*, *Limosilactobacillusreuteri*, *Bacillus megaterium*, *B. pumilus*, showed hydrolysis of gluten to non-immunogenic and non-toxic peptides under GI conditions. These findings state that both microbial consortia can detoxify immunogenic gluten peptides and may be used to improve the intestinal digestion of CeD and gluten-sensitive patients [55].

A curative measure of probiotics can help by preventing and treating conditions like IBD (e.g., Crohn's disease and ulcerative colitis), autoimmune diseases (e.g., rheumatoid arthritis), CeD and lactose intolerance, IBS, vaginal infections (e.g., candida or thrush), and atopic dermatitis [60]. Probiotic consumption also helps to reduce diarrhea and allergies. Probiotics found in dairy and meats reduced low-density lipoprotein (LDL) levels, killed the bacteria that caused tooth decay, and lessened the harmful effects of gingivitis. Probiotics also stimulate, modulate, and regulate the host's immune response, gastrointestinal hormone release, and brain-behavior through bidirectional neuronal signaling [61][62]. Probiotics have physiological functions that improve the host environment's health, regulate microbes, and are also supportive in combating obesity and being overweight [63]. There are some examples where probiotic prophylaxis was given to patients with severe acute pancreatitis and the probiotics caused significantly more severe side effects [64]. Thus, the exact mechanisms of the health-promoting effects of probiotics remain elusive. However, it would be of great significance to explore membrane and extracellular proteins/enzymes and other biomolecules of probiotics [65]. These bacteria produce diverse compounds such as organic acids, enzymes, bacteriocins, antimicrobial compounds, exopolysaccharides, secreted low-calorie sweetening molecules, and nutraceuticals [66]. Probiotics are now a rising field for food manufacturers with remarkable growth potential. As it involves the ingestion of live probiotic bacterial cultures, it enhances the

intestinal microflora. The importance and success of probiotics in the overall market will depend on the effectiveness of the probiotic strain or cultures used. The food products which contain probiotics and prebiotics affect the functionality of the foods, which results in the enhancement of the microflora that promotes gut health [67].

4. Prebiotics

Prebiotics are defined as a substrate that is selectively utilized by host microorganisms to confer a health benefit, for example, by stimulating one or more groups of gut-friendly microbes, mainly *Bifidobacterium* and *Lactobacillus*. Examples are substances in foods such as garlic, onions, artichokes, and others. Eating adequate amounts of these dietary foods might be necessary to have the beneficial “bifidogenic” effect. Another alternative is to take a prebiotic supplement to achieve the most favorable levels. In addition, prebiotics are resistant to hydrolysis by digestive enzymes and are not absorbed in the upper part of the gastrointestinal tract, reaching the large intestine where they stimulate certain microorganisms’ growth [68]. Different compounds have been tested to determine their function as prebiotics. Fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and trans-galacto-oligosaccharides (TOS) are the most common examples of prebiotics. The fermentation of prebiotics by gut microbiota produces short-chain fatty acids (SCFAs), including lactic acid, butyric acid, and propionic acid [69].

5. Synbiotics

Synbiotics are a combination of probiotics and prebiotics. These synbiotics contain probiotics, which are beneficial bacteria, and prebiotics, which are indigestible products for improving the growth of beneficial bacteria. In the sense that a product in which a prebiotic is specifically added favors the wanted probiotic’s growth. For example, fermented dairy products such as yogurt are synbiotic food products. The most common synbiotics include FOS and *Bifidobacteria;* inulins and *Lactobacillus*; and *Bifidobacteria*, and *Lactobacilli* with FOS [70][71]. Wilms et al. reported that synbiotic dietary strategies might be used to improve intestinal barrier functions. They reported that when 20 healthy adult individuals were supplemented with synbiotic supplementation Ecologic® 82S + 10 g Fructo-oligosachharides P6(FOS P6) every day for two weeks, the individuals reported increased stool frequency. The intestinal permeability under basal and indomethacin-induced stressed conditions was determined, showing that these synbiotics neither affect the intestinal permeability, immune function, or gastrointestinal symptoms under basal or indomethacin-induced conditions [72].

6. Postbiotics

Postbiotics are products secreted by living bacteria or released after their lysis, for instance, molecules such as SCFAs, lactic acid, and bioactive peptides, among other metabolites. It can also be extended to protein compounds, hydrogen peroxide (H_2O_2), bacteriocins, organic acids, exopolysaccharides, and enzymes [73][74]. When postbiotics are administered in adequate amounts, they help improve the host’s health. Nevertheless, to date, the exact mechanisms of improvement have not yet been completely unfolded. The advantage of using

postbiotics rather than probiotics concerns higher stability and safety: postbiotics do not contain any living bacteria and hence harbor no risk of microbial infection and translocation [75].

Postbiotics have been used in in vitro experiments in Caco-2 cells to analyze their ability to prevent gliadin and gliadin peptides' effects on Caco-2 cells. Sarno et al. reported that postbiotics CBA L74, supernatant from *Lactobacillus paracasei*, could reduce gliadin peptides' entrance into Caco-2 cells [76]. In this direction, recently, Conte et al. investigated the beneficial postbiotic effect from *Lactobacillus paracasei* CBA L74, both in Caco-2 cells and in vitro on CeD organoids after stimulation with pepsin-trypsin gliadin (PTG) digest or the cytotoxic 13-mer gliadin peptide. The postbiotic prevented the gliadin-induced activation of the inflammatory response as measured by activation markers NfkB and ERK phosphorylation and activation of mTOR signaling, and it was capable of inhibiting the gliadin-induced reduction of the autophagy pathway. Hence, *Lactobacillus paracasei* CBA L74 postbiotics decreased the gliadin-induced inflammatory response and stimulated autophagy, which has an important role in intestinal homeostasis [77]. Another selected report from Freire et al. used an in vitro model of organoids from non-celiac individuals and celiac patients to study the pathogenesis of CeD. In this study, they also investigated the effects of three postbiotics, butyrate, lactate, and polysaccharide A from *B. fragilis*. They found that these molecules could modulate the intestinal responses to gluten. The authors showed an increase in paracellular permeability that was already present at baseline in CeD organoids. In particular, butyrate and polysaccharide A could restore CeD barrier function through increased expression of the tight junction sealing molecule claudin-18. Likewise, incubation of the CeD organoids with gliadin induced immune activation (expression of IL-15 and IFN gamma) that was decreased by butyrate and lactate [78].

7. Viruses

Numerous publications exist on the human microbiome and the place of the corresponding dysbiota in specific human chronic conditions. The community of viruses in the gastrointestinal tract is named virome, and its role in health and disease is a fascinating new area of research [79].

The knowledge about the ecology of gut viruses is limited yet. Still, gut viruses outnumber microbes in a ratio of 10:1 [80]. The microbiome cannot maintain a homeostatic equilibrium without the gut phageome (a collection of bacteriophages).

The gastrointestinal virome biodiversity changes along with the human life cycle. With aging, the phage load decreases, while the abundance and complexity of the microbial populations increase substantially. It seems that intestinal bacterial composition and diversification occur at the expense of the virome communities [81]. In humans, viral dysbiosis in IBD has been reported, so it is not a surprise that children with CeD, which is also an inflammatory enteropathy, show a statistically significant viral dysbiosis by metagenomic analysis. In this sense, it was found recently that viral dysbiosis in children newly diagnosed with CeD before starting the GFD [82]. It was already reported that the lower initial diversity of the human gut virome leads to a more pronounced effect of the GFD on its composition [83], showing the impact of the GFD on the dynamics of the gut virome.

Some phages have been proposed as new prebiotics and are undergoing clinical trials to prove safety, tolerability, and efficacy. In a short intervention of 28 days, phages did not globally disrupt the microbiota. However, in response to the intervention, specific populations were altered as the members of the butyrate-producing genera increased. The authors concluded that bacteriophages could selectively reduce target organisms without causing global gut microbiome disruption [84].

In this direction, it has been hypothesized that phage therapy may represent a new strategy for treating CeD. Their role could be to select microbes that digest gluten or lack glutenase capacity, thus modifying the luminal gluten load or modifying the transglutaminase activity. Lerner et al. have presented different potential interventions [85]. Studies with functional analyses to define the relationship of bacteriophages to bacteria and to clarify the role of viruses in CeD might lead to the development of additional treatment options. A funded proof of concept project focusing on altering human gut microbes to treat gluten-related disorders is now advancing in this direction. The project's objectives include engineering *Bifidobacterium*-targeting templated bacteriophages capable of infecting *B. longum* to express a gluten-degrading enzyme from *Sphingomonas capsulata* and the introduction of the glutenase-expressing phage into a *B. longum* in an in-vitro biofilm model [86]. The technology readiness levels (TRLs) is a validated method of 9 stages to estimate the maturity of technologies [87]. The use of viruses for therapeutic interventions in gastroenterology is currently in stage 1, when the basic principles are observed. Any actual clinical application of viruses in CeD therapy is still quite far away, but it is worth investigating what is going on and monitoring the advent of potential "viromebiotics".

References

1. Rey, M.; Yang, M.; Lee, L.; Zhang, Y.; Sheff, J.G.; Sensen, C.W.; Mrazek, H.; Halada, P.; Man, P.; McCarville, J.; et al. Addressing proteolytic efficiency in enzymatic degradation therapy for celiac disease. Sci. Rep. 2016, 6, 30980.
2. Cavaletti, L.; Taravella, A.; Carrano, L.; Carenzi, G.; Sigurtà, A.; Solinas, N.; De Caro, S.; Di Stasio, L.; Picascia, S.; Laezza, M.; et al. E40, a novel microbial protease efficiently detoxifies gluten proteins, for the dietary management of gluten intolerance. Sci. Rep. 2019, 9, 13147.
3. Shan, L.; Molberg, O.; Parrot, I.; Hausch, F.; Filiz, F.; Gray, G.; Sollid, L.; Khosla, C. Structural basis for gluten intolerance in celiac sprue. Science 2002, 27, 2275–2279.
4. Herrera, M.; Dodero, V. Gliadin proteolytical resistant peptides: The interplay between structure and self-assembly in gluten-related disorders. Biophys. Rev. 2021, 13, 1147–1154.
5. Wei, G.; Tian, N.; Valery, A.; Zhong, Y.; Schuppan, D.; Helmerhorst, E. Identification of Pseudolysin (lasB) as an Aciduric Gluten-Degrading Enzyme with High Therapeutic Potential for Celiac Disease. Am. J. Gastroenterol. 2015, 110, 899–908.

6. Wagh, S.; Gadge, P.; Padul, M. Significant Hydrolysis of Wheat Gliadin by Bacillus tequilensis (10bT/HQ223107): A Pilot Study. Probiotics Antimicrob. Prot. 2018, 10, 662–667.
7. De Angelis, M.; Rizzello, C.; Fasano, A.; Clemente, M.; Simone, C.; Silano, M.; De Vincenzi, M.; Losito, I.; Gobbetti, M. VSL#3 probiotic preparation has the capacity to hydrolyze gliadin polypeptides responsible for Celiac Sprue probiotics and gluten intolerance. Biochim. Acta Mol. Basis Dis. 2006, 1762, 80–93.
8. Duar, R.M.; Clark, K.; Patil, P.B.; Hernández, C.; Brüning, S.; Burkey, T.; Madayiputhiya, N.; Taylor, S.; Walter, J. Identification and characterization of intestinal lactobacilli strains capable of degrading immunotoxic peptides present in gluten. J. Appl. Microbiol. 2014, 118, 515–527.
9. Shan, L.; Marti, T.; Sollid, M.; Gary, M.; Khosla, C. Comparative biochemical analysis of three bacterial prolyl endopeptidases: Implications for coeliac sprue. Biochem. J. 2004, 383, 311–318.
10. Prabucka, B.; Bielawski, W. Purification and partial characterization of a major gliadin-degrading cysteine endopeptidase from germinating triticale seeds. Acta Physiol. Plant. 2004, 26, 383–392.
11. Krishnareddy, S.; Stier, K.; Recanati, M.; Lebwohl, B.; Green, P. Commercially available glutenases: A potential hazard in celiac disease. Ther. Adv. Gastroenterol. 2017, 10, 473–481.
12. Cornell, H.; Stelmasiak, T. A unified hypothesis of coeliac disease with implications for management of patients. Amino Acids 2007, 33, 43–49.
13. Cornell, H.; Doharti, W.; Stelmasiak, T. Papaya latex enzymes capable of detoxification of gliadin. Amino Acids 2010, 38, 155–165.
14. Gerez, C.; Dallagnol, A.; Rollán, G.; de Valdez, F. A combination of two lactic acid bacteria improves the hydrolysis of gliadin during wheat dough fermentation. Food Microbiol. 2012, 32, 427–430.
15. Tack, J.; van de Water, M.; Bruins, M.; Kooy-Winkelaar, E.M.; van Bergen, J.; Bonnet, P.; Vreugdenhil, A.C.E.; Korponay-Szabo, I.; Edens, L.; von Blomberg, B.M.E.; et al. Consumption of gluten with gluten-degrading enzyme by coeliac patients: A pilot-study. World J. Gastroenterol. 2013, 19, 5837–5847.
16. Rizzello, G.; Curiel, A.; Nionelli, L.; Vincentini, O.; Di Cagno, R.; Silano, M.; Gobbetti, M.; Coda, R. Use of fungal proteases and selected sourdough lactic acid bacteria for making wheat bread with an intermediate content of gluten. Food Microbiol. 2014, 37, 59–68.
17. Brzozowski, B. Impact of food processing and simulated gastrointestinal digestion on gliadin immunoreactivity in rolls. J. Sci. Food Agric. 2018, 987, 3363–3375.
18. Gass, J.; Khosla, C. Prolyl endopeptidases. Cell. Mol. Life Sci. 2007, 64, 345–355.
19. Tye-Din, A.; Anderson, P.; Ffrench, A.; Brown, G.J.; Hodsman, P.; Siegel, M.; Botwick, W.; Shreeniwas, R. The effects of ALV003 pre-digestion of gluten on immune response and

symptoms in celiac disease in vivo. Clin. Immunol. 2010, 134, 289–295.

20. Syage, J.; Green, P.; Khosla, C.; Adelman, D.; Sealey-Voyksner, J.; Murray, A. Latiglutenase Treatment for Celiac Disease: Symptom and Quality of Life Improvement for Seropositive Patients on a GlutenFree Diet. GastroHep 2019, 2, 371.
21. Lahdeaho, M.; Kaukinen, K.; Laurila, K.; Vuotikka, P.; Koivurova, O.-P.; Kärjä-Lahdensuu, T.; Marcantonio, A.; Adelman, D.C.; Mäki, M. Glutenase ALV003 Attenuates Gluten-Induced Mucosal Injury in Patients With Celiac Disease. Gastroenterology 2014, 146, 1649–1658.
22. Murray, A.; Kelly, P.; Green, P.; Marcantonio, A.; Wu, T.; Mäki, M.; Adelman, C. CeliAction Study Group of Investigators. No Difference Between Latiglutenase and Placebo in Reducing Villous Atrophy or Improving Symptoms in Patients With Symptomatic Celiac Disease. Gastroenterology 2017, 152, 787–798.e2.
23. Wolf, C.; Siegel, B.; Tinberg, C.; Camarca, A.; Gianfrani, C.; Paski, S.; Guan, R.; Montelione, G.; Baker, D.; Pultz, S. Engineering of Kuma030: A Gliadin Peptidase That Rapidly Degrades Immunogenic Gliadin Peptides in Gastric Conditions. J. Am. Chem. Soc. 2015, 137, 13106–13113.
24. Pultz, S.; Hill, M.; Vitanza, M.; Wolf, C.; Saaby, L.; Liu, T.; Winkle, P.; Leffler, A. Gluten Degradation, Pharmacokinetics, Safety, and Tolerability of TAK-062, an Engineered Enzyme to Treat Celiac Disease. Gastroenterology 2021, 161, 81–93.e3.
25. Catassi, C.; Fabiani, E.; Iacono, G.; D'Agate, C.; Francavilla, R.; Biagi, F.; Volta, U.; Accomando, S.; Picarelli, A.; De Vitis, I.; et al. A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. Am. J. Clin. Nutr. 2007, 85, 160–166.
26. Leonard, M.M.; Silvester, J.A.; Leffler, D.; Fasano, A.; Kelly, C.P.; Lewis, S.K.; Goldsmith, J.D.; Greenblatt, E.; Kwok, W.W.; McAuliffe, W.J.; et al. Evaluating Responses to Gluten Challenge: A Randomized, Double-Blind, 2-Dose Gluten Challenge Trial. Gastroenterology 2021, 160, 720–733.e8.
27. Kunz, C.; Kuntz, S.; Rudloff, S. Intestinal flora. Adv. Exp. Med. Biol. 2009, 639, 67–79.
28. Morelli, L. Postnatal development of intestinal microflora as influenced by infant nutrition. J. Nutr. 2008, 138, 1791–1795.
29. Neish, S. Microbes in gastrointestinal health and disease. Gastroenterology 2009, 136, 65–80.
30. Sekirov, I.; Shannon, L.; Russell, L.; Caetano, M.; Antunes, B.; Finlay, B. Gut Microbiota in Health and Disease. Physiol. Rev. 2009, 90, 859–904.
31. Zhang, Y.-J.; Li, S.; Gan, R.-Y.; Zhou, T.; Xu, D.-P.; Li, H.-P.; , Yu-Jie. Impacts of Gut Bacteria on Human Health and Diseases. Int. J. Mol. Sci. 2015, 16, 7493–7519.

32. Mtasher, S.; Abdulhussein, J.; Mutlag, H. Probiotics and Prebiotics. Int. J. Curr. Res. 2018, 10, 75341–77535.
33. Chiller, K.; Selkin, A.; Murakawa, J. Skin microflora and bacterial infections of the skin. J. Investig. Dermatol. Symp. Proc. 2001, 6, 170–174.
34. Lewis, B.; Buffie, G.; Carter, R.; Leiner, I.; Toussaint, N.C.; Miller, L.C.; Gobourne, A.; Ling, L.; Pamer, E.G. Loss of microbiota-mediated colonization resistance to clostridium difficile infection is greater following oral vancomycin as compared with metronidazole. J. Infect. Dis. 2015, 212, 1656–1665.
35. Perez-Cobas, E.; Moya, A.; Gosalbes, J.; Latorre, A. Colonization resistance of the gut microbiota against clostridium difficile. Antibiotics 2015, 4, 337–357.
36. Pecora, F.; Persico, F.; Gismondi, P.; Fornaroli, F.; Iuliano, S.; de'Angelis, G.; Esposito, S. Gut Microbiota in Celiac Disease: Is There Any Role for Probiotics? Front. Immunol. 2020, 11, 957.
37. Laterza, L.; Rizzatti, G.; Gaetani, E.; Chiusolo, P.; Gasbarrini, A. The Gut Microbiota and Immune System Relationship in Human Graft-versus-Host Disease. Mediterr. J. Hematol. Infect. Dis. 2016, 8, e2016025.
38. Rinninella, E.; Raoul, P.; Cintoni, M.; Franceschi, F.; Miggiano, G.; Gasbarrini, A.; Mele, M. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms 2019, 7, 14.
39. Verdu, F.; Galipeau, J.; Jabri, B. Novel players in coeliac disease pathogenesis: Role of the gut microbiota. Nat. Reviews. Gastroenterol. Hepatol. 2015, 12, 497–506.
40. Wu, X.; Qian, L.; Liu, K.; Wu, J.; Shan, Z. Gastrointestinal microbiome and gluten in celiac disease. Ann Med. 2021, 53, 1797–1805.
41. Elsouri, K.; Arboleda, V.; Heise, S.; Kesselman, M.; Demory-Beckler, M. Microbiome in Rheumatoid Arthritis and Celiac Disease: A Friend or Foe. Cureus 2021, 9, e15543.
42. Caminero, A.; Meisel, M.; Jabri, B.; Verdu, E. Mechanisms by which gut microorganisms influence food sensitivities. Nat. Rev. Gastroenterol. Hepatol. 2019, 16, 7–18.
43. Caminero, A.; Galipeau, H.; McCarville, J.; Johnston, C.W.; Bernier, S.P.; Russell, A.K.; Jury, J.; Herran, A.R.; Casqueiro, J.; Tye-Din, J.A.; et al. Duodenal bacteria from patients with celiac disease and healthy subjects distinctly affect gluten breakdown and immunogenicity. Gastroenterology 2016, 151, 670–683.
44. Hiippala, K.; Jouhten, J.; Ronkein, A.; Hartikainen, A.; Kainulainen, V.; Jalanka, J.; Satokari, R. The potential of gut commensals in reinforcing intestinal barrier function and alleviating inflammation. Nutrients 2018, 10, 988.

45. Huibregtse, I.; Marrieta, E.; Rashtak, S.; Koning, F.; Rottiers, P.; David, C.S.; van Deventer, S.J.H.; Murray, J.A. Induction of antigen-specific tolerance by oral administration of Lactococcus lactis delivered immunodominant DQ8-restricted gliadin peptide in sensitized nonobese diabetic Abo Dq8 transgenic mice. J. Immunol. 2009, 183, 2390–2396.

46. Helmerhorst, E.; Wei, G. Experimental Strategy to Discover Microbes with Gluten-degrading Enzyme Activities. Conference paper at international society of optical engineering. In Proceedings of the SPIE 9112, Sensing Technologies for Global Health, Military Medicine, and Environmental Monitoring IV, Baltimore, MD, USA, 5 June 2014.

47. Girbovan, A.; Sur, G.; Samasca, G.; Lupan, I. Dysbiosis a risk factor for celiac disease. Med Microbiol Immunol. 2017, 206, 83–91.

48. D'Argenio, V.; Casaburi, G.; Precone, V.; Pagliuca, C.; Colicchio, R.; Sarnataro, D.; Discepolo, V.; Kim, S.M.; Russo, I.; Blanco, G.D.V.; et al. Metagenomics reveals dysbiosis and a potentially pathogenic N. flavescens strain in duodenum of adult celiac patients. Am. J. Gastroenterol. 2016, 111, 879–890.

49. Iaffaldano, L.; Granata, I.; Pagliuca, C.; Esposito, M.V.; Casaburi, G.; Salerno, G.; Colicchio, R.; Piccirillo, M.; Ciacci, C.; Blanco, G.D.V.; et al. Oropharyngeal microbiome evaluation highlights Neisseria abundance in active celiac patients. Sci. Rep. 2018, 8, 1–10.

50. Di Biase, A.; Marasco, G.; Ravaioli, F.; Dajti, E.; Colecchia, L.; Righi, B.; D'Amico, V.; Festi, D.; Iughetti, L.; Colecchia, A. Gut microbiota signatures and clinical manifestations in celiac disease children at onset: A pilot study. J. Gastroenterol. Hepatol. 2021, 36, 446–454.

51. Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint, H.J.; Salminen, S.; et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol. 2014, 11, 506–514.

52. Gagliardi, A.; Totino, V.; Cacciotti, F.; Iebba, V.; Neroni, B.; Bonfiglio, G.; Trancassini, M.; Passariello, C.; Pantanella, F.; Schippa, S. Rebuilding the Gut Microbiota Ecosystem. Int. J. Environ. Res. Public Health 2018, 15, 1679.

53. Wang, X.; Zhang, P.; Zhang, X. Probiotics Regulate Gut Microbiota: An Effective Method to Improve Immunity. Molecules 2021, 26, 6076.

54. Hemarajata, P.; Versalovic, J. Effects of probiotics on gut microbiota: Mechanisms of intestinal immunomodulation and neuromodulation. Ther. Adv. Gastroenterology. 2013, 6, 39–51.

55. De Angelis, M.; Siragusa, S.; Vacca, M.; Di Cagno, R.; Cristofori, F.; Schwarm, M.; Pelzer, S.; Flügel, M.; Speckmann, B.; Francavilla, R.; et al. Selection of Gut-Resistant Bacteria and Construction of Microbial Consortia for Improving Gluten Digestion under Simulated Gastrointestinal Conditions. Nutrients 2021, 13, 992.

56. Kim, S.; Guevarra, B.; Kim, T.; Kwon, J.; Kim, H.; Cho, H.; Kim, M.; Lee, H. Role of probiotics in the human gut microbiome-associated diseases. J. Microbiol. Biotechnol. 2019, 29, 1335–1340.
57. Rubio, R.; Jofre, A.; Martin, B.; Aymerich, T.; Garriga, M. Characterization of lactic acid bacteria isolated from infant faeces as potential probiotic starter cultures for fermented sausages. Food Microbiol. 2014, 38, 303–331.
58. Khochamit, N.; Siripornadulsil, S.; Sukon, P.; Siripornadulsi, W. Antibacterial activity and genotypic-phenotypic characteristics of bacteriocin-producing Bacillus subtilis KKU213: Potential as a probiotic strain. Microbiol. Res. 2015, 170, 36–50.
59. Swain, R.; Anandharaj, M.; Ray, C.; Rani, P. Fermented fruits and vegetables of Asia: A potential source of probiotics. Biotechnol. Res. Int. 2014, 2014, 1–19.
60. Francavilla, R.; Cristofori, F.; Tripaldi, E.; Indro, F. Intervention for disbiosis in children born by C-section. Ann. Nutr. Metab. 2018, 73, 33–39.
61. Scott, P.; Antoine, M.; Midtvedt, T.; Hemert, V. Manipulating the gut microbiota to maintain health and treat disease. Microb. Ecol. Health Dis. 2015, 26, 25877.
62. Kristensen, B.; Bryrup, T.; Allin, H.; Nielsen, T.; Hansen, T.H.; Pedersen, O. Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: A systematic review of randomized control trials. Genome Med. 2016, 8, 1–11.
63. Kobyliak, N.; Conte, C.; Cammarota, G.; Haley, A.P.; Styriak, I.; Gaspar, L.; Fusek, J.; Rodrigo, L.; Kruzliak, P. Probiotics in prevention and treatment of obesity: A critical view. Nutr. Metab. 2016, 13, 1e13.
64. Besselink, M.G.; Van Santvoort, H.C.; Buskens, E.; Boermeester, M.A.; Van Goor, H.; Timmerman, H.M.; Nieuwenhuijs, V.B.; Bollen, T.L.; van Ramshorst, B.; Witterman, B.J.; et al. Dutch Acute Pancreatitis Study Grp (2008). Probiotic prophylaxis in predicted severe acute pancreatitis: A randomised, double-blind, placebo-controlled trial. Lancet 2008, 371, 651–659.
65. Plaza- Diaz, J.; Rulz- Ozeda, F.; Gil-Campos, M.; Gil, A. Mechanisms of action of Probiotics. Am. Soc. Nutr. 2019, 10 (Suppl. 1), S49–S66.
66. Capozzi, V.; Russo, P.; Duenas, M.; Lopez, P.; Spano, G. Lactic acid bacteria producing B-group vitamins: A great potential for functional cereals products. Appl. Microbiol. Biotechnol. 2012, 96, 1383–1394.
67. Terpou, A.; Papadaki, A.; Lappa, I.; Kachrimanidou, V.; Bosnea, L.; Kopsahelis, N. Probiotics in food systems: Significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. Nutrients 2019, 11, 1591.
68. Gibson, R.; Roberfroid, B. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J. Nutr. 1995, 125, 1401–1412.

69. Davani-Davari, D.; Negahdaripour, M.; Karimzadeh, I.; Seifan, M.; Mohkam, M.; Masoumi, S.; Berenjian, A.; Ghasemi, Y. Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications. Foods 2019, 8, 92.

70. Ahmadi, A.; Milani, E.; Madadlou, A.; Mortazavi, S.A.; Mokarram, R.R.; Salarbashi, D. Synbiotic yogurt-ice cream produced via incorporation of microencapsulated lactobacillus acidophilus (la-5) and fructooligosaccharide. J. Food Sci. Technol. 2014, 51, 1568–1574.

71. Markowiak, P.; Śliżewska, K. Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. Nutrients 2017, 15, 1021.

72. Wilms, E.; Gerritsen, J.; Smidt, H.; Besseling-van der Vaart, I.; Rijkers, G.T.; Fuentes, A.R.G.; Masclee, A.A.M.; Troost, F.J. Effects of Supplementation of the Synbiotic Ecologic® 825/FOS P6 on Intestinal Barrier Function in Healthy Humans: A Randomized Controlled Trial. PLoS ONE 2016, 11, e0167775.

73. Rad, A.; Aghebati-Maleki, L.; Kafil, H.; Gilani, N.; Abbasi, A.; Khani, N. Postbiotics, as dynamic biomolecules, and their promising role in promoting food safety. Biointerface Res. Appl. Chem. 2021, 11, 14529–14544.

74. Salminen, S.; Collado, M.; Endo, A.; Hill, C.; Lebeer, S.; Quigley, E.; Sanders, M.; Shamir, R.; Swann, J.; Szajewska, H.; et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Nat. Rev. Gastroenterol. Hepatol. 2021, 18, 649–667.

75. Conte, M.; Porpora, M.; Nigro, F.; Nigro, R.; Budelli, L.; Barone, V.; Nanayakkara, M. Pro-Pre and Postbiotic in Celiac Disease. Appl. Sci. 2021, 11, 8185.

76. Sarno, M.; Lania, G.; Cuomo, M.; Nigro, F.; Passannanti, F.; Budelli, A.; Fasano, F.; Troncone, R.; Auricchio, S.; Barone, M.V.; et al. Lactobacillus paracasei CBA L74 interferes with gliadin peptides entrance in Caco-2 cells. Int. J. Food Sci. Nutr. 2014, 65, 953–959.

77. Conte, M.; Nigro, F.; Porpora, M.; Bellomo, C.; Furone, F.; Budelli, A.L.; Nigro, R.; Barone, M.V.; Nanayakkara, M. Gliadin Peptide P31–43 Induces mTOR/NFkβ Activation and Reduces Autophagy: The Role of Lactobacillus paracasei CBA L74 Postbiotc. Int. J. Mol. Sci. 2022, 23, 3655.

78. Freire, R.; Ingano, L.; Serena, G.; Cetinbas, M.; Anselmo, A.; Sapone, A.; Sadreyev, R.; Fasano, A.; Senger, S. Human gut derived-organoids provide model to study gluten response and effects of microbiota-derived molecules in celiac disease. Sci. Rep. 2019, 9, 7029.

79. Cao, Z.; Sugimura, N.; Burgermeister, E.; Ebert, M.; Zuo, T.; Lan, P. The gut virome: A new microbiome component in health and disease. EBioMedicine 2022, 81, 104113.

80. Mills, S.; Shanahan, F.; Stanton, C.; Hill, C.; Coffey, A.; Ross, R. Movers and shakers: Influence of bacteriophages in shaping the mammalian gut microbiota. Gut Microbes 2013, 4, 4–16.

81. Vitetta, L.; Vitetta, G.; Hall, S. Immunological Tolerance and Function: Associations Between Intestinal Bacteria, Probiotics, Prebiotics, and Phages. Front. Immunol. 2018, 9, 2240.
82. El Mouzan, M.; Assiri, A.; Al Sarkhy, A.; Alasmi, M.; Saeed, A.; Al-Hussaini, A.; AlSaleem, B.; Al Mofarreh, M. Viral dysbiosis in children with new-onset celiac disease. PLoS ONE 2022, 17, e0262108.
83. Garmaeva, S.; Gulyaeva, A.; Sinha, T.; Shkoporov, A.N.; Clooney, A.G.; Stockdale, S.R.; Spreckels, J.E.; Sutton, T.D.; Draper, L.A.; Dutilh, B.E.; et al. Stability of the human gut virome and effect of gluten-free diet. Cell Rep. 2021, 35, 109132.
84. Febvre, H.; Rao, S.; Gindin, M.; Goodwin, N.; Finer, E.; Vivanco, J.; Lu, S.; Manter, D.; Wallace, T.; Weir, T. PHAGE Study: Effects of Supplemental Bacteriophage Intake on Inflammation and Gut Microbiota in Healthy Adults. Nutrients 2019, 11, 666.
85. Lerner, A.; Ramesh, A.; Matthias, T. The Revival of the Battle between David and Goliath in the Enteric Viruses and Microbiota Struggle: Potential Implication for Celiac Disease. Microorganisms 2019, 7, 173.
86. SBIR Phase I: Bacteriophage-Based Microbial Gene Therapy Platform for In Situ Engineering of Microbiomes. Available online: https://www.sbir.gov/sbirsearch/detail/1705577 (accessed on 20 September 2022).
87. Heder, M. From NASA to EU: The evolution of the TRL scale in Public Sector Innovation. Innov. J. 2017, 22, 1.