

Review Article

Microbiota, Bacterial Carbonic Anhydrases, and Modulators of Their Activity: Links to Human Diseases?

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The involvement of the human microbiome is crucial for different host functions such as protection, metabolism, reproduction, and especially immunity. However, both endogenous and exogenous factors can affect the balance of the microbiota, creating a state of dysbiosis, which can start various gastrointestinal or systemic diseases. The challenge of future medicine is to remodel the intestinal microbiota to bring it back to healthy equilibrium (eubiosis) and, thus, counteract its negative role in the diseases' onset. The shaping of the microbiota is currently practiced in different ways ranging from diet (or use of prebiotics, probiotics, and synbiotics) to phage therapy and antibiotics, including microbiota fecal transplantation. Furthermore, because microbiota modulation is a capillary process, and because many microbiota bacteria (both beneficial and pathogenic) have carbonic anhydrases (specifically the four classes α , β , γ , and ι), we believe that the use of CA inhibitors and activators can open up new therapeutic strategies for many diseases associated with microbial dysbiosis, such as the various gastrointestinal disorders and the same colorectal cancer.

1. Introduction

The term microbiome refers to the whole habitat, including the different microorganisms (bacteria, archaea, eukaryotes, and viruses) that define the so-called microbiota, their genomes, and the environmental conditions. Lederberg and McCray first specified the expression microbiota, who pointed out the importance of microorganisms inhabiting the human body in health and disease [1]. However, we have only recently started to recognize that the human body is home to much more than human cells [2]: we shelter at least 100 trillion (10^{14}) microbial cells and a quadrillion viruses [3]. As said before, this intricate community includes bacteria, eukaryotes, viruses, and at least one archaeon that interact with each other and with the host, resulting in a significant impact on human health and physiology. Only a tiny portion of these can be cultured, and high-throughput

sequencing has recently seriously increased the range of known microbes in our bodies and the environment [2, 4]. The gut microbiota (GM) composition mirrors the natural selection at both microbial and host levels, fostering mutual interplay and functional stability of this complex ecosystem [5]. Acid and pancreatic secretions usually prevent bacterial colonization of the stomach and proximal small intestine. Bacterial density, however, rises in the distal small intestine and increases to an estimated 10^{11} - 10^{12} bacteria per gram of colonic matter in the large intestine, contributing to 60% of the fecal mass [5, 6]. The foetal gut seems sterile, but colonization starts immediately after birth and is affected by the delivery mode, infant diet, hygiene levels, and antibiotic intake [7]. In the environment, humans coevolved with microbes, and each body habitat has in its microbiota a unique set of microorganisms that are established in the first 1-3 years of life and remain relatively stable over the entire life span [8]. The

microbiota was generally characterized using molecular methods primarily based on the analysis of 16S rRNA genes or other marker genes and genomic regions, amplified and sequenced from the biological samples provided [9]. Several tools that assign each sequence to a microbial taxon (bacteria, archaea, or lower eukaryotes) may be used to perform taxonomic assignments at different taxonomic levels according to phyla, groups, orders, families, genera, and organisms [9]. Just a few phyla are represented in each body district, accounting for hundreds of species of bacteria [10].

For body physiology, the human microbiome is crucial, producing an enormous number of molecules able to communicate with the host. In particular, gut bacteria are a natural protection against pathogens, and, in addition, they break down indigestible dietary components (e.g., vegetal polysaccharides) [11]. The metabolic functions of residential microbes are involved in host functions, such as protection, metabolism, reproduction, and immunity [12]. It consists of two predominant phyla, *Firmicutes* and *Bacteroidetes* (about 90% of the total bacteria), while the remaining 10% is split between *Verrucomicrobia*, *Proteobacteria*, and *Actinobacteria* [13]. Notably, if, on the one hand, the microbiota participates in the maturation of the host immunity and its functionality, on the other hand, it is modulated by the host's immune system [6]. The GM role is crucial for the proper development of the gut-associated lymphoid tissue (GALT) and the expected evolution of the innate and specific immune system [14]. The microbiota-immunity axis enables the optimal arrangement of the innate and adaptive immune response in eubiosis conditions to modulate the most suitable reaction [15]. The recent increase of microbiome studies sheds light on its contributing impact on etiology and the progression of many diseases. A microbiota imbalance, named "dysbiosis," can cause significant effects on the host [16]. Understanding the link between illness and dysbiosis could let researchers sufficiently define the development of an increasing number of human diseases and discover innovative treatments, modulating the microbiota composition to restore its eubiosis status and so the host health.

2. Link between Dysbiosis and Pathologies

Gut dysbiosis has a drastic impact on gut health. As reported by the American Gastroenterologist Association's journal, Crohn's disease (CD), ulcerative colitis (UC), and pouchitis are the results of the pathogenic immune response following gut microbiota antigenic stimulation consequently to mucosal barrier defects [17]. Recently, we have described a dissimilarity of cytokines' distribution and microbiota composition within the CD and the adjacent healthy ileal tissue layers and, in addition, between the first operation and surgical relapse [18]. Another relevant disease deeply correlated with gut dysbiosis is *Clostridioides difficile* Infection (CDI), caused by opportunistic bacteria responsible for infectious colitis in hospitalized patients [19]. Considering that CDI occurs in patients with disrupted gut microbiota, it seems easy to hypothesize that healthy gut microbiota can prevent *Clostridioides difficile* colonization [20]; in fact, the fecal

microbiota transplant can contrast the infection restoring a functional microbiota, as recently documented by a systematic study [21]. Microbiota composition in cancer biology has been increasingly accepted as an environmental factor favoring colorectal cancer (CRC) development. Microbial dysbiosis associated with CRC can alter the delicate balance between the gut microbiota and the host's immune system, leading to cancer initiation and/or progression [22]. As a result, CRC can be avoided by converting the microbiome to a noncarcinogenic microbiome. In this context, probiotics are being explored for their potential function in CRC prevention and treatment and as an adjunct to conventional therapy [23]. The role of *Fusobacterium nucleatum* is very emblematic. It promotes CRC by the induction of epithelial cell proliferation [24], enabling a proinflammatory microenvironment [25] and producing proteins able to stop the antitumoral activity of T and NK cells [26, 27]. The data of our recent study [28] suggest that microbial communities can drive and modulate the antitumor immune response. We have shown for the first time that in CRC, *Prevotella* and *Bacteroides* species are correlated positively and negatively, respectively, with the secretion of IL-9, which has a fascinating and still debated role in tumor immunity.

In the context of cancer, the microbiota is also involved in other tumor types. As previously reported, changes in microbiome composition seem to induce or exacerbate chronic inflammation, leading to immune surveillance disruption. Besides, concerning the intestinal microbiota, the link between local microbiota and some cancers has been elucidated, connecting local dysbiosis and carcinogenesis. Hayes et al. have demonstrated that increased relative abundance of *Corynebacterium* and *Kingella bacteria* in the oral microbiome has been associated with reduced incidence of oral cavity carcinomas [29]. Different gut microbiome composition has been shown among patients with pancreatic malignancies compared to healthy controls [30].

Finally, chronic inflammation, related to altered lung microbiota, could explain local carcinogenesis in lung cancer. It has been demonstrated that several bacteria species have been enriched in lung cancer patients compared with healthy individuals [31, 32]. Lung microbiota modifications induced by antibiotics could explain the higher incidence of lung cancer among users of antibiotics as reported in a recent meta-analysis [33].

Still, regarding cancer, the development of immune checkpoint inhibitors (ICIs) has transformed the therapeutic view for many malignancies. Several lines of evidence have indicated that the gut microbiome plays a crucial role in modulating immune checkpoint blockade response across a range of cancer types [34–36]. In responders to anti-PD1 antibody, a higher gut microbial alpha diversity was highlighted, as the relative abundance of the order Clostridiales, the *Ruminococcaceae* family, and the species *Faecalibacterium prauznitzii* [36]. Regarding other pathologies, Novakovic [37] and Oikonomou [38], in two recent reviews, analyzed some studies, focused on the interplay between microbiota-immune response with cardiovascular diseases, for which hypertension represents the leading risk factor. Several data [39, 40] have highlighted that atherosclerosis

TABLE 1: The genome of Gram-positive and Gram-negative bacteria of the human microbiome encodes for CAs belonging to different classes. Some of the probiotics considered in the present study play an essential role in human health. Others, due to the changes in the microbial composition, can be considered pathogens for the host.

| Microorganism | CA class | | | | Disease | Through the production of antimicrobial ^a |
|---------------------------------------|----------|---------|----------|---|---|---|
| | α | β | γ | ? | | |
| Gram-positive | | | | | | |
| <i>Lactobacillus reuteri</i> | - | - | + | - | | Intestinal beneficial effects through the production of antimicrobial molecules, organic acids, ethanol, and reuterin, showing antimicrobial activity. |
| <i>Clostridium butyricum</i> | - | + | + | - | | Involved positively in antiprogrammed cell death protein-1 (PD-1) treatment, in homeostasis and anti-inflammatory response in inflammatory gut disease. |
| <i>Clostridium difficile</i> | - | + | + | - | Associated with a history of unrelated diarrheal illnesses, such as food poisoning or laxative abuse. Production of toxin, such as enterotoxin and cytotoxin. | |
| <i>Ruminococcus gnavus</i> | - | - | - | - | Associated with Crohn's disease, an inflammatory bowel disease, through the production of an inflammatory polysaccharide. | |
| <i>Ruminococcus torques</i> | - | + | + | - | Bacterial species known to decrease gut barrier integrity. | |
| Gram-negative | | | | | | |
| <i>Fusobacterium nucleatum</i> | - | - | - | - | Involved in periodontal disease and colon-rectal cancer (CRC) development. | |
| <i>Faecalibacterium prausnitzii</i> * | - | + | + | - | | Involved positively in antiprogrammed cell death protein-1 (PD-1) treatment. Potentially important role in promoting gut health. |
| <i>Akkermansia muciniphila</i> | - | - | + | - | | Intestinal beneficial effects acting as anti-inflammatory. |
| <i>Prevotella melaninogenica</i> | - | + | - | - | Associated with many types of infection, including oral abscesses and infections in the intestinal tract, the female genitalia tract, and the upper and lower respiratory tracts and in the bone marrow. This species interferes with the host inflammatory response. | |
| <i>Prevotella copri</i> | - | + | + | - | Associated with inflammatory diseases, interfering with the host inflammatory response. | |
| <i>Prevotella intermedia</i> | - | - | + | - | Involved in periodontal infections. Interferes with the host inflammatory response. | |
| <i>Bacteroides fragilis</i> | - | + | + | - | Involved in abscess formation and bacteremia. | |
| <i>Bacteroides uniformis</i> | - | + | + | - | Associated with human infections. | |
| <i>Bacteroides vulgatus</i> | - | + | + | - | Associated with human infections. | |
| <i>Bacteroides stercoris</i> | - | + | + | - | Associated with human infections. | |
| <i>Bacteroides thetaiotaomicron</i> | - | + | + | - | Associated with human infections. | |
| <i>Kingella oralis</i> | - | + | - | - | Associated with periodontitis. | Reduces/reduced incidence of oral cavity carcinomas. |
| <i>Kingella kingae</i> | - | + | + | + | Associated with respiratory or urinary tract infections. | Reduces/reduced incidence of oral cavity carcinomas. |

^a[98]. * *Faecalibacterium prausnitzii* stains like a Gram-negative bacterium but exhibits dermis characteristics that resemble Gram-positive bacteria; - absent; + present.

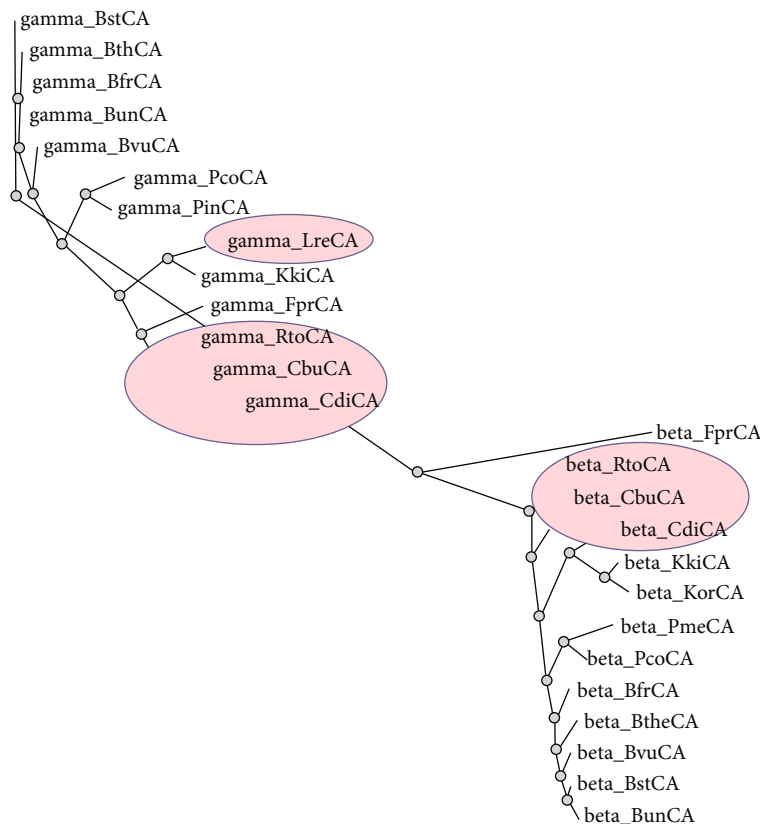


FIGURE 1: Dendrogram obtained by the phylogenetic analysis carried out on γ - and β -CAs identified in the genome of the Gram-negative and Gram-positive bacteria indicated in Table 1. The tree was constructed using the program PhyML 3.0. Accession numbers of the amino acid sequences used in the phylogenetic analysis are given in Table 2. Blue circles indicate the Gram-positive bacteria.

development, the dominant cause of cardiovascular diseases, is linked with trimethylamine N-oxide (TMAO) levels on that the changes in GM composition have marked effects.

The gut-brain-microbiome axis is one of the principal pathways for the interplay between the microbiome and disease. Although most studies are in preclinical stages, evidence suggests continuous communication along this axis. In particular, the brain responds to gut microbiome signals in order to change gut motility and permeability, influencing the microbiota functionality [41]. In this scenario, increasing studies focus on the link between the gut-brain-microbiome axis and neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS). Marizzoni and his group [42] reported that gut microbiota-related products, such as lipopolysaccharides and short-chain fatty acids, and systemic inflammation are related to brain amyloidosis presence in older human subjects. In addition, a recent study by Mandrioli et al. [43] proposed a trial to evaluate the biological basis of a potential treatment for ALS using the FMT. In ALS, GM dysbiosis may favor the disease onset or drive its progression and related outcomes in the presence of other risk factors. Otherwise, ALS presence could further alter GM dysbiosis and, in some individuals, lead to disease progression and prognosis and affect treatment response [44].

3. Microbiota Shaping: Focus on the Antibiotic Therapy

The imbalance of the gut microbiota of the bacterial species has been demonstrated to be prevalent among various debilitating diseases. Diet, prebiotics, probiotics, symbiotics, FMT, phage therapy, and antibiotics are some of the new emerging therapeutic options. All of these are aimed at restoring gut homeostasis, microbiota composition, and physical barrier defense.

3.1. Diet. Diet is probably the most readily modifiable environmental factor, but few studies have accurately investigated the link between diet and GM composition [45–47]. Increasing evidence suggests that diets low in animal protein and high in vegetable and fiber intake are related to the prevention of cardiovascular disease [48]. A fascinating study of Pagliai et al. evaluated the functional composition of the fecal microbiota in a short-term, fully controlled low-calorie Mediterranean and vegetarian diet. They found that the short-term Mediterranean or vegetarian dietary pattern does not cause significant modification in the GM composition, implying that nutritional interventions should be sustained for more extended periods to scratch GM resilience [49].

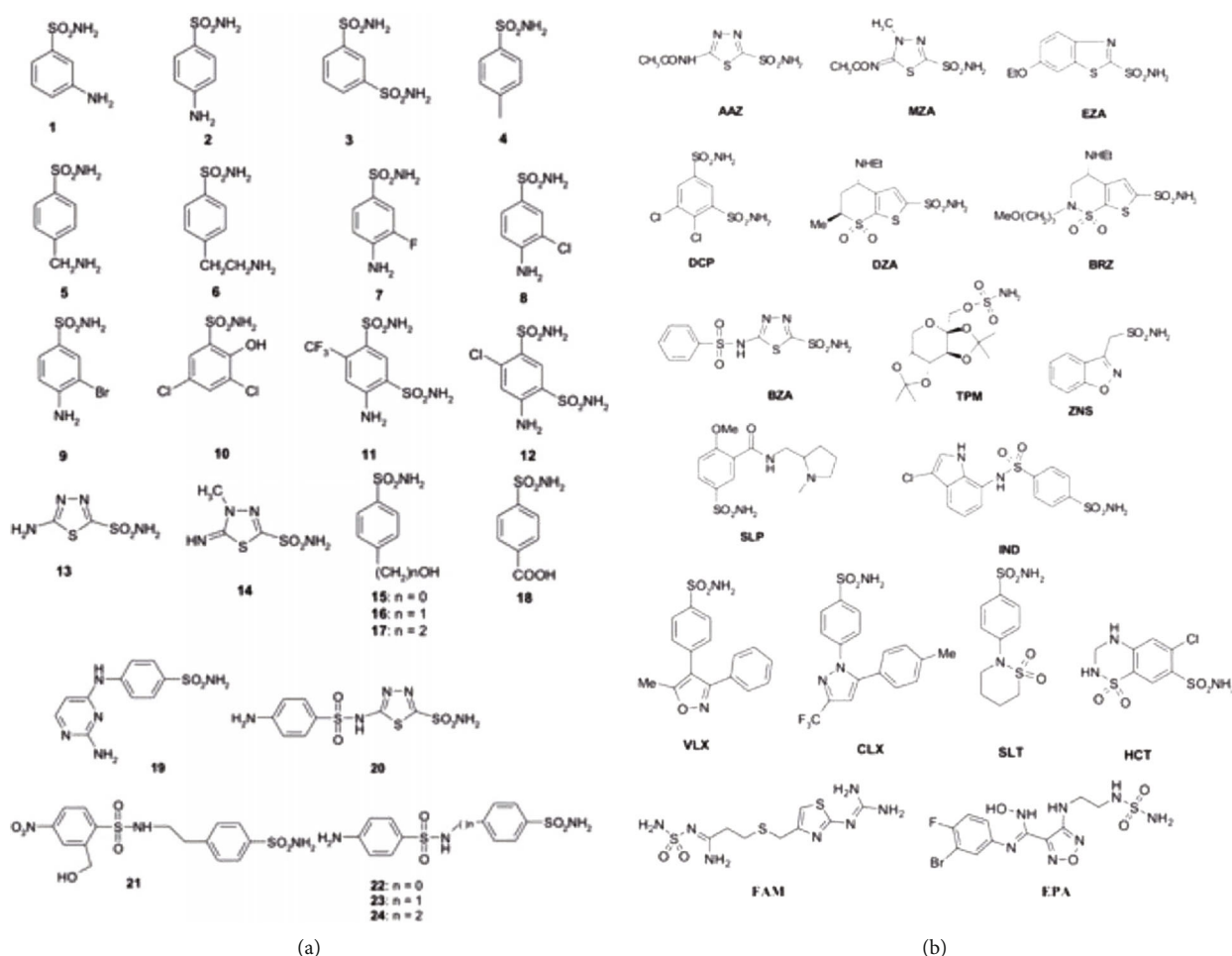


FIGURE 2: Sulfonamides and their isostere classes (sulfamates and sulfamides) as CAIs. Simple aromatic/heterocyclic derivatives 1-24 (a); clinically used drugs or agents in clinical development (b). Legend: AAZ: acetazolamide; MZA: methazolamide; EZA: ethoxzolamide; DCP: dichlorophenamide; DZA: dorzolamide; BRZ: brinzolamide; BZA: benzolamide; TPM: topiramate; SLT: sulthiame; ZNS: zonisamide; SLP: sulpiride; IND: indisulam; CLX: celecoxib; VLX: valdecoxib; HCT: hydrochlorothiazide; FAM: famotidine; EPA: epacadostat.

3.2. Probiotics, Prebiotics, and Synbiotics. Probiotics are live microorganisms that, when given in sufficient amounts, provide health benefits to the host. Probiotics have been shown in multiple studies to be effective in alleviating diarrhea and other gut-related side effects associated with anticancer therapy, restoring a healthy GM composition [50]. In detail, *Bifidobacterium* spp., *Lactobacillus* spp., *Lactococcus* spp., and *Saccharomyces boulardii* are the most routinely employed probiotics [51]. Prebiotics include nondigestible polysaccharides and oligosaccharides, among which inulin, lactulose, fructooligosaccharides, and galactooligosaccharides that are fermented by colonic bacteria, resulting in specific changes in the GM composition and functions. Prebiotic fibers can be found in various foods, primarily in vegetables like asparagus, garlic, leeks, and onions [52]. Prebiotics promote the growth of protective bacteria in the intestine, especially *Bifidobacterium* and *Lactobacilli*, and reduce intestinal permeability and metabolic endotoxemia [53]. Finally, synbiotics are a blend of prebiotics and probiotics that can help the host.

3.3. Fecal Microbiota Transplant. The injection by different ways (via colonoscopy, enema, orogastric tube, or by mouth in the form of a capsule) of feces from a healthy donor into the gastrointestinal tract of a recipient patient is known as FMT [54]. As a result, there is a chance to restore the complexity and diversity of the intestinal microbiota, even though probiotics are beneficial [55]. As previously reported, FMT is effective in treating recurrent CDI, with a cure rate of about 90%. FMT tends to be healthy in a short-term follow-up, with the most common recorded side effects including abdominal pain, diarrhea, constipation, and low-grade fever. Long-term consequences could include the transmission of undiagnosed infections that can cause disease years later and lead to chronic diseases like obesity, diabetes, NAFLD, asthma, and autism, as reported in case reports [56, 57]. Recent studies suggest a potential FMT role in improving anticancer therapy efficacy and adverse events. This therapeutic role of FMT was shown with some chemotherapy agents, immunotherapy, and radiotherapy [58, 59].

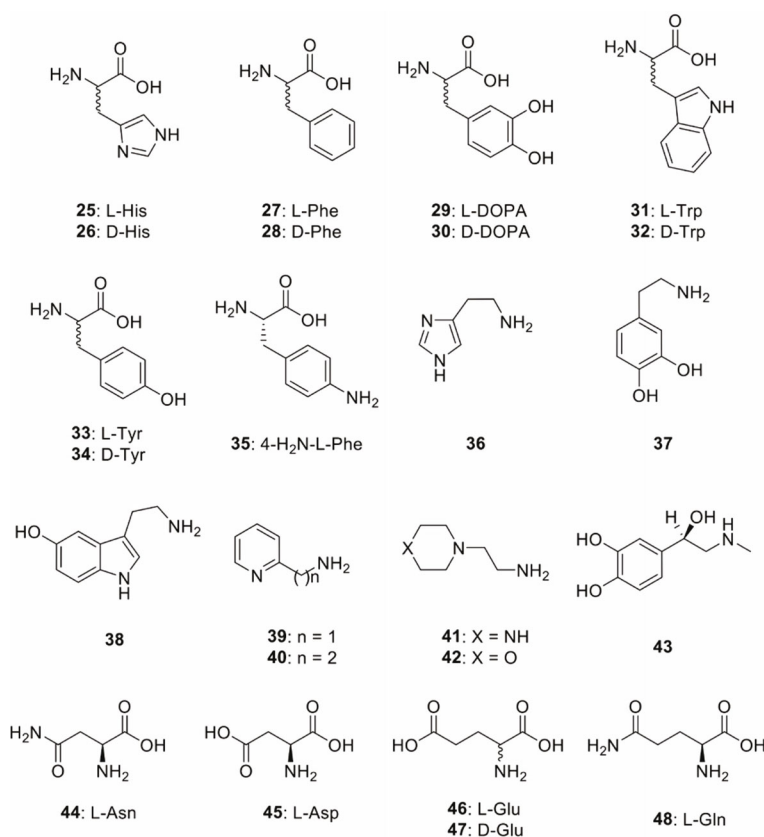


FIGURE 3: Amino acid and amine activators 25-48 investigated for the activation of several bacterial CAs.

3.4. Phage Therapy. Phages have been used as therapeutic instruments since their discovery more than a century ago. Despite their success in the first trials, the use of phage therapy was controversial and not generally accepted [60]. In the last few years, thanks to scientific progress in metagenomics and the consciousness of the intestinal microbiota importance to maintain human health, research on the intestinal phagome has brought up interest [61]. Phages have been mainly explored as promising tools in infectious diseases, among which cholera and *Clostridioides difficile* colitis and the eradication of adherent invasive *Escherichia coli* (AIEC) in Crohn's disease [62, 63]. The most critical aspect of phage therapeutic development is determining the phages' safety and efficacy. Currently, in vivo animal models or a suitable in vitro system is used in this research field [64].

3.5. Antibiotics. Antibiotics are a strong weapon against pathogenic bacteria but can also damage commensal organisms, leading to the loss of microbial diversity and reduced colonization resistance against pathogens [65]. A few days after antibiotic treatment, profound and swift modifications occur in the GM composition. Despite this, antibiotic therapy plays a fundamental role in microbiota manipulation. Small intestinal bacterial overgrowth (SIBO) is characterized by an abnormal number of bacteria in the small intestine and is followed by numerous gastrointestinal symptoms. The target in SIBO patients is to relieve symptoms through bacteria eradication [66], and antibiotics are widely used. However, some patients remain symptomatic after care,

meaning that other underlying disorders and/or antibiotic-resistant bacteria are to blame [67]. Currently, rifaximin, a nonsystemic antibiotic, is the most studied drug for SIBO patients. Numerous studies demonstrated its efficacy, even if the dose, treatment duration, diagnostic methods, and patients vary among studies [68–73]. A meta-analysis of rifaximin (dose range: 600–1,600 mg/d; duration of treatment: 5–28 days) documented that SIBO was eradicated in 70.8% of patients (26 studies; 95% CI, 61.4–78.2). Adverse events were rare, occurring in just 4.6 percent of 815 patients in 17 studies that reported safety [74]. Also, systemic antibiotics as ciprofloxacin, norfloxacin, and metronidazole reported SIBO eradication with both the breath test or bacterial culture [75–78]. Antibiotic GM regulation was only partially investigated in CRC, with only a few reports in the literature [79]. Cefoxitin is a semisynthetic and broad-spectrum cephalosporin that induced a complete and lasting clearance of enterotoxigenic *B. fragilis* colonization in previously ETBF-inoculated mice with a reduction in median adenoma formation [80]. Erythromycin can suppress the transcriptional activity of NF- κ B, the activator protein-1 (AP-1), and its downstream targets, IL-6 and cyclooxygenase-2 (COX-2), in human CRC cells [81]. As reviewed by Elkrief et al., antibiotics with a wide action range harm the outcomes of patients receiving ICIs [82]. Nevertheless, there could be present specific antibiotics that might induce favorable alterations in the host immune system even if the problem of antibiotics with a spectrum narrow enough to ensure a fine depletion remains. Some patients, in fact, could show an abundance of species that promote immune

TABLE 2: Microorganisms, CA accession numbers, and protein acronyms of the amino acid sequences used in the phylogenetic analysis.

| Microorganism | Accession number | ?-class | Acronym | Accession number | ?-class | Acronym |
|-------------------------------------|------------------|---------|-------------|------------------|---------|-------------|
| <i>Lactobacillus reuteri</i> | | | | WP_163622737.1 | | gamma_LreCA |
| <i>Clostridium butyricum</i> | ALP89146.1 | | beta_CbuCA | WP_035762541.1 | | gamma_CbuCA |
| <i>Clostridium difficile</i> | WP_003423380.1 | | beta_CdiCA | WP_004454132.1 | | gamma_CdiCA |
| <i>Ruminococcus gnavus</i> | | | | | | |
| <i>Ruminococcus torques</i> | WP_144366732.1 | | beta_RtoCA | CUN19994.1 | | gamma_RtoCA |
| <i>Fusobacterium nucleatum</i> | | | | | | |
| <i>Faecalibacterium prausnitzii</i> | WP_158402608.1 | | beta_FprCA | MBD9046903.1 | | gamma_FprCA |
| <i>Prevotella melaninogenica</i> | WP_120175219.1 | | beta_PmeCA | | | |
| <i>Prevotella copri</i> | WP_203055371.1 | | beta_PcoCA | WP_089544874.1 | | gamma_PcoCA |
| <i>Prevotella intermedia</i> | | | | WP_014708543.1 | | gamma_PinCA |
| <i>Bacteroides fragilis</i> | EEZ25097.1 | | beta_BfrCA | WP_005814348.1 | | gamma_BfrCA |
| <i>Bacteroides uniformis</i> | WP_005828510.1 | | beta_BunCA | WP_118132341.1 | | gamma_BunCA |
| <i>Bacteroides vulgatus</i> | ABR38061.1 | | beta_BvuCA | CDF19756.1 | | gamma_BvuCA |
| <i>Bacteroides stercoris</i> | WP_005652261.1 | | beta_BstCA | RGZ94434.1 | | gamma_BstCA |
| <i>Bacteroides thetaiotaomicron</i> | WP_118307725.1 | | beta_BtheCA | WP_008765423.1 | | gamma_BthCA |
| <i>Kingella oralis</i> | WP_040558280.1 | | beta_KorCA | | | |
| <i>Kingella kingae</i> | WP_019389503.1 | | beta_KkiCA | WP_019390101.1 | | gamma_KkiCA |

suppression through the activation and expansion of the regulatory T cells (Tregs). A recent clinical trial highlights the depletion of vancomycin-sensitive bacteria resulting in boosted radiotherapy's antitumor immune response and inhibition of tumor growth [83].

4. Bacterial Carbonic Anhydrases and Their Modulation

As mentioned above, it is evident that (a) microbiota metabolism has a crucial impact on the human intestine, acting as a self-regulating system and influencing those districts known as gut-brain, gut-liver, gut-kidney, and gut-heart; (b) microbiota tunes negatively or positively the host health.

Here, we focused on a superfamily of enzymes named carbonic anhydrases (CAs, EC 4.2.1.1) encoded by the genome of pathogenic and nonpathogenic bacteria [84–86], which are involved in the metabolic balance of the carbon dioxide (CO₂), bicarbonate (HCO₃⁻), and protons (H⁺), catalyzing the physiologically crucial reversible reaction of CO₂ hydration to HCO₃⁻ and H⁺, according to the following chemical reaction: CO₂ + H₂O ⇌ HCO₃⁻ + H⁺. Until now, eight CA classes indicated with α , β , γ , δ , ζ , η , θ , and ι have been described in all kingdoms of living organisms [84, 87–90]. All CA classes strictly conserve the CO₂ hydration and HCO₃⁻ dehydration mechanisms, showing an evident convergent evolution phenomenon, having a very low sequence similarity, and different 3D molecular folds and structures [86, 91]. In bacteria, four CA classes (α , β , γ , and ι) regulate the CO₂ and HCO₃⁻ balance, being the only CA classes encoded by the bacterial genome [84, 91–96].

Our groups started to explore the genome of both Gram-positive and Gram-negative probiotics for searching CA

genes belonging to four different classes (α , β , γ , and ι). Some of these bacteria play an essential role in human health, such as *Lactobacillus reuteri*, *Clostridium butyricum*, *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, *Kingella oralis*, and *K. kingae*. Due to the changes in the microbial composition, others can be considered pathogens for the host, such as *Clostridium difficile*, *Ruminococcus gnavus*, *Prevotella melaninogenica*, and *Bacteroides fragilis*. As reported in Table 1, the considered microorganisms show a CA gene distribution very varied. Some of these bacteria, such as *Lactobacillus reuteri*, *Akkermansia muciniphila*, and *Prevotella intermedia*, show only one CA class, the γ -CA. In contrast, the genome of bacteria, like *Prevotella melaninogenica* and *Kingella oralis*, contained only genes for β -CAs. At the same time, most of them have γ - and β -CAs. Again, *Kingella kingae* is the only species among all whose genome is characterized by the presence of the new recently identified class, the θ -class. *Ruminococcus gnavus*, which is generally associated with Crohn's disease, and *Fusobacterium nucleatum*, a bacterium involved in the periodontal disease, are typified by genomes that do not encode for any CA class (Table 1). Intriguingly, the bacteria of the human microbiome here considered resulted in the absence of genes encoding for α -CAs. We want to stress that a common feature of the α -CAs known to date is the presence of an N-terminal signal peptide, which suggests a periplasmic or extracellular location and a possible physiological role in CO₂ uptake processes [85, 90]. The lack of α -CAs in Gram-negative bacteria could be compensated by the presence of γ - or β -CAs characterized by a signal peptide, which may have a periplasmic localization and a role similar to that described for the α -forms [97]. Interestingly, we constructed a phylogenetic tree to investigate the evolutionary relationship of β - and γ -CAs

identified in the microorganisms reported in Table 1 (Figure 1). As a result, the two CA classes (β and γ) are grouped in two clusters well separated from each other, indicating how phylogenetically different they are. The γ -CA amino acid sequences can be considered transition amino acid sequences from which the β -CAs have originated (Figure 1).

4.1. Inhibition of Bacterial CAs. CAs, with their activity, continually provide the indispensable CO_2 and HCO_3^- /protons to microbial biosynthetic pathways. Thus, their inhibition might impair the survival of microbes [7]. However, it is important to stress that the inhibition of the human microbiome CAs will not interfere with the human CAs since the mammalian genome encodes only for α -CAs, which are phylogenetically and structurally well separated by the bacterial β - and γ -CAs [7]. Fortunately, many CA inhibitors (CAIs) exist and belong to many chemical classes, such as substituted benzene-sulfonamides, inorganic metal-complexing anions, dithiocarbamates, and carboxylic acids [99–101]. Among them, the sulfonamides shown in Figure 2 are the most potent investigated CA inhibitors (CAIs) (simple derivatives 1–24 and clinically used drugs or agents in clinical development) [87, 98, 102–119]. All of them were shown to also act as CAI primary sulfonamides as these inhibit CAs by binding to the Zn^{2+} ion from the enzyme active site, in a tetrahedral geometry of the metal, whereas the sulfonamide is deprotonated at the SO_2NH_2 moiety. The nitrogen atom of the SO_2NH_2 group then coordinates the Zn^{2+} ion and participates in a network of H-bonds, which involve conserved amino acid residues (Thr199 and Glu106), which in this way anchor the inhibitor molecule to the enzyme very strongly. This has been demonstrated by X-ray crystallographic studies of many adducts of such sulfonamides with various CA isoforms. The scaffold of the inhibitor (aromatic/heterocyclic moiety) also interacts with amino acid residues from the active site, either in the hydrophilic or within the hydrophobic part of the catalytic cleft.

4.2. CA Activators. It is possible to assume that the resident gastric microflora responsible for human wellbeing can be reinforced and improved through the increase of the CO_2 and HCO_3^- produced by enhancing the activity of their bacterial CAs. To accomplish this, the CA activity can be specifically intensified with molecules known as “activators” (CAA), which can bind within the middle-exit part of the enzyme active site. The modulation of the CAs encoded by the human microbiome can be considered a possible new pharmacological treatment since selective CAIs can interfere with the growth of those bacteria responsible for human illness, while the use of selective CAAs could improve the action of the microbiome having a beneficial effect on the host. The CAAs are biogenic amines (histamine, serotonin, and catecholamines—see Figure 3), amino acids, oligopeptides, or small proteins, such as compounds 25–48 shown in Figure 3 [120–122]. The X-ray crystal structure of the human isoforms (hCA I and II) bound to activators, such as histamine, L-/D-histidine, L-/D-phenylalanine, and D-tryptophan, allowed the comprehension of the activation

mechanism and the structure-activity relationship governing it [118, 123–129]. CAAs do not influence the binding of CO_2 to the CA active site but mediate the rate-determining step of the catalysis hurrying the transfer of protons from the active site to the environment. The final result is an overall increase in the catalytic turnover. Thus, the CA activators enhance the k_{cat} of the enzyme up to 10^6 s^{-1} with no effect on K_M [118, 120, 121]. CAAs may have pharmacologic applications in therapy memory, in neurodegenerative diseases (Alzheimer’s disease), or in the treatment of genetic CA deficiency syndromes [118, 120, 121]. On the other hand, the activation of bacterial CAs was poorly investigated [130, 131]. For this reason, Vullod et al. and Akdemir et al. investigated the activation profile of the bacterial CAs identified in the genome of the pathogenic and nonpathogenic bacteria to understand better the role of the CAs in the life-cycle and virulence of bacteria [130, 131].

5. Conclusion

It is now well established that the gut microbiome in the eubiosis status plays an important role in human physiology by producing numerous molecules and mediators that influence various host functions such as digestion, vitamin production, energy intake, pathogen protection, and immune system maturation/modulation [132]. However, various factors (endogenous and exogenous) can affect the compositional/functional balance of the microbiota, creating a state of dysbiosis, which is the starting point of various gastrointestinal diseases (IBD, CRC, celiac disease, etc.) and not metabolic disorders, immunological dysregulations, mental illnesses, etc. The challenge for modern medicine is to figure out how to reconstruct the gut microbiota to restore it to a healthy balance (eubiosis) and counterbalance its negative involvement in illness onset. This is undoubtedly a difficult challenge as the microbiota is a complex ecosystem that interfaces with an equally complex universe called host/human. The microbiota shaping is, therefore, a delicate process that falls in precision (personalized) medicine [133] and that is currently practiced in different ways, as previously discussed, ranging from the diet (or use of prebiotics, probiotics, and synbiotics) up to the phage therapy and antibiotics, including finally the microbiota fecal transplantation. Because microbiota modulation is a capillary process, and because many microbiota bacteria (both beneficial and pathogenic) have carbonic anhydrases (specifically the four classes α , β , γ , and ι), the use of CA inhibitors and activators can open up new therapeutic strategies for many of the diseases related to a maximum microbial dysbiosis, such as the various gastrointestinal disorders and the same colorectal cancer [134], which is currently one of the most common tumors in the world with an age-standardized worldwide incidence of 19.7 and mortality of 8.9 per 100000 person-year [133]. Surgical resection is the golden standard of CRC management. However, according to the clinical and pathological stage and if appropriate, this treatment should be integrated with neoadjuvant and/or adjuvant therapies, such as CA inhibitors and activators that we propose as future integration.

Conflicts of Interest

The authors declare no conflicts of interest.

References

- [1] B. J. Lederberg and A. T. McCray, "Ome SweetOmics—A genealogical treasury of words," *The scientist*, vol. 15, no. 7, p. 8, 2000.
- [2] W. B. Whitman, D. C. Coleman, and W. J. Wiebe, "Prokaryotes: the unseen majority," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 12, pp. 6578–6583, 1998.
- [3] M. Haynes and F. Rohwer, "The human virome," *Metagenomics of the human body*, pp. 63–77, 2011.
- [4] J. Shendure and H. Ji, "Next-generation DNA sequencing," *Nature Biotechnology*, vol. 26, no. 10, pp. 1135–1145, 2008.
- [5] A. M. O'Hara and F. Shanahan, "The gut flora as a forgotten organ," *EMBO Reports*, vol. 7, no. 7, pp. 688–693, 2006.
- [6] P. B. Eckburg, E. M. Bik, C. N. Bernstein et al., "Diversity of the human intestinal microbial flora," *Science*, vol. 308, no. 5728, pp. 1635–1638, 2005.
- [7] M.-M. Grönlund, O.-P. Lehtonen, E. Eerola, and P. Kero, "Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery," *Journal of pediatric gastroenterology and nutrition*, vol. 28, no. 1, pp. 19–25, 1999.
- [8] T. Yatsunencko, F. E. Rey, M. J. Manary et al., "Human gut microbiome viewed across age and geography," *Nature*, vol. 486, no. 7402, pp. 222–227, 2012.
- [9] J. R. Marchesi and J. Ravel, "The vocabulary of microbiome research: a proposal," *Microbiome*, vol. 3, no. 1, p. 31, 2015.
- [10] L. Laterza, G. Rizzatti, E. Gaetani, P. Chiusolo, and A. Gasbarrini, "The gut microbiota and immune system relationship in human graft-versus-host disease," *Mediterranean journal of hematology and infectious*, vol. 8, no. 1, article e2016025, 2015.
- [11] J. L. Sonnenburg, L. T. Angenent, and J. I. Gordon, "Getting a grip on things: how do communities of bacterial symbionts become established in our intestine?," *Nature Immunology*, vol. 5, no. 6, pp. 569–573, 2004.
- [12] M. Kumar, P. Babaei, B. Ji, and J. Nielsen, "Human gut microbiota and healthy aging: recent developments and future prospective," *Nutrition and Healthy aging*, vol. 4, no. 1, pp. 3–16, 2016.
- [13] E. Russo, G. Bacci, C. Chiellini et al., "Preliminary comparison of oral and intestinal human microbiota in patients with colorectal cancer: a pilot study," *Frontiers in Microbiology*, vol. 8, p. 2699, 2018.
- [14] J. J. Cebra, "Influences of microbiota on intestinal immune system development," *The American journal of clinical nutrition*, vol. 69, no. 5, pp. 1046s–1051s, 1999.
- [15] L. Zitvogel, M. Ayyoub, B. Routy, and G. Kroemer, "Microbiome and anticancer immunosurveillance," *Cell*, vol. 165, no. 2, pp. 276–287, 2016.
- [16] N. Weinstein, B. Garten, J. Vainer, D. Minaya, and K. Czaja, "Managing the microbiome: how the gut influences development and Disease," *Nutrients*, vol. 13, no. 1, pp. 74–114, 2021.
- [17] R. B. Sartor, "Microbial influences in inflammatory bowel diseases," *Gastroenterology*, vol. 134, no. 2, pp. 577–594, 2008.
- [18] E. Russo, F. Giudici, F. Ricci et al., "Diving into inflammation: a pilot study exploring the dynamics of the immune-microbiota axis in ileal tissue layers of patients with Crohn's disease," *Journal of Crohn's and Colitis*, vol. 15, no. 9, pp. 1500–1516, 2021.
- [19] C. P. Kelly and J. T. LaMont, "*Clostridium difficile* — more difficult than ever," *The New England Journal of Medicine*, vol. 359, no. 18, pp. 1932–1940, 2008.
- [20] S. L. Revolinski and L. S. Munoz-Price, "Clostridium difficile exposures, colonization, and the microbiome: implications for prevention," *Infection Control and Hospital Epidemiology*, vol. 39, no. 5, pp. 596–602, 2018.
- [21] E. N. Tixier, E. Verheyen, Y. Luo et al., "Systematic review with meta-analysis: fecal microbiota transplantation for severe or fulminant Clostridioides difficile," *Digestive Diseases and Sciences*, vol. 66, no. 3, 2021.
- [22] S. H. Wong and J. Yu, "Gut microbiota in colorectal cancer: mechanisms of action and clinical applications," *Nature Reviews. Gastroenterology & Hepatology*, vol. 16, no. 11, pp. 690–704, 2019.
- [23] B. Kumar, S. Harilal, S. Carradori, and B. Mathew, "A comprehensive overview of colon cancer- a grim reaper of the 21st century," *Current Medicinal Chemistry*, vol. 28, no. 14, pp. 2657–2696, 2021.
- [24] M. R. Rubinstein, X. Wang, W. Liu, Y. Hao, G. Cai, and Y. W. Han, "*Fusobacterium nucleatum* Promotes Colorectal Carcinogenesis by Modulating E-Cadherin/ β -Catenin Signaling via its FadA Adhesin," *Cell Host & Microbe*, vol. 14, no. 2, pp. 195–206, 2013.
- [25] A. D. Kostic, E. Chun, L. Robertson et al., "*Fusobacterium nucleatum* Potentiates Intestinal Tumorigenesis and Modulates the Tumor-Immune Microenvironment," *Cell Host & Microbe*, vol. 14, no. 2, pp. 207–215, 2013.
- [26] T. Wu, L. Cui, Z. Liang, C. Liu, Y. Liu, and J. Li, "Elevated serum IL-22 levels correlate with chemoresistant condition of colorectal cancer," *Clinical Immunology*, vol. 147, no. 1, pp. 38–39, 2013.
- [27] C. Gur, Y. Ibrahim, B. Isaacson et al., "Binding of the Fap2 Protein of *Fusobacterium nucleatum* to Human Inhibitory Receptor TIGIT Protects Tumors from Immune Cell Attack," *Immunity*, vol. 42, no. 2, pp. 344–355, 2015.
- [28] E. Niccolai, E. Russo, S. Baldi et al., "Significant and conflicting correlation of IL-9 with *Prevotella* and *Bacteroides* in human colorectal cancer," *Frontiers in immunology*, vol. 11, pp. 1–14, 2021.
- [29] R. B. Hayes, J. Ahn, X. Fan et al., "Association of oral microbiome with risk for incident head and neck squamous cell cancer," *JAMA Oncology*, vol. 4, no. 3, pp. 358–365, 2018.
- [30] X. Zhang, Q. Liu, Q. Liao, and Y. Zhao, "Pancreatic cancer, gut microbiota, and therapeutic efficacy," *Journal of Cancer*, vol. 11, no. 10, pp. 2749–2758, 2020.
- [31] Q. Mao, F. Jiang, R. Yin et al., "Interplay between the lung microbiome and lung cancer," *Cancer Letters*, vol. 415, pp. 40–48, 2018.
- [32] A. Fabbri, A. Amedei, F. Lavorini, T. Renda, and G. Fontana, "The lung microbiome: clinical and therapeutic implications," *Internal and Emergency Medicine*, vol. 14, no. 8, pp. 1241–1250, 2019.
- [33] F. Petrelli, M. Ghidini, A. Ghidini et al., "Use of antibiotics and risk of cancer: a systematic review and meta-analysis of observational studies," *Cancers*, vol. 11, no. 8, p. 1174, 2019.

- [34] V. Matson, J. Fessler, R. Bao et al., “The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients,” *Science*, vol. 359, no. 6371, pp. 104–108, 2018.
- [35] B. Routy, E. le Chatelier, L. Derosa et al., “Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors,” *Science*, vol. 359, no. 6371, pp. 91–97, 2018.
- [36] V. Gopalakrishnan, C. N. Spencer, L. Nezi et al., “Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients,” *Science*, vol. 359, no. 6371, pp. 97–103, 2018.
- [37] M. Novakovic, A. Rout, T. Kingsley et al., “Role of gut microbiota in cardiovascular diseases,” *World Journal of Cardiology*, vol. 12, no. 4, pp. 110–122, 2020.
- [38] E. Oikonomou, M. Leopoulou, P. Theofilis et al., “A link between inflammation and thrombosis in atherosclerotic cardiovascular diseases: clinical and therapeutic implications,” *Atherosclerosis*, vol. 309, pp. 16–26, 2020.
- [39] R. A. Koeth, Z. Wang, B. S. Levison et al., “Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis,” *Nature Medicine*, vol. 19, no. 5, pp. 576–585, 2013.
- [40] P. Illiano, R. Brambilla, and C. Parolini, “The mutual interplay of gut microbiota, diet and human disease,” *The FEBS Journal*, vol. 287, no. 5, pp. 833–855, 2020.
- [41] C. R. Martin, V. Osadchiy, A. Kalani, and E. A. Mayer, “The brain-gut-microbiome axis,” *Cellular and Molecular Gastroenterology and Hepatology*, vol. 6, no. 2, pp. 133–148, 2018.
- [42] M. Marizzoni, A. Cattaneo, P. Mirabelli et al., “Short-chain fatty acids and lipopolysaccharide as mediators between gut dysbiosis and amyloid pathology in Alzheimer’s disease,” *Journal of Alzheimer’s Disease*, vol. 78, no. 2, pp. 683–697, 2020.
- [43] J. Mandrioli, A. Amedei, G. Cammarota et al., “FETR-ALS study protocol: a randomized clinical trial of fecal microbiota transplantation in amyotrophic lateral sclerosis,” *Frontiers in Neurology*, vol. 10, p. 1021, 2019.
- [44] H. Tremlett, K. C. Bauer, S. Appel-Cresswell, B. B. Finlay, and E. Waubant, “The gut microbiome in human neurological disease: a review,” *Annals of Neurology*, vol. 81, no. 3, pp. 369–382, 2017.
- [45] G. D. Wu, J. Chen, C. Hoffmann et al., “Linking long-term dietary patterns with gut microbial enterotypes,” *Science*, vol. 334, no. 6052, pp. 105–108, 2011.
- [46] C. de Filippo, D. Cavalieri, M. di Paola et al., “Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 33, pp. 14691–14696, 2010.
- [47] F. de Filippis, N. Pellegrini, L. Vannini et al., “High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome,” *Gut*, vol. 65, no. 11, pp. 1812–1821, 2016.
- [48] F. Sofi, M. Dinu, G. Pagliai et al., “Low-calorie vegetarian versus Mediterranean diets for reducing body weight and improving cardiovascular risk profile,” *Circulation*, vol. 137, no. 11, pp. 1103–1113, 2018.
- [49] G. Pagliai, E. Russo, E. Niccolai et al., “Influence of a 3-month low-calorie Mediterranean diet compared to the vegetarian diet on human gut microbiota and SCFA: the CARDIVEG study,” *European Journal of Nutrition*, vol. 59, no. 5, pp. 2011–2024, 2020.
- [50] M. Mego, V. Holec, L. Drgona, K. Hainova, S. Ciernikova, and V. Zajac, “Probiotic bacteria in cancer patients undergoing chemotherapy and radiation therapy,” *Complementary Therapies in Medicine*, vol. 21, no. 6, pp. 712–723, 2013.
- [51] S. Rampelli, M. Candela, M. Severgnini et al., “A probiotics-containing biscuit modulates the intestinal microbiota in the elderly,” *The Journal of Nutrition, Health & Aging*, vol. 17, no. 2, pp. 166–172, 2013.
- [52] M. Roberfroid, “Functional food concept and its application to prebiotics,” *Digestive and Liver Disease*, vol. 34, pp. S105–S110, 2002.
- [53] J. Vulevic, A. Juric, G. Tzortzis, and G. R. Gibson, “A mixture of trans-galactooligosaccharides reduces markers of metabolic syndrome and modulates the fecal microbiota and immune function of overweight adults,” *The Journal of Nutrition*, vol. 143, no. 3, pp. 324–331, 2013.
- [54] G. Cammarota, G. Ianiro, H. Tilg et al., “European consensus conference on faecal microbiota transplantation in clinical practice,” *Gut*, vol. 66, no. 4, pp. 569–580, 2017.
- [55] D. Shahinas, M. Silverman, T. Sittler et al., “Toward an understanding of changes in diversity associated with fecal microbiome transplantation based on 16S rRNA gene deep sequencing,” *MBio*, vol. 3, no. 5, pp. e00338–e00412, 2012.
- [56] J.-W. Wang, C. H. Kuo, F. C. Kuo et al., “Fecal microbiota transplantation: Review and update,” *Journal of the Formosan Medical Association*, vol. 118, pp. S23–S31, 2019.
- [57] H. H. Choi and Y.-S. Cho, “Fecal microbiota transplantation: current applications, effectiveness, and future perspectives,” *Clinical endoscopy*, vol. 49, no. 3, pp. 257–265, 2016.
- [58] M. Cui, H. Xiao, Y. Li et al., “Faecal microbiota transplantation protects against radiation-induced toxicity,” *EMBO molecular medicine*, vol. 9, no. 4, pp. 448–461, 2017.
- [59] X. Wu, T. Zhang, X. Chen, G. Ji, and F. Zhang, “Microbiota transplantation: targeting cancer treatment,” *Cancer Letters*, vol. 452, pp. 144–151, 2019.
- [60] K. Moelling, F. Broecker, and C. Willy, “A wake-up call: we need phage therapy now,” *Viruses*, vol. 10, no. 12, p. 688, 2018.
- [61] A. N. Shkoporov and C. Hill, “Bacteriophages of the Human Gut: The “Known Unknown” of the Microbiome,” *Cell Host & Microbe*, vol. 25, no. 2, pp. 195–209, 2019.
- [62] J. Sabino, R. P. Hirten, and J.-F. Colombel, “Review article: bacteriophages in gastroenterology—from biology to clinical applications,” *Alimentary Pharmacology & Therapeutics*, vol. 51, no. 1, pp. 53–63, 2020.
- [63] M. Maronek, R. Link, L. Ambro, and R. Gardlik, “Phages and their role in gastrointestinal disease: focus on inflammatory bowel disease,” *Cells*, vol. 9, no. 4, p. 1013, 2020.
- [64] B. Gutiérrez and P. Domingo-Calap, “Phage therapy in gastrointestinal diseases,” *Microorganisms*, vol. 8, no. 9, p. 1420, 2020.
- [65] K. Lange, M. Buerger, A. Stallmach, and T. Bruns, “Effects of antibiotics on gut microbiota,” *Digestive Diseases*, vol. 34, no. 3, pp. 260–268, 2016.
- [66] S. S. C. Rao and J. Bhagatwala, “Small intestinal bacterial overgrowth: clinical features and therapeutic management,” *Clinical and translational gastroenterology*, vol. 10, no. 10, article e00078, 2019.

- [67] S. C. Shah, L. W. Day, M. Somsouk, and J. L. Sewell, "Meta-analysis: antibiotic therapy for small intestinal bacterial overgrowth," *Alimentary Pharmacology & Therapeutics*, vol. 38, no. 8, pp. 925–934, 2013.
- [68] M. Majewski and R. McCallum, "Results of small intestinal bacterial overgrowth testing in irritable bowel syndrome patients: clinical profiles and effects of antibiotic trial," *Advances in Medical Sciences*, vol. 52, pp. 139–142, 2007.
- [69] D. L. Franco, M. B. Disbrow, A. Kahn et al., "Duodenal aspirates for small intestine bacterial overgrowth: yield, PPIs, and outcomes after treatment at a tertiary academic medical center," *Gastroenterology Research and Practice*, vol. 2015, Article ID 971582, 5 pages, 2015.
- [70] A. Greco, G. P. Caviglia, P. Brignolo et al., "Glucose breath test and Crohn's disease: diagnosis of small intestinal bacterial overgrowth and evaluation of therapeutic response," *Scandinavian Journal of Gastroenterology*, vol. 50, no. 11, pp. 1376–1381, 2015.
- [71] D. Boltin, T. T. Perets, E. Shporn et al., "Rifaximin for small intestinal bacterial overgrowth in patients without irritable bowel syndrome," *Annals of Clinical Microbiology and Antimicrobials*, vol. 13, no. 1, p. 49, 2014.
- [72] S. Bae, K. J. Lee, Y.-S. Kim, and K.-N. Kim, "Determination of rifaximin treatment period according to lactulose breath test values in nonconstipated irritable bowel syndrome subjects," *Journal of Korean Medical Science*, vol. 30, no. 6, pp. 757–762, 2015.
- [73] I. G. Moraru, P. Portincasa, A. G. Moraru, M. Diculescu, and D. L. Dumitraşcu, "Small intestinal bacterial overgrowth produces symptoms in irritable bowel syndrome which are improved by rifaximin. A pilot study," *Romanian journal of internal medicine= Revue roumaine de medecine interne*, vol. 51, no. 3–4, pp. 143–147, 2013.
- [74] L. Gatta and C. Scarpignato, "Systematic review with meta-analysis: rifaximin is effective and safe for the treatment of small intestine bacterial overgrowth," *Alimentary Pharmacology & Therapeutics*, vol. 45, no. 5, pp. 604–616, 2017.
- [75] A. R. Khalighi, M. R. Khalighi, R. Behdani et al., "Evaluating the efficacy of probiotic on treatment in patients with small intestinal bacterial overgrowth (SIBO)—a pilot study," *The Indian journal of medical research*, vol. 140, no. 5, pp. 604–608, 2014.
- [76] A. Sajjad, M. Mottershead, W. K. Syn, R. Jones, S. Smith, and C. U. Nwokolo, "Ciprofloxacin suppresses bacterial overgrowth, increases fasting insulin but does not correct low acylated ghrelin concentration in non-alcoholic steatohepatitis," *Alimentary Pharmacology & Therapeutics*, vol. 22, no. 4, pp. 291–299, 2005.
- [77] F. Castiglione, A. Rispo, E. di Girolamo et al., "Antibiotic treatment of small bowel bacterial overgrowth in patients with Crohn's disease," *Alimentary Pharmacology & Therapeutics*, vol. 18, no. 11–12, pp. 1107–1112, 2003.
- [78] A. M. Madrid, C. Hurtado, M. Venegas, F. Cumsille, and C. Defilippi, "Long-term treatment with cisapride and antibiotics in liver cirrhosis: effect on small intestinal motility, bacterial overgrowth, and liver function," *The American Journal of Gastroenterology*, vol. 96, no. 4, pp. 1251–1255, 2001.
- [79] T. Van Raay and E. Allen-Vercoe, "Microbial interactions and interventions in colorectal cancer," *Bugs as Drugs*, vol. 5, no. 3, pp. 99–130, 2018.
- [80] C. E. DeStefano Shields, S. W. van Meerbeke, F. Housseau et al., "Reduction of murine colon tumorigenesis driven by enterotoxigenic *Bacteroides fragilis* using cefoxitin treatment," *The Journal of Infectious Diseases*, vol. 214, no. 1, pp. 122–129, 2016.
- [81] T. Hamoya, S. Miyamoto, S. Tomono et al., "Chemopreventive effects of a low-side-effect antibiotic drug, erythromycin, on mouse intestinal tumors," *Journal of Clinical Biochemistry and Nutrition*, vol. 60, no. 3, pp. 199–207, 2017.
- [82] A. Elkrief, L. Derosa, G. Kroemer, L. Zitvogel, and B. Routy, "The negative impact of antibiotics on outcomes in cancer patients treated with immunotherapy: a new independent prognostic factor?," *Annals of Oncology*, vol. 30, no. 10, pp. 1572–1579, 2019.
- [83] M. A. Jackson, S. Verdi, M. E. Maxan et al., "Gut microbiota associations with common diseases and prescription medications in a population-based cohort," *Nature communications*, vol. 9, no. 1, p. 2655, 2018.
- [84] C. Capasso and C. Supuran, "An overview of the carbonic anhydrases from two pathogens of the oral cavity: *Streptococcus mutans* and *Porphyromonas gingivalis*," *Current topics in medicinal chemistry*, vol. 16, no. 21, pp. 2359–2368, 2016.
- [85] C. T. Supuran and C. Capasso, "Biomedical applications of prokaryotic carbonic anhydrases," *Expert Opinion on Therapeutic Patents*, vol. 28, no. 10, pp. 745–754, 2018.
- [86] C. T. Supuran and C. Capasso, "An overview of the bacterial carbonic anhydrases," *Metabolites*, vol. 7, no. 4, p. 56, 2017.
- [87] G. Annunziato, A. Angeli, F. D'Alba et al., "Discovery of new potential anti-infective compounds based on carbonic anhydrase inhibitors by rational target-focused repurposing approaches," *ChemMedChem*, vol. 11, no. 17, pp. 1904–1914, 2016.
- [88] O. Ozensoy Guler, C. Capasso, and C. T. Supuran, "A magnificent enzyme superfamily: carbonic anhydrases, their purification and characterization," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 31, no. 5, pp. 689–694, 2016.
- [89] S. del Prete, D. Vullo, V. de Luca et al., "Sulfonamide inhibition studies of the β -carbonic anhydrase from the pathogenic bacterium *Vibrio cholerae*," *Bioorganic & medicinal chemistry*, vol. 24, no. 5, pp. 1115–1120, 2016.
- [90] R. Perfetto, S. del Prete, D. Vullo et al., "Cloning, expression and purification of the α -carbonic anhydrase from the mantle of the Mediterranean mussel, *Mytilus galloprovincialis*," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 32, no. 1, pp. 1029–1035, 2017.
- [91] C. Capasso and C. T. Supuran, "An overview of the alpha-, beta- and gamma-carbonic anhydrases from bacteria: can bacterial carbonic anhydrases shed new light on evolution of bacteria?," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 30, no. 2, pp. 325–332, 2015.
- [92] C. Capasso and C. T. Supuran, "Bacterial, fungal and protozoan carbonic anhydrases as drug targets," *Expert Opinion on Therapeutic Targets*, vol. 19, no. 12, pp. 1689–1704, 2015.
- [93] C. T. Supuran and C. Capasso, "The η -class carbonic anhydrases as drug targets for antimalarial agents," *Expert Opinion on Therapeutic Targets*, vol. 19, no. 4, pp. 551–563, 2015.
- [94] C. Capasso and C. T. Supuran, "An overview of the selectivity and efficiency of the bacterial carbonic anhydrase inhibitors," *Current Medicinal Chemistry*, vol. 22, no. 18, pp. 2130–2139, 2015.
- [95] C. Capasso and C. T. Supuran, "Sulfa and trimethoprim-like drugs – antimetabolites acting as carbonic anhydrase,

- dihydropteroate synthase and dihydrofolate reductase inhibitors," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 29, no. 3, pp. 379–387, 2014.
- [96] C. Capasso and C. T. Supuran, "Anti-infective carbonic anhydrase inhibitors: a patent and literature review," *Expert Opinion on Therapeutic Patents*, vol. 23, no. 6, pp. 693–704, 2013.
- [97] C. T. Supuran and C. Capasso, "New light on bacterial carbonic anhydrases phylogeny based on the analysis of signal peptide sequences," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 31, no. 6, pp. 1254–1260, 2016.
- [98] A. Maccelli, S. Carradori, V. Puca et al., "Correlation between the antimicrobial activity and metabolic profiles of cell free supernatants and membrane vesicles produced by *Lactobacillus reuteri* DSM 17938," *Microorganisms*, vol. 8, no. 11, p. 1653, 2020.
- [99] C. T. Supuran, "How many carbonic anhydrase inhibition mechanisms exist?," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 31, no. 3, pp. 345–360, 2016.
- [100] C. T. Supuran, "Advances in structure-based drug discovery of carbonic anhydrase inhibitors," *Expert opinion on drug discovery*, vol. 12, no. 1, pp. 61–88, 2017.
- [101] H. Otten, "Domagk and the development of the sulphonamides," *The Journal of Antimicrobial Chemotherapy*, vol. 17, no. 6, pp. 689–690, 1986.
- [102] C. T. Supuran, "Structure and function of carbonic anhydrases," *The Biochemical Journal*, vol. 473, no. 14, pp. 2023–2032, 2016.
- [103] C. T. Supuran, "Carbonic anhydrase inhibition and the management of neuropathic pain," *Expert Review of Neurotherapeutics*, vol. 16, no. 8, pp. 961–968, 2016.
- [104] C. T. Supuran, "Drug interaction considerations in the therapeutic use of carbonic anhydrase inhibitors," *Expert Opinion on Drug Metabolism & Toxicology*, vol. 12, no. 4, pp. 423–431, 2016.
- [105] D. Vullo, S. del Prete, G. M. Fisher et al., "Sulfonamide inhibition studies of the η -class carbonic anhydrase from the malaria pathogen *Plasmodium falciparum*," *Bioorganic & Medicinal Chemistry*, vol. 23, no. 3, pp. 526–531, 2015.
- [106] D. Vullo, V. de Luca, S. del Prete et al., "Sulfonamide inhibition studies of the γ -carbonic anhydrase from the Antarctic bacterium *Pseudoalteromonas haloplanktis*," *Bioorganic & medicinal chemistry letters*, vol. 25, no. 17, pp. 3550–3555, 2015.
- [107] N. Dedeoglu, V. DeLuca, S. Isik et al., "Sulfonamide inhibition study of the β -class carbonic anhydrase from the caries producing pathogen *Streptococcus mutans*," *Bioorganic & medicinal chemistry letters*, vol. 25, no. 11, pp. 2291–2297, 2015.
- [108] A. M. Alafeefy, M. Ceruso, A. M. S. al-Tamimi, S. Del Prete, C. T. Supuran, and C. Capasso, "Inhibition studies of quinazoline-sulfonamide derivatives against the γ -CA (PgiCA) from the pathogenic bacterium, *Porphyromonas gingivalis*," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 30, no. 4, pp. 592–596, 2015.
- [109] A. M. Alafeefy, H. A. Abdel-Aziz, D. Vullo et al., "Inhibition of human carbonic anhydrase isozymes I, II, IX and XII with a new series of sulfonamides incorporating aroylhydrazono-, [1,2,4]triazolo[3,4-b][1,3,4]thiadiazinyl- or 2-(cyanophenylmethylene)-1,3,4-thiadiazol-3(2H)-yl moieties," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 30, no. 1, pp. 52–56, 2015.
- [110] J. R. A. Diaz, M. Fernández Baldo, G. Echeverría et al., "A substituted sulfonamide and its Co (II), Cu (II), and Zn (II) complexes as potential antifungal agents," *Journal of enzyme inhibition and medicinal chemistry*, vol. 31, no. sup2, pp. 51–62, 2016.
- [111] S. del Prete, D. Vullo, V. de Luca et al., "Comparison of the sulfonamide inhibition profiles of the α -, β - and γ -carbonic anhydrases from the pathogenic bacterium *Vibrio cholerae*," *Bioorganic & Medicinal Chemistry Letters*, vol. 26, no. 8, pp. 1941–1946, 2016.
- [112] S. Del Prete, V. De Luca, G. De Simone, C. T. Supuran, and C. Capasso, "Cloning, expression and purification of the complete domain of the η -carbonic anhydrase from *Plasmodium falciparum*," *Journal of enzyme inhibition and medicinal chemistry*, vol. 31, no. sup4, pp. 54–59, 2016.
- [113] N. M. Abdel Gawad, N. H. Amin, M. T. Elsaadi et al., "Synthesis of 4-(thiazol-2-ylamino)-benzenesulfonamides with carbonic anhydrase I, II and IX inhibitory activity and cytotoxic effects against breast cancer cell lines," *Bioorganic & medicinal chemistry*, vol. 24, no. 13, pp. 3043–3051, 2016.
- [114] C. T. Supuran, "Legionella pneumophila carbonic anhydrases: underexplored antibacterial drug targets," *Pathogens*, vol. 5, no. 2, p. 44, 2016.
- [115] I. Nishimori, D. Vullo, T. Minakuchi, A. Scozzafava, C. Capasso, and C. T. Supuran, "Sulfonamide inhibition studies of two β -carbonic anhydrases from the bacterial pathogen *Legionella pneumophila*," *Bioorganic & medicinal chemistry*, vol. 22, no. 11, pp. 2939–2946, 2014.
- [116] D. Vullo, R. S. S. Kumar, A. Scozzafava, J. G. Ferry, and C. T. Supuran, "Sulphonamide inhibition studies of the β -carbonic anhydrase from the bacterial pathogen *Clostridium perfringens*," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 33, no. 1, pp. 31–36, 2018.
- [117] D. Vullo, R. S. Sai Kumar, A. Scozzafava, C. Capasso, J. G. Ferry, and C. T. Supuran, "Anion inhibition studies of a β -carbonic anhydrase from *Clostridium perfringens*," *Bioorganic & Medicinal Chemistry Letters*, vol. 23, no. 24, pp. 6706–6710, 2013.
- [118] I. Nishimoria, T. Minakuchia, A. Marescab, F. Cartab, A. Scozzafava, and C. Supuran, "The β -carbonic anhydrases from *Mycobacterium tuberculosis* as drug targets," *Current Pharmaceutical Design*, vol. 16, no. 29, pp. 3300–3309, 2010.
- [119] F. Carta, A. Maresca, A. S. Covarrubias, S. L. Mowbray, T. A. Jones, and C. T. Supuran, "Carbonic anhydrase inhibitors. Characterization and inhibition studies of the most active β -carbonic anhydrase from *Mycobacterium tuberculosis*, Rv3588c," *Bioorganic & Medicinal Chemistry Letters*, vol. 19, no. 23, pp. 6649–6654, 2009.
- [120] E. Licsandru, M. Tanc, I. Kocsis, M. Barboiu, and C. T. Supuran, "A class of carbonic anhydrase I - selective activators," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 32, no. 1, pp. 37–46, 2017.
- [121] C. T. Supuran, "Carbonic anhydrase inhibitors and activators for novel therapeutic applications," *Future Medicinal Chemistry*, vol. 3, no. 9, pp. 1165–1180, 2011.
- [122] C. T. Supuran, "Carbonic anhydrases: from biomedical applications of the inhibitors and activators to biotechnological use for CO₂ capture," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 28, no. 2, pp. 229–230, 2013.
- [123] F. Briganti, S. Mangani, P. Orioli, A. Scozzafava, G. Vernagione, and C. T. Supuran, "Carbonic anhydrase activators: X-ray crystallographic and spectroscopic investigations for the interaction

- of isozymes I and II with histamine,” *Biochemistry*, vol. 36, no. 34, pp. 10384–10392, 1997.
- [124] C. Temperini, A. Scozzafava, D. Vullo, and C. T. Supuran, “Carbonic anhydrase activators. Activation of isozymes I, II, IV, VA, VII, and XIV with L- and D-Histidine and crystallographic analysis of their adducts with isoform II: engineering proton-transfer processes within the active site of an enzyme,” *Chemistry—A European Journal*, vol. 12, no. 27, pp. 7057–7066, 2006.
- [125] C. Temperini, A. Scozzafava, L. Puccetti, and C. T. Supuran, “Carbonic anhydrase activators: X-ray crystal structure of the adduct of human isozyme II with L-histidine as a platform for the design of stronger activators,” *Bioorganic & medicinal chemistry letters*, vol. 15, no. 23, pp. 5136–5141, 2005.
- [126] C. T. Supuran, “Carbonic anhydrase activators,” *Future Medicinal Chemistry*, vol. 10, no. 5, pp. 561–573, 2018.
- [127] A. Bhatt, U. K. Mondal, C. T. Supuran, M. A. Ilies, and R. McKenna, “Crystal structure of carbonic anhydrase II in complex with an activating ligand: implications in neuronal function,” *Molecular Neurobiology*, vol. 55, no. 9, pp. 7431–7437, 2018.
- [128] C. T. Supuran, “Carbonic anhydrase inhibition/activation: trip of a scientist around the world in the search of novel chemotypes and drug targets,” *Current Pharmaceutical Design*, vol. 16, no. 29, pp. 3233–3245, 2010.
- [129] C. Temperini, A. Scozzafava, and C. T. Supuran, “Carbonic anhydrase activation and the drug design,” *Current Pharmaceutical Design*, vol. 14, no. 7, pp. 708–715, 2008.
- [130] D. Vullo, S. del Prete, S. M. Osman et al., “*Burkholderia pseudomallei* γ -carbonic anhydrase is strongly activated by amino acids and amines,” *Bioorganic & Medicinal Chemistry Letters*, vol. 27, no. 1, pp. 77–80, 2017.
- [131] A. Akdemir, D. Vullo, V. D. Luca et al., “The extreme- α -carbonic anhydrase (CA) from *Sulfurihydrogenibium azorense*, the fastest CA known, is highly activated by amino acids and amines,” *Bioorganic & Medicinal Chemistry Letters*, vol. 23, no. 4, pp. 1087–1090, 2013.
- [132] F. Boem and A. Amedei, “Healthy axis: towards an integrated view of the gut-brain health,” *World Journal of Gastroenterology*, vol. 25, no. 29, pp. 3838–3841, 2019.
- [133] A. Amedei and F. Boem, “I’ve got a feeling: microbiota impacting the conceptual and experimental perspectives of personalized medicine,” *International journal of molecular sciences*, vol. 19, no. 12, p. 3756, 2018.
- [134] I. Bartolini, M. Risaliti, M. N. Ringressi et al., “Role of gut microbiota-immunity axis in patients undergoing surgery for colorectal cancer: focus on short and long-term outcomes,” *World Journal of Gastroenterology*, vol. 26, no. 20, pp. 2498–2513, 2020.