Review

Effects of Microbiota Imbalance in Anxiety and Eating Disorders: Probiotics as Novel Therapeutic Approaches

Elisabet Navarro-Tapia 1,4, Laura Almeida-Toledano 2,3,†, Giorgia Sebastiani 4, Mariona Serra-Delgado 2,3, Oscar García-Algar 1,4,‡ and Vicente Andreu-Fernández 1,3,5,*,‡

Abstract: Anxiety and eating disorders produce a physiological imbalance that triggers alterations in the abundance and composition of gut microbiota. Moreover, the gut–brain axis can be altered by several factors such as diet, lifestyle, infections, and antibiotic treatment. Diet alterations generate gut dysbiosis, which affects immune system responses, inflammation mechanisms, the intestinal permeability, as well as the production of short chain fatty acids and neurotransmitters by gut microbiota, which are essential to the correct function of neurological processes. Recent studies indicated that patients with generalized anxiety or eating disorders (anorexia nervosa, bulimia nervosa, and binge-eating disorders) show a specific profile of gut microbiota, and this imbalance can be partially restored after a single or multi-strain probiotic supplementation. Following the PRISMA methodology, the current review addresses the main microbial signatures observed in patients with generalized anxiety and/or eating disorders as well as the importance of probiotics as a preventive or a therapeutic tool in these pathologies.

Keywords: probiotics; gut microbiota; dysbiosis; gut–brain axis; anorexia nervosa; bulimia nervosa; binge-eating disorder; anxiety

1. Introduction

The interest in mental health has increased in recent years. Anxiety and mood disorders are associated with many disabilities and individual suffering. The overall prevalence of anxiety ranges from 5% to 30%, and of mood disorders from 5% to 15% [1,2]. Moreover, the COVID-19 pandemic has increased these percentages, as demonstrated by a recent meta-analysis conducted in the general population and in healthcare workers, showing a prevalence for anxiety of 30% and 23.2%, respectively [3,4]. Furthermore, there is a close relationship between eating disorders (EDs) and anxiety. The most prevalent EDs are anorexia nervosa (AN), bulimia nervosa (BN), and binge eating disorder (BED), with a lifetime prevalence of 0.48%, 0.51%, and 1.12%, respectively. All of them usually start between 10 and 20 years old, predominantly in females [5]. The lifetime prevalence of any ED is 2.5% in the European population, these patients have a prevalence of 33–40% of any anxiety disorder and 19–50% of any mood disorder [5]. However, the pathophysiology of anxiety...
and EDs is poorly understood due to the complexity of analyzing the genetic, metabolic, and environmental factors that are involved in the appearance of these disorders.

The altered neural mechanisms in EDs are mainly related to the reward, behavioral control, and decision-making paths. The current bibliography suggests that amygdala, hippocampus, and medial prefrontal cortex are functionally compromised in anxiety disorder [6]. These brain regions are involved in the generation and regulation of emotions and fear. In anorexia and BN, there is a greater connectivity between insula, orbitofrontal cortex, and ventral striatum, but lower connectivity from orbitofrontal cortex and amygdala to the hypothalamus [7]. In BED, there is a diminished activity in the ventromedial prefrontal cortex, inferior frontal gyrus, and insula [8], areas that are involved in self-regulation and impulse control. Moreover, the alteration of the dopamine pathway is an important contributor to the development of any ED [8-10]. In anorexia and BN, harm avoidance mechanisms related to serotonin receptor availability and to dopamine receptor binding are also altered [11]. Otherwise, the neuuropeptides that manage the signal of hunger (ghrelin) and satiety (leptin) interact with the mesolimbic dopamine system, being altered in EDs. Leptin is an anorexigenic peptide released from adipose tissue that is diminished in AN. Ghrelin peptide with orexigenic functions is elevated in AN and does not respond correctly after food intake [12]. Sensitivity to insulin (anorexigenic pancreatic hormone) is also increased in AN [13]. Stress, hyperactivity, and appetite are also modulated by the cortisol awakening response of hypothalamic–pituitary–adrenal (HPA) axis, being imbalanced in anxiety and AN [14].

One of the factors that influence the pathophysiology of anxiety and EDs is the composition of gut microbiota due to the strong association between the microbial signature and the brain function. The gut microbiota includes the phyla Firmicutes (including Lactobacillus, Enterococcus, and Clostridium genera) and Bacteroidetes (including Bacteroides genus), which represent more than 90% of the intestinal community in healthy adults as well as Actinobacteria and Proteobacteria [15,16]. The gut–brain axis refers to the bidirectional interaction between the gut microbiota and the central nervous system (CNS). This interaction has been shown an increasing interest in recent years due to the harmful effects of dysbiosis on brain function. Gut bacteria interact with the CNS by synthesizing neurotransmitters such as serotonin, dopamine, gamma aminobutyric acid (GABA), acetylcholine, and glutamate and respond to hormones. Moreover, gut microbial diversity is also associated with dysregulation of appetite due to ability to influence the intestinal satiety pathways [17].

Diet influences the microbial composition and richness. The fermentation of indigestible carbohydrates by the colon microbiota produce short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, implicated on the maintenance of homeostasis, the regulation of appetite, and anti-inflammatory processes [18]. Diet alterations can generate an imbalance of microbial diversity and richness (alpha-diversity), which reduces gut Firmicutes and increases Bacteroides phyla [19]. Moreover, alterations of gut microbiota decrease the intake of calories from the diet [20], altering the immunological response. The innate immune system is activated under dysbiosis by the increase of bacterial lipopolysaccharides (LPS). These endotoxins trigger the release of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) in plasma, and downregulate synaptic proteins through the subdiaphragmatic vague nerve [21]. Moreover, recent studies show that the gut microbiota modulates the reactivity of the HPA axis, which influences the endocrine pathway. Therefore, its imbalance can produce abnormal glucocorticoid levels and promote behavioral changes [22].

Due to the role of gut microbiota on modulation of neuronal circuits through the gut–brain axis, the analysis of microbial profiles in patients with anxiety and/or EDs is of vital importance. The knowledge of the changes in the microbial communities of these patients could help in the development of novel therapeutic tools based on the modulation of the microbiome.
2. Results

2.1. Role of Microbiota in Anxiety

Anxiety is considered one of the most frequent mental disorders and is highly comorbid with other mental disorders and EDs [23,24]. A 2004 study found that about two-thirds of 672 individuals with AN, BN, or both had one or more lifetime anxiety disorders [25]. Moreover, anxiety disorders, especially obsessive–compulsive disorder (OCD) in childhood, could be a risk factor for EDs later in life [26]. Although the etiology of anxiety has traditionally been focused on genetic factors, evidences indicate that the gut microbiota and its metabolites are closely linked to the host’s central nervous system though a bidirectional communication. The microbiome plays an important role in the programming early in life of the HPA axis, a primary component of the stress response, and its reactivity in adult life [27]. Recent studies showed that prenatal stress in mice is related to alterations in the microbiome, cytokines levels, and reduction of the brain derived neurotrophic factor (BDNF) in the offspring, suggesting a link between the microbiome at early stages and adult behavioral changes [28]. Moreover, a transfer of vaginal microbiome from stressed mothers to offspring that were not stressed resulted in higher corticosterone levels and an altered gene expression in the para-ventricular nucleus (PVN) of the hypothalamus after stress exposure in adulthood [29].

Stress decreases specific intestinal epithelial tight junction proteins such as claudin 1 (CLDN1) and damages the integrity of the gut epithelium [30], altering gut motility, secretions, and mucin production [31,32]. These changes in the habitat of resident bacteria will promote changes in microbial composition and intestinal permeability. One of the most relevant is the translocation of certain gram-negative bacteria and allergens to the bloodstream that will trigger the inflammasome signaling and neuroinflammatory processes [33–35]. Inflammatory and neuromodulatory cytokines in the systemic circulation will reach the brain due to an altered permeability of the blood–brain barrier (BBB), enhancing mood disorders such as anxiety [36,37]. Therefore, an altered intestinal microbiota can alter behavior, immunity, and endocrinology, and has been associated with central nervous disorders, such as autism, and depression or anxiety behaviors.

Preclinical models highlight how gut microbiota disturbances due to antibiotics, delivery mode, or fecal microbiota transplantation (FMT) from AN patients correlated with anxiety-like behaviors [38–41]. Lach et al., assessed the long-term effects of transient gut microbiota depletion using a cocktail of five antibiotics in mice. Results showed a decrease of members of the family Lachnospiraceae and Bacteroides genus, which are also associated with anxiety in humans [42]. Furthermore, expression of genes involved in neurotransmission (such as Gabra1, Gabbr1, Npy, or Nr3c1), SCFA, and tight-junction proteins were more drastically affected during adolescence than adulthood [43]. These results highlight the consequences of gut microbiota manipulation during adolescence in mood disorders and the need for this kind of study in humans.

Given the role of gut microbiota in the gut–brain axis due its capacity to produce neurotransmitters (e.g., GABA by Bifidobacterium [44]) and its precursors as well as cytokines, BDNF, and SCFA [45], several efforts have been carried out to establish a microbiological signature in individuals with anxiety disorders. Unfortunately, only a few studies in humans achieved the establishment of taxonomic differences between anxiety disorders relative to controls [42,46,47]. In all three studies analyzed, participants with generalized anxiety disorders (GAD) showed lower microbial richness, measured by the number of observed operational taxonomic units (OTUs), compared to controls. In a cross-sectional study of 36 healthy controls and 40 patients with GAD, Jiang et al., observed a significant decrease of Firmicutes spp., specially Butyricoccus, Lachnospira, and the anti-inflammatory commensal bacterium Faecalibacterium in GAD patients. Conversely, Fusobacteria, a pathobiont with invasive and proinflammatory properties, and Bacteroidetes spp. increased significantly. Interestingly, the abundance of Ruminococcus gnarus, related to Crohn’s disease and the producer of an inflammatory polysaccharide [48], was significantly higher in GAD patients. This study showed that gender or remission had no effect on the relative abundance of
the different taxa; however, the medication did. An enrichment of *Escherichia–Shigella* in treatment-naïve GAD was observed after a subgroup analysis [42].

Another study in a Chinese population with GAD published a year later also showed a greater enrichment in *Escherichia–Shigella* and *Bacteroides* [47]. Other groups such as *Enterobacteriaceae*, *Enterobacteriales*, and *Bacteroidaceae* were also increased in these patients, highlighting again the relationship between the presence of pathogens in the gut and anxiety. On the contrary, *Firmicutes*, *Dialister*, *Prevotellaceae*, *Coprococcus*, *Clostridium innocuum* group, *Buchnera*, *Tenericutes*, *Mollicutes*, and *Acinetobacter* were more abundant in healthy controls. Interestingly, abundances of *Bacteroides* and *Escherichia–Shigella* were positively associated with anxiety severity, whereas *Eubacterium coprostanoligenes* group, *Ruminococcaceae_UCG-014*, and *Prevotella_9* correlated positively with anxiety reduction. These authors observed association between gender and the gut-microbial community composition in patients with GAD (contrary to Jiang et al.). Unfortunately, they did not examine the effects of drugs on gut microbiota profiles. Only two case-control studies have been carried out for the study of a microbiological signature related exclusively to GAD, both being carried out in the Asian population. Only one study was performed in the Caucasian population, although the subjects exclusively diagnosed with anxiety was very low (*n* = 8) and all females were under medication [46]. In this study, Mason et al., associated negatively the abundance of *Eubacterium rectale/Closteridium* group (*Clostridium* cluster XIVa) and *Clostridium leptum* group (*Clostridium* cluster IV) with the severity of anxiety (measured as GAD-7 score). Neither α-diversity nor β-diversity were significantly associated with this score, contrary to the other two studies where GAD was associated with a lower fecal bacterial α-diversity. Likewise, the abundance of *Bacteroides* was also inconsistent with previous studies, since, in this case, reductions in *Bacteroides* were associated with the presence of anxiety. However, due to the bias in sex and the limited sample size, these conclusions should be analyzed with caution [46].

Overall these studies identified an imbalance of microbial communities in GAD patients, suggesting that microbiome modulation may be a preventive and therapeutic tool for anxiety.

### 2.2. Microbiota and Anorexia Nervosa

The intestinal microbiota plays a crucial role in metabolic function, immunomodulation, and weight regulation. AN represents a severe mental illness characterized by severe weight loss associated with biochemical, metabolic, and immunologic disabilities, as well as high mortality rates [49].

The gut microbiota exerts a critical role in weight gain and energy intake from the diet. Dietary changes may also provoke an intense microbiota shift [50]. Furthermore, the microbiota impacts satiety pathways through interaction with peptide signaling and may be included in the etiology of AN [51]. The interactions between microbiota and the CNS are regulated by neuroendocrine and metabolic pathways, adjusting the balance between anorexigenic hormones like alpha melanocyte stimulating hormone (αMSH) and orexigenic peptides (ghrelin, leptine, and orexin) originated from the gastrointestinal tract [52]. *Bifidobacterium* spp. and *Lactobacillus* spp. produce GABA, related to anxiety control; *Enterococcus* spp., *Escherichia* spp., *Streptococcus* spp., and *Candida* spp. produce serotonin, a neurotransmitter involved in mood regulation, as well as dopamine produced by *Bacillus* spp. [53,54]. In general, gram-negative bacteria produces bacterial lipopolysaccharides, involved in the regulation of food intake through the activation of the enteroendocrine cells expressing toll-like receptors (TLRs) [55]. An imbalance of such molecules may impair feeding behaviors and weight loss [56]. Moreover, disorders in microbiota composition have been linked to anxiety and depression typical of AN patients, and psychological stress may lead to microbial translocation that enhance gut inflammation [57].

The first case studies in people with AN showed 11 new bacterial species classified in *Firmicutes* (*Soleaferrea massiliensis*, *Stoquefichus massiliensis*, *Dorea massiliensis*, *Hodelmania massiliensis*, *Clostridium anorexicus*, *Clostridium anorexicamassiliensi*, *Bacillus*...
marseilloanorexicus), Bacteroidetes (Bacteroides timonensis, Alistipes marseilloanorexicus), and Actinobacteria (Streptomyces massiliensis, Blastococcus massiliensis) phyla and four new micro-eukaryote species (Tetratrichomonas sp., Aspergillus ruber, Penicillium solitum, and Cladosporium bruhnei), respectively, in a single fecal sample, demonstrating an abnormal state in the intestinal tract of people with AN [58,59] (Table 1).

Complex carbohydrates are metabolized by intestinal microorganisms into SCFA with neuroactive involvement (n-butyrate, acetate, and propionate) [60,61]. Recent clinical studies (Table 1) conducted in the AN population showed that the total amount of bacteria including Clostridium coccoides, Clostridium leptum, and Bacteroides fragilis was significantly decreased in AN patients [62]. In addition, Streptococcus, Lactobacillus plantarum, and the genera butyrate-producing Roseburia, carbohydrate-fermenter Ruminococcus, and Clostridium, belonging to Firmicutes, were reduced with the subsequent reduced acetic, propionic, and butyrate acid concentration in the feces of patients with AN [62–65]. Propionate exhibited a positive correlation with insulin concentrations and with the relative depletion of the propionate producer Roseburia inulinivorans, whereas butyrate levels were negatively correlated with anxiety and depression [63]. This may explain the reduced insulin levels and the increased anxiety in AN individuals. Additionally, a reduction of the butyrate-producing Roseburia spp. was identified in AN compared with healthy controls leading to elevated branched-chain fatty acid concentrations and products of protein fermentation, which may impair gut physiology and motility [66,67]. In contrast, mucin-degraders and members of Clostridium clusters I, XI, and XVIII; Actinobacteria (mainly Bifidobacteria) [66], Enterobacteriaceae, and the methan-producing archeon Methanobrevibacter smithii [63], as well as Coribacteriaceae, were increased in AN compared with healthy controls. Methanobrevibacter smithii improves the efficiency of microbial fermentation, and its richness optimizes calorie extraction from a diet with very low calorie content. The development of Methanobrevibacter in AN patients might be associated with an adaptive mechanism to optimize the absorption of a hypocaloric diet [64]. The nutrient-deficient environment, together with a delayed colonic transit in AN patients, favors the increase of mucin-degrading microorganisms. This will contribute to a disrupted gut barrier and a chronic state of low-grade inflammation, exacerbating the disease [68]. Therefore, the microbiota profile has been linked to gut inflammation and impaired structure of epithelial layer [69], as demonstrated by the increased levels of IL-6 found in AN patients and IL-6 and IL-1α in obese individuals [70]. LPS also produces an increase in blood–brain barrier permeability with the elevation of plasma circulating cytokines responsible for the anorexigenic response [71]. Although there is an association between cytokine production and specific gut microbiota in a healthy population [72], more studies are needed in ED patients.

Morkl et al., found that athletes displayed the most diverse gut microbiota, while obese participants and AN patients displayed less diversity [69]. Nevertheless, Mack et al., did not find differences in microbiota diversity between AN patients and normal weight (NW) controls, probably because the high fiber intake of AN patients may have protected against the estimated reduction of alpha diversity [66]. Moreover, impaired microbiota, SCFA profiles, and gastrointestinal complaints remained persistent after weight gain, whereas overall species richness increased [66]. Significant differences in the composition of intestinal microbiota were found in patients with AN during renourishment. The Ruminococcaceae, a family associated with bowel inflammation, were prevalent in AN patients [73].

Distinct alterations in microbiota were observed for individuals with restrictive and binge/purging AN-subtypes. During weight gain, microbial richness increased; however, perturbations in intestinal microbiota and SCFA profiles in addition to several gastrointestinal symptoms did not recover [66].

A recent report suggested that Enterobacteriaceae, in particular Escherichia coli species, can produce an anorexigenic and anxiogenic protein, the caseinolytic protease b (ClpB), which may impair αMSH involved in satiety and anxiety signaling. Consequently, the
increased abundance of Gram negative bacteria might be linked to a higher production of neuropeptide ClpB, which could be a mediator with the gut–brain axis in AN subjects [63].

Borgo et al., demonstrated that the most suitable predictor for intestinal dysbiosis and metabolic changes was the body mass index (BMI) [63]. In contrast, in a case-series study with three AN patients, no associations between the composition of intestinal microbiota and BMI were observed despite significant weight gain during the treatment [74].
Table 1. Recent studies focused on the role of microbiota in anorexia nervosa.

| Author (Year) | Aim of Study | Type of Study/Population | Methods | Primary Outcomes | Conclusions | Quality of Evidence |
|---------------|--------------|--------------------------|---------|-----------------|-------------|-------------------|
| Morita 2015   | To compare the fecal microbiota of female patients with AN with those of age-matched healthy female controls | Cross-sectionalFemale patients with AN (n = 25), including restrictive (ANR, n = 14) and binge-eating (ANBP, n = 11) subtypes, compared with age-matched healthy female controls (n = 21) | Using the Yakult Intestinal Flora-SCAN based on 16S or 23S rRNA–targeted RT–quantitative PCR technology | - AN patients had: lower amounts of total bacteria and obligate anaerobes including Clostridium cocoides group, Clostridium leptum subgroup, and Bacteroides fragilis group; lower numbers of Streptococcus |- In the analysis based on AN subtypes, the counts of the Bacteroides fragilis group in the ANR and ANBP groups and the counts of the Clostridium cocoides group in the ANR group were lower than those in the control group. - The detection rate of the Lactobacillus plantarum subgroup was significantly lower in the AN group. - The AN group had lower acetic and propionic acid concentrations in the feces. - The subtype analysis showed that the fecal concentrations of acetic acid were lower in the ANR group than in the control group. | The analysis confirmed a clear difference in the bacterial components between the AN patients and healthy women. Collectively, these results clearly indicate the existence of dysbiosis in the gut of AN patients. | ++ |
| Borgo 2017    | To elucidate the possible relationship between nutritional status, and the microbiota-gut-brain axis in AN | Prospective Case-control 15 AN women 15 age-, sex-, and ethnicity-matched healthy controls | Collection of stool sample, dietary evaluation with a three-day food record, psychopathology assessment. | - AN diet: significant lower energy intake, but macronutrient analysis highlighted a restriction only in fats and carbohydrates consumption. - AN intestinal microbiota showed a significant increase of Enterobacteriaceae, and of the archeon Methanobrevibacter smithii compared with healthy controls. - In contrast, the genera Roseburia, Ruminococcus, and Clostridium were depleted, in line with the observed reduction in AN of total short chain fatty acids, butyrate, and propionate. - Butyrate concentrations inversely correlated with anxiety levels, whereas propionate directly correlated with insulin levels and with the relative abundance of Roseburia inulinivorans, a known propionate producer. - BMI represented the best predictive value for gut dysbiosis and metabolic alterations | The gut dysbiosis could take part in the AN neurobiology, in particular in sustaining the persistence of alterations that eventually result in relapses after renourishment and psychological therapy, but causality still needs to be proven. | ++ |
| Author (Year) | Aim of Study | Type of Study/Population | Methods | Primary Outcomes | Conclusions | Quality of Evidence |
|--------------|-------------|--------------------------|---------|------------------|-------------|-------------------|
| **Mack 2016**<br>Germany [66] | To explore if the intestinal microbiota of AN patients is perturbed in comparison to NW participants and whether these perturbations are recovered after weight gain and/or normalization of eating behavior. | Prospective Case-control<br>AN patients before (n = 55) and after weight gain (n = 44). Control group: normal-weight participants (NW, n = 55) | Authors investigated the fecal microbiota and SCFA in these patients before (n = 55) and after weight gain (n = 44) in comparison to normal-weight participants (NW, n = 55) along with dietary intake and gastrointestinal complaints. | AN patients:<br>- Higher levels of mucin-degraders and members of *Clostridium* clusters I, XI, and XVIII and reduced levels of the butyrate-producing *Roseburia* spp.<br>- Elevated branched-chain fatty acid concentrations, being markers for protein fermentation.<br>- Distinct perturbations in microbial community compositions were observed for individual restrictive and binge/purging AN-subtypes.<br>- Upon weight gain, microbial richness increased; however, perturbations in intestinal microbiota and SCFA profiles in addition to several gastrointestinal symptoms did not recover. | The authors showed profound microbial perturbations in AN patients as compared to NW participants. These insights provide new leads to modulate the intestinal microbiota in order to improve the outcomes of the standard therapy. | ++ |
| **Morkl 2017**<br>Austria [69] | To investigate the gut microbiota composition of a large female cohort including different BMI groups and activity levels along with body composition parameters | Cross-sectional study of 106 female participants: AN patients (n = 18), athletes (n = 20), normal weight (n = 26), overweight (n = 22), and obese women (n = 20) | DNA was extracted from stool samples and subjected to 16S rRNA gene analysis. QIIME was used to analyze data. Anthropometric assessments, ultrasound, bioimpedance analysis, administered depression inventories, laboratory parameters, and dietary intakes | Alpha diversity was particularly lower in AN patients and obese participants compared to other groups, while athletes showed highest alpha diversity. Several categories significantly associated with community structure were identified: body fat parameters, serum lipids, CRP, depression scales, and smoking. Comparative analysis revealed *Coriobacteriaceae* as the only enriched phylotype in AN compared to other entities (LDA score >3.5) | This study provides further evidence of intestinal dysbiosis in AN and sheds light on characteristics of the gut microbiome in different BMI and physical activity groups. These insights point to new modulation possibilities of the gut microbiota that could improve the standard therapy of AN | ++ |
| **Armougom 2009**<br>France [64] | To assess the relative abundance of *Lactobacillus*, *Methanobrevibacter smithii*, *Bacteroidetes*, and *Firmicutes* divisions in the microbiota of obese subjects, lean subjects, and AN patients using a real-time PCR assay. | Case-control study20 obese subjects, nine patients with AN, 20 normal-weight healthy controls.Age range: 19-36 years | Authors developed an efficient and robust real-time PCR tool that includes a plasmid-based internal control and allows for quantification of the bacterial divisions *Bacteroidetes*, *Firmicutes*, and *Lactobacillus* as well as the methanogen *M. smithii*. | - Reduction in the *Bacteroidetes* community in obese patients (p < 0.01).<br>- Significantly higher *Lactobacillus* species concentration in obese patients than in lean controls (p = 0.0197) or anorexic patients (p = 0.0332).<br>- *M. smithii* was higher in anorexic patients than in the lean population (p = 0.0171) | *Lactobacillus* species are linked to obesity in humans. Increase of *M. smithii* in anorexic patients. This increase might represent an adaptive use of nutrients in this population. | ++ |
| Author (Year)          | Aim of Study                                                                                                                                                                                                 | Type of Study/Population | Methods                                                                                                                                                                                                 | Primary Outcomes                                                                                                                                                                                                 |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Kleiman 2017 USA [74] | To characterize daily changes in the intestinal microbiota in three acutely ill patients with AN over the entire course of hospital-based renutrition                                                                           | n = 3 AN patients No controls | Fecal samples were collected on a daily basis from all participants. All samples were collected by unit nurses and nursing assistants trained in collection protocols.                                               | - Significant changes in composition and diversity of the intestinal microbiota over time at the phylum (n = 4), class (n = 8), order (n = 14), family (n = 28), and genus (n = 68) levels.                                      |
|                       |                                                                                                                                                                                                           |                          | - REE increased during treatment, in parallel with energy intake and BMI.                                                                                                                                  | - REE was not related to composition or diversity of gut microbiota.                                                                                                                                             |                                                                                                                                                                                                                     |
|                       |                                                                                                                                                                                                           |                          | - Diet-induced thermogenesis reached a peak after 2–3 weeks of treatment                                                                                                                                       | This preliminary case series suggests that even in a state of pathology, individual microbial signatures persist in accounting for the majority of intestinal microbial variation.                                           |
| Pfleider 2013 France [58] | To study for the first time an anorexia nervosa stool sample by culturomics                                                                                                                                   | AN female single patient (21 years) | The stool sample was collected on her first day of hospitalization, before the introduction of tube feeding. The dietary habits of the patient were surveyed                                                  | Nineteen bacterial species never isolated from the human gut before were found, including 11 new bacterial species for which the genome has been sequenced, *Firmicutes*, *Bacterioides*, and *Actinobacteria*                             |
|                       |                                                                                                                                                                                                           |                          |                                                                                                                                                                                                          | This study revealed new bacterial species participating significantly to the extension of the gut microbiota repertoire, which is the first step before being able to connect the bacterial composition with the geographic or clinical status.                 |
| Gouba 2014 France [59] | The diversity of microeukaryotes in the gut microbiota of an anorexic patient was investigated using molecular and culture approaches                                                                            | A 21-year-old Caucasian woman was admitted in an intensive care unit for severe malnutrition in AN                                               | One stool specimen was collected from the anorexic patient                                                                                                                                                | Culture and PCR-based explorations yielded a restricted diversity of fungi but four microeukaryotes, *Tetrahymena sp.*, *Aspergillus ruber*, *Penicillium solitum*, and *Cladosporium bruhnei*, previously undescribed in the human gut. |
|                       |                                                                                                                                                                                                           |                          |                                                                                                                                                                                                          | Establishing microeukaryote repertoire in gut microbiota contributes to the understanding of its role in human health.                                                                                  |
| Hanachi 2018 France [67] | Authors aimed to determine an association between FIDs severity and dysbiosis of the gut microbiota in a severely malnourished patients with AN undergoing enteral nutrition.                                               | 33 AN patients (BMI: 11.7 ± 1.5; Age: 32 ± 12) and 22 healthy controls (BMI: 21 ± 2; age: 36 ± 12)                                           | Fecal microbiota of AN (DSM IV criteria) female inpatients were collected and compared to healthy controls based on 16S rRNA profiling. The severity of FIDs was evaluated in patients and healthy controls using Francis Score. | - Some potentially pathogenic bacterial genera (*Klebsiella, Salmonella*) were more abundant in AN patients, whereas bacterial genera *Eubacterium* and *Roseburia* involved in immune balance were significantly less abundant in patients than controls. |
|                       |                                                                                                                                                                                                           |                          |                                                                                                                                                                                                          | - Severity of FIDs was strongly correlated with several microbial genera (r = −0.581 for an unknown genus belonging to *Peptostreptococcaceae* family; r = 0.392 for *Dialister*; r = 0.444 for *Robinsoniella*; and r = 0.488 for *Enterococcus*). Other associations between dysbiosis, clinical, and biological characteristics were identified including severity of undernutrition. |
|                       |                                                                                                                                                                                                           |                          |                                                                                                                                                                                                          | - A marked dysbiosis was identified in AN patients compared to healthy controls.                                                                                                                                  |                                                                                                                                                                                                                     |
|                       |                                                                                                                                                                                                           |                          |                                                                                                                                                                                                          | - Observed gut microbiota dysbiosis in malnourished patients with AN is correlated with the severity of FIDs and other metabolic disturbances, which strongly suggests an altered host–microbe symbiosis.                   |                                                                                                                                                                                                                     |
| Author (Year)                  | Aim of Study                                                                 | Type of Study/Population                  | Methods                                                                                   | Primary Outcomes                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Conclusions                                                                                                                                                                                                                                                                                                                                 | Quality of Evidence |
|-------------------------------|------------------------------------------------------------------------------|------------------------------------------|-------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| Prochazkova 2019              | The change in the gut microbiome and microbial metabolites in a patient suffering from severe and enduring AN and diagnosed with SIBO was investigated. | FMT in a single AN patients              | This study assessed the effects of FMT on gut barrier function, microbiota composition, and the levels of bacterial metabolic products. | - Very low bacterial alpha diversity, a lack of beneficial bacteria, together with a great abundance of fungal species were observed in the patient stool sample before FMT.  - After FMT, both bacterial species richness and gut microbiome evenness increased in the patient, while the fungal alpha diversity decreased. The total SCFA levels gradually increased after FMT. Contrarily, one of the most abundant intestinal neurotransmitters, serotonin, tended to decrease throughout the observation period.                                                                 | The patient treatment with FMT led to the improvement of gut barrier function, which was altered prior to FMT.                                                                                                                                                                                                                                                                          | +                   |
| De Clercq 2019                | To describe FMT in a single patient with AN                                  | 26-year-old female following clinical recovery from AN (restricting type) | FMT was performed with feces from an unrelated healthy female donor with a BMI of 25. Dietary intake was reported through online application seven days prior to each visit. Changes in metabolic parameters and body composition were assessed at baseline, six, 12, and 36 weeks. | - The patient gained 6.3 kg in bodyweight (from 45.8 to 52.1 kg), mostly due to a 55% increase in body fat and despite a reported stable caloric intake. Resting energy expenditure was decreased on all post-measurements compared to baseline.  - Gut microbial composition showed an increase in weighted phylogenetic diversity at six and 12 weeks with an especially marked increase in the number of Verrucomicrobia.  - The gut microbiota composition slowly changed back towards the patients’ initial personal core microbial composition. No side effects from FMT were reported or observed during the entire study period. | Authors showed for the first time that FMT induced weight gain in a patient with recurrent AN, suggesting that gut dysbiosis may be one of the causal factors in the etiology of persistent underweight in AN.                                                                                                                                                                                                                       | +                   |

Abbreviations. AN: anorexia nervosa; ANBP: anorexia nervosa binge eating/purging type; ANR: anorexia nervosa restricting type; BMI: body mass index; CRP: C-Reactive Protein; DSM: diagnosis and statistical manual of mental disorders; FIDs: functional intestinal disorders; FMT: fecal microbiota transplantation; LDA: linear discriminant analysis; NW: normal weight; QUIME: quantitative insights into microbial ecology; REE: resting energy expenditure; SIBO: small intestinal bacterial overgrowth; Quality of evidence grades: low (++), very low (+).
2.3. Microbiota Involvement in Bulimia Nervosa and Binge Eating Disorder

BN is characterized by recurrent episodes of binge eating and compensatory behaviors such as self-induced vomiting, laxative or diuretic abuse, fasting, or intensive exercise designed to undo or compensate for the effects of binge eating [77]. Binge-eating disorder shares some characteristics with BN: recurrent episodes of eating large quantities of food; a feeling of a loss of control during the binge; experiencing shame, distress, or guilt afterwards; and not regularly using unhealthy compensatory measures to counter the binge eating [77]. The etiopathogenesis of both disorders is poorly understood. Genetic factors [78–80], neurotransmitters, and neurohormonal peptide secretion disturbances [80,81] have been involved in BN and BED. Recently, the gut microbiota has been considered a modulator of host metabolome, inflammation processes, and brain function [80]. Despite gut microbiota in EDs has acquired increased interest in recent years, there are scarce studies on the microbiota in BN and BEDs.

The gut microbiota of BED obese patients has a specific composition and differs from that of obese subjects without BED. A cross-sectional study of a 101-patient cohort using the microbial 16S rDNA sequencing showed decreased Akkermansia and Intestinimonas, and elevated Bifidobacterium, Roseburia, and Anaerostipes in BED obese patients (Table 1) [82]. Changes in the profile of gut microbiota entail biological consequences, sometimes related to the eating behavior. Akkermansia muniphila produces SCFAs (propionate, an important regulator of satiety) and acetate and increases the intestinal levels of several acylglycerols (2-OG, 2-arachidonylglycerol, and 2-palmitoyl glycerol) involved in the regulation of the inflammation and immunity reactions. Therefore, Akkermansia has an impact on food intake behavior through the modulation of gut peptides [83,84]. Moreover, this genus is associated with improved insulin-resistance and obesity. The decreased Akkermansia observed in BED obese patients may be harmful. Bifidobacterium and Roseburia are related to cardiometabolic benefits, such as the reduction of hypertension and atherogenesis [82]. Anaerostipes is suggested to regulate human behavior. This genus is increased in psychiatric disorders such as depression and bulimia nervosa. Intestinimonas can metabolize toxic products from processed foods, such as Amadori products. This bacteria can convert lysine into butyrate and acetate, involved in the maintenance of a proper gut function [85]. Therefore, the decrease observed in Intestinimonas in BED patients may be negative. Hence, gut microbiota may be a modulator factor of metabolic profile of obese BED patients.

As mentioned previously, one of the molecular pathways involved in the regulation of anxiety and satiety is mediated by α-MSH. The caseinolytic protease B produced by Escherichia coli is a conformational antigen-mimetic protein of α-MSH with anorexigenic effect. Plasma levels of ClpB depend on the ClpB concentration in gut microbiota [86]. Preclinical models in Wistar rats showed sex-related different response to E. coli feeding. Females had E. coli in gut microbiota before the intervention, but not males. After E. coli feeding, males presented an increased production of α-MSH Ig M compared to females. However, females respond to the intervention by producing higher α-MSH Ig G levels. Females also presented a higher weight gain associated with Ig G and more efficient stimulation of α-MSH. Furthermore, food restriction was associated with ClpB production in the gut microbiota [87]. ED patients have elevated ClpB concentrations in plasma compared to healthy population. ClpB plasma concentrations in the three subgroups of ED patients (AN, BN, and BED) were associated with α-MSH-reactive Ig G, but not statistically significant differences were found when compared the ClpB plasma levels in the subgroups of ED patients. In BED patients, the ClpB concentration correlated with disorder duration, but no association was found with the frequency of binge-eating episodes in BN and BED patients. ClpB plasma concentrations also have been associated with psychopathological traits of these patients [86]. These data support the relation between the E. coli ClpB production and ED diagnosis and sex-related differences.

Different factors are involved in the etiopathogenesis of BN and BED, but there is a lack of studies offering a global analysis of the etiopathogenic factors of these disorders. The Binge Eating Genetics Initiative (BEGIN) arises with the aim of expanding the knowledge...
about the etiopathogenesis of BN and BED. This study will include 1000 patients diagnosed with BN and BED in order to characterize the disease through the collection of saliva, feces, and the register of behavior traits. The study of the genome, the gut microbiota, and the behavioral factors of this cohort will allow exploring the etiology, risk factors, natural history, and response to treatment of patients diagnosed with BN and BED [88]. The phenotyping of these patients will allow in the future the offering of personalized therapeutic options, in many cases related to gut microbiota (Table 2).
Table 2. Main studies based on microbiota composition in Bulimia nervosa and Binge Eating Disorder and microbiota studies.

| Author (Year) | Objective | Type of Study and Sample Size | Inclusion and Exclusion Criteria | Interventions | Outcomes | Conclusion | Quality of Evidence |
|---------------|-----------|------------------------------|----------------------------------|---------------|----------|------------|---------------------|
| Raevuori (2016) [89] | To examine the use of antimicrobials as an indicator for infection prior to the onset of the ED. | Case control study. ED patients (n = 1592) and controls (n = 6368). | IC: patients treated in the Eating Disorder Unit at the Helsinki University Central Hospital form January 2000 to September 2010. Diagnosis of the ED according the ICD-10 criteria. Four controls for each patient matched for age, sex, and place of residence. | Assessment of antimicrobial medication (systemic antibacterial, antifungal, and antiviral therapy) through registries of the Social Insurance Institution of Finland. | BN and BED patients had received more antimicrobial prescription than controls (OR: 1.7, 95% CI: 1.3–2.1 and 2.6, 95% CI: 1.4–4.6, respectively), no differences in AN patients were found. Antibacterial and antifungal therapy. Mean DDD use of antibacterial therapy were increased in BN (p < 0.001) and BED (p < 0.001) compared to controls. No differences were found in the use of antivirals in any of the EDs. Mean DDD of antifungal medication was increased in BED (p < 0.001). Use of antimicrobials was higher in BN and BED (OR: 2.1, 95% CI: 1.6–2.8 and 2.9, 95% CI: 1.6–5.4, respectively) when compared to AN. The use of antibacterial and antifungal was increased in BN and BED compared to AN. The use of antiviral was increased only in BN group compared to AN. | The increased use of antimicrobial therapy in patients with BN and BED indicates a higher number of infections in these groups prior to the onset of the disorder. Infections and antibacterial therapy may contribute to the proxy of the EDs through inflammation and changes in gut microbiota. | +++ |
| Breton (2016) [86] | To verify if ClpB produced by *Escherichia coli* is present in human plasma of patients with ED (AN, BN, and BED). | Case control study. Female patients with AN (n = 24), BN (n = 29), and BED (n = 13) and controls (n = 29). | EC: participants with EDs (according to EDI-2) and other psychiatric disorders (according to MADRS) | ClpB immunoassay in plasma | ClpB was detectable in plasma of ED patients and controls. ClpB plasma concentrations were increased in ED patients compared to controls. No statistically significant differences in patients’ subgroups. Positive correlation of ClpB with α-MSH-reactive-IgG in all subgroups of ED patients. | ClpB is present in human plasma. ClpB increased concentration in ED patients supports a link between bacterial ClpB and ED diagnosis. | ++ |
| Author (Year) | Objective | Type of Study and Sample Size | Inclusion and Exclusion Criteria | Interventions | Outcomes | Conclusion | Quality of Evidence |
|--------------|-----------|-------------------------------|---------------------------------|--------------|---------|-----------|---------------------|
| Leyrolle (2020) [82] | To study the fecal microbiome and non-targeted plasma metabolomics of obese BED patients. | Cross-sectional study in obese population (n = 101 obese patients) from Food4Gut cohort. | IC: male and female patients, aged 18-65 years, BMI > 30 kg/m², Caucasian ethnicity, presence of metabolic obesity-related disorder. EC: use of antibiotics, pre- or probiotics, dietary fibers, or any drug that modifies intestinal transit time within six weeks before the study, pregnancy, heavy psychiatric disorders, use of antipsychotics, current particular diets, excessive alcohol intake, type 1 diabetes, general dislike for vegetables. | Q-EDD Microbial 16S rDNA sequencing, Non-targeted metabolomics (liquid chromatography–mass spectrometry) | Subjects with BED showed a decrease in Akkermansia (p = 0.01), Desulfovibrio (p = 0.04), and Intestimonas (p = 0.01), and an increase in Anaerostipes (p = 0.03). Metabolomics revealed higher level of Bisphenol A (p = 0.011) and Isovalerylcarntine (p = 0.006) in BED individuals. | Omics approaches allow the characterization of gut microbiota and plasma metabolites of BED obese subjects. | ++ |
| Tennoune (2015) [90] | To compare the effects of E. coli on autoantibodies against α-MSH and ACTH according to gender in rats | Preclinical model in Wistar rats (n = 48). | Male and female Wistar rats, body weight 220 to 250 g. | Wistar rats received daily E. coli K12 in a culture medium by intragastric gavage over a three-week period. Control rats received only a culture medium. Plasma autoantibody assay against α-MSH, ACTH, and ClpB. Locomotor activity and anxiety tests. | E. coli was present only in females before gavage. Body weight increase in females and decrease in males after E. coli gavage. Plasma levels of anti-α-MSH and ACTH IgG were higher in females independent of the gavage. After E. coli gavage, α-MSH IgG was increased in females, and α-MSH IgM in males. E. coli affects feeding and anti-MSH antibodies in a different way according to gender. Sex-related levels of the different gut bacteria may represent a risk factor for developing an ED. | ++ |
| Author (Year) | Objective | Type of Study and Sample Size | Inclusion and Exclusion Criteria | Interventions | Outcomes | Conclusion | Quality of Evidence |
|--------------|-----------|-------------------------------|----------------------------------|---------------|----------|------------|---------------------|
| Tennoune (2014) [51] | To study the effect of α-MSH antigen-mimetic protein on α-MSH auto-antibodies production and food intake. To see the association of plasma antibodies of α-MSH levels of patients diagnosed with AN, BN, and BED with EDI-2 score. | Preclinical model and case-control study. | C57Bl6 male mice (n = 32). Human subjects (women): controls and cases of AN, BN, and BED disorders (diagnosis according DSM-IV). | E. coli K12 culture and protein extraction. Chronic intragastric delivery of E. coli in mice. Study of plasma levels of anti-ClpB IgG α-MSH. Locomotor activity and anxiety tests in mice. EDI-2 scores. | Production of anti-ClpB IgG crossreactive with α-MSH influences food intake, body weight, anxiety, and melanocortin receptor 4 signaling in mice. Intragastric gavage of E. coli decreased food intake and stimulated formation of ClpB- and α-MSH-reactive antibodies in mice. Patients with AN, bulimia, and BED have increased plasma levels of anti-ClpB IgG crossreactive with α-MSH and correlates with EDI-2 scores. | Bacterial ClpB protein, responsible for the production of auto-Ab crossreactive with α-MSH, is associated with pathologic feeding and emotion in humans diagnosed with EDs. | ++ |
| Breton (2020) [87] | To study if ClpB production by enterobacteria can be altered by chronic food restriction and female sex. | Preclinical model in Sprague–Dawley rats (n = 24) | 12 male and 12 female Sprague–Dawley rats. | Wistar rats received free access to food and water for seven days. Food access was limited during 1.5 h for one week. Plasma collection and feces. ClpB DNA analysis, ClpB, and α-MSH reactive antibody assay. Bacterial culture. | Food restriction increased ClpB levels in feces and plasma in both females and males. Females had higher levels of basal ClpB in plasma and gut and increased levels of ClpB-reactive IgG and IgM. ClpB concentration after the use of estradiol in E.coli cultures were lower and testosterone had no effect. | Enterobacterial ClpB antigen may be associated with risk for developing an ED. | + |

**Abbreviations.** ACTH: adrenocorticotrophic hormone; AN: anorexia nervosa; BED: binge eating disorder; BMI: body mass index; BN: bulimia nervosa; ClpB: caseinolytic protease B; DDD: defined daily dose; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th Ed; EC: exclusion criteria; ED: eating disorder; EDI-2: Eating Disorder Inventory-2 scores; IC: inclusion criteria; MADRS: Montgomery–Asberg Depression Rating Scale; MSH: melanocyte-stimulating hormone; Q-EDD: Questionnaire for Eating Disorder Diagnosis. Quality of evidence grades: moderate (+++), low (++), very low (+).
2.4. Therapeutic Tools in Anxiety and Eating Disorders

Although current pharmacological treatments for anxiety disorders are safer than a few decades ago, the effectiveness in some of them has not improved and can generate addiction problems. Benzodiazepines (BZs) or serotonergic anti-depressants (ADs) are the most widespread therapeutic options, BZs being more efficacious than ADs for reducing GAD symptoms [91]. However, BZs are not recommended for patients with a history of drug abuse, nor can it be prescribed indefinitely. Moreover, BZs are related to some side effects, and high doses may be associated with dementia [92]. Therefore, the development of new tools to look after or restore mental health without the undesired effects discussed above are necessary. A psychobiotic is a live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness [93]. The growing preference for preventive medicine coupled with a growing portfolio of products and a greater understanding of their mechanism of action will make psychobiotics a key player in the area of nutritional supplements.

Preclinical studies show that probiotics can alter the cognitive and emotional processes modulating behaviors and brain processes via the gut–brain axis in animal models, such as zebrafish, mice, or piglets [94–96]. As previously stated, anxiety is significantly more frequent in subjects with EDs than the general community and may predispose subjects to developing AN or BN [94,97]. For this reason, it was necessary to review all the clinical studies to date of the use of probiotics or postbiotics in patients that suffered anxiety or worked in stressful environments. For that, those studies in which the population had some disease concomitant (irritable bowel syndrome, major depression, autism, autoimmune disorders, etc.), were discarded.

A total of 14 studies published until 2020 were included in the analysis: twelve of them referred to a probiotic treatment, while the remaining two used a postbiotic [98] or a symbiotic [99] (Table 3). Regarding the duration of the treatments, these varied from 28 days [100] to six months [98], three months being the most used duration. In reference to probiotic intake, only three studies with the same strain (Lactobacillus casei Shirota YIT9029, LeS) in fourth grade medical students in exam period did not detect a significant decrease in anxiety according to the State–Trait Anxiety Inventory (STAI) or the Hospital Anxiety and Depression Scale-Anxiety (HADS-A) scores compared to placebo [101–103]. Neither did it improve depression rates according to the Hospital Anxiety and Depression Scale-Depression (HADS-D) and Zung Self-Rating Depression Scale (SDS) or decreased salivary cortisol [102]. However, in their last study, they observed a significant positive effect of LeS treatment on sleep scores related to sleepiness on rising and increased sleep length. In addition, overnight single-channel electroencephalography (EEG) recordings showed that LeS strain suppressed sleep latency and increased sleep intensity [101]. The symbiotic mixture composed of 10 g of resistant maize starch and nine probiotic strains also failed to significantly reduce anxiety levels according to the HADS-A scale after six weeks of treatment. Nonetheless, the authors observed a relevant improvement in these female healthcare workers, since after probiotic supplementation all anxiety, depression, and fatigue scores fell into normal ranges [99]. In all studies identified, only one used a single strain of Bifidobacterium [104]; by contrast, five studies used a mixture of probiotics strains [99,100,105–107] and eight studies used Lactobacillus, being L. plantarum and L. rhamnosus the most used species [98,101–103,108–111]. Bifidobacterium longum 1714 strain was evaluated under a cold-pressor test in 22 healthy volunteers and after a month of treatment, the anxiety score (STAI) did not significantly increase under the stressor, in contrast to placebo group [104]. This finding is important, since the study was carried out with individuals who did not have baseline anxiety, so these results open the door to the use of probiotics for the prevention of anxiety states. Although more studies would be needed with other different stressors to corroborate this preventive effect. The longest treatment (six months) corresponded to the only study that was done with postbiotics (heat treated Lactobacillus gasseri CP2305). In this randomized double-blind placebo controlled trial with medical students under final examination pressure,
authors showed a significantly reduction of State Trait Anxiety Inventory (STAI)-trait scores after postbiotic treatment compared to placebo (−1.9 vs. +1.1). Students also increased significantly their sleep quality (according to Pittsburg Sleep Quality Index (PSQI) score) and showed lower depression scores using the General Health Questionnaire (GHQ-28), although there was no significant difference in the global GHQ-28 scores between groups. The probiotic group also showed a non-significant improvement of anxiety and depressive moods (HADS) [98]. This study, moreover, was the only one among the 14 selected that analyzed the change in the microbiota after treatment. Results showed that stress in the students significantly decreased *Bifidobacterium* and increased *Streptococcus* in placebo group. By contrast, the heat treated probiotic significantly mitigated the reduction in *Bifidobacterium* and prevented the elevation of *Streptococcus*. These promising results call for the need to increase the number of clinical studies with postbiotics for the prevention and treatment of anxiety.

Recently, Ma et al., (2021) analyzed, in a follow-up work, the gut microbiota from the study of Lew et al., (2019) to elucidate the mechanism behind the clinical efficacy of *L. plantarum* P8 in significantly reducing some stress and anxiety symptoms [112]. Like the *L. plantarum* DR7 strain, P8 strain significantly decreased TNF-α and interferon-gamma (IFN-γ) after treatment. However, DR7 strain also significantly decreased plasma cortisol levels, in addition to enhancing the serotonin pathway and increasing IL-10 levels [109]. The analysis of the fecal metagenomes from the study with *L. plantarum* P8 showed a significant decrease of the Shannon diversity index in the placebo group but not the probiotic group after 12 weeks. Moreover, the prevalence of some species-level genome bins (SGBs) related to neuroprotective properties significantly increased (e.g., *B. adolescentis*, *B. longum*, and *F. prausnitzii*) after the treatment with *L. plantarum* P8 [112].

The interactions between eating behavior and microbiome modulate host biology. The microbiota has the ability to modulate HPA axis, behavior, neuronal, and immune system. Thus, manipulation of the gut microbiota may be useful to alter the natural history of the EDs. Increased use of antibiotics in patients with BN and BED prior to the onset of the ED indicates the existing dysbiosis in these patients [89]. Intestinal microbiota in ED patients are different from the healthy population. Microbial gut composition of AN patients shows specific characteristics; butyrate-producers (such as *Roseburia* spp.) are decreased and mucin-degrading bacteria (for example, *Akknarnansia muciniphila* and *Methanobrevibacter smithii*) are increased compared to controls [20]. Moreover, the study of intestinal microbiota in the obese population found a different pattern of intestinal microbiota in patients diagnosed with BED (decreased *Akkermania*, *Desulfovibrio*, and *Intestimonas*, and increased *Anaerostipes*) [82]. Additionally, ClpB produced by *Escherichia coli* (a conformational antigen-mimetic protein of α-MSH with anorexigenic effect) is present in human plasma of patients with EDs [86]. On the other hand, studies in specific populations with malnutrition showed a nutritional recovery of the subgroup treated with antibiotics [113]. Therefore, antibiotics may be a therapeutic target for the existing dysbiosis in patients with EDs. However, researchers have to be cautious in using this therapeutic strategy, because antibiotics can also produce intestinal dysbiosis. More studies are necessary to clarify the effect of antimicrobial therapy on the course of the EDs. Future research should address the search for therapeutic target molecules for the eradication of the desired bacteria.
Table 3. Studies focused on the use of probiotics in anxiety disorder.

| Author (Year) | Subjects | Design/ Country | Strain (s)/Dose/Duration | Questionnaires/ Techniques Used | Main Outcomes | Conclusions | Quality of Evidence |
|---------------|----------|----------------|--------------------------|--------------------------------|---------------|-------------|-------------------|
| Tran et al., (2019) [100] | Healthy college students; \( n = 86 \); 75.6% Hispanic non-white: 27.9% African/African American: 26.7% Caucasian: 23.3% Asian/Pacific Islanders: 16.3% | Randomized DBPC/US | Group A: 18 probiotic species; \( 5 \times 10^{10} \) CFU/d \( (n = 11) \) Group B: 10 probiotic species; \( 5 \times 10^{10} \) CFU/d \( (n = 13) \) Group C: placebo \( (n = 11) \) Group D: 18 probiotic species; \( 1 \times 10^{10} \) CFU/d \( (n = 15) \) | BAI; ACQ-R; PANAS; NMR; PSWQ | Group A: ↑ positive affect \**\ and anxiety control \*\; ↓ in worry \*\ Group B: ↓ in panic anxiety \*\ and negative affect \*\; ↑ in positive mood regulation \*\ Group C: NS changes Group D: ↓ in neurophysiological anxiety \*\ Group E: ↓ in negative affect \*\ | Gender and ethnicity might be a covariance to anxiety CFU and species count may not be equally applicable to the probiotics' effectiveness Species combination, not species count, may be the critical factor in the effectiveness of probiotics Probiotics can have a greater effect on the higher the initial stress level | ++ |

By gender: ♂: ↓ in worry \*\ and negative affect \**\ ♂: ↓ in autonomic anxiety \*\ By ethnicity: African/African American: ↓ in negative affect \*\, neurophysiological anxiety \**, subjective anxiety \*, panic anxiety \*, and BAI total score \**\ By CFU count: High CFU (A and B groups): ↓ panic anxiety \*\ and worry \*\; ↑ positive affect \**, and anxiety control \*\ Low CFU (A and B groups): NS changes By number of strains: High (A and D groups): NS changes Low (B and E groups): ↓ negative affect \**\ By distress level at baseline: High: ↓ BAI \*, PSWQ \*, PANAS Negative affect \*\; ↑ ACQ \**, ANAS Positive affect \*, and NMR \*\ Normative level: NS change |
Table 3. Cont.

| Author (Year)          | Subjects                                      | Design/Country | Strain (s)/Dose/Duration                                                                 | Questionnaires/Techniques Used | Main Outcomes                                                                                     | Conclusions                                                                                      | Quality of Evidence |
|------------------------|-----------------------------------------------|----------------|------------------------------------------------------------------------------------------|-------------------------------|--------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|---------------------|
| Smith-Ryan et al., (2019) [99] | Female healthcare workers employed on a rotating-shift schedule; n = 41 Caucasian: 93.9%; African American: 6% | Randomized DBPC/US | Ecologic® BARRIER (Bifidobacterium bifidum W23, B. lactis W51, B. lactis W52, Lactobacillus acidophilus W37, L. brevis W63, L. casei W56, L. salivarius W24, and L. lactis (W19 and W58); 1 × 10^{10} / d +10 g of resistant maize starch; 6 w | HADS; CFQ                      | HADS-A; HADS-D and CFQ; NS differences between groups Clinically relevant decrease in HADS-A scores (change (\(\Delta\)) = −2.3 ± 2.6) and CFQ-11 scores (\(\Delta\)) = −4.8 ± 5.5) in PG | Potential beneficial effect on anxiety and mental fatigue in a shift-working population. Probiotics can have a greater effect the higher the initial stress level | +++                 |
| Nishida et al., (2019) [98] | Medical students in exam period; n = 60; 18% | Randomized DBPC/Japan | Heat treated Lactobacillus gasseri CP2305; 1 × 10^{10} cells/d; 6 m | STAI; PSQI; HADS; GHQ-28 | 6 m: significantly reduction of STAI-trait scores in PG compared to CG (−1.9 vs. +1.1) and increase of sleep quality (PSQI) CP2305 ameliorated anxiety and depressive moods vs. placebo (HADS) NS changes in salivary cortisol between groupsSignificant \(\Delta\) Bifidobacterium \(\Delta\) Streptococcus in CG vs. PG after 6 m n-Valeric acid significantly increased after postbiotic intake. NS changes in other SCFAs | Long-term use of the probiotic may improve the mental state, sleep quality, and gut microbiota of healthy adults under stressful conditions | +++                 |
| Lew et al., (2019) [108] | Stressed adults; n=103; 49.5% | Randomized DBPC/ Malaysia | Lactobacillus plantarum P8; 1.2 × 10^{10} CFU/d; 3 m | DASS-42; PSS-10 | Total score for stress (PSS-10); NS differences between groups Total score for stress (DASS-42); significant reduction vs. placebo after 4, 8, and 12 w Total score for anxiety (DASS-42); significant reduction vs. placebo at 4 and 12 w. Changes from moderate to normal levels in PG. P8 strain significantly reduced breathlessness **, abnormal heart beats *, and fear * NS effects against reduction of depression and salivary cortisol between groups Significant changes IFN-\(\gamma\) (PG: −0.62 ± 2.98 vs. 7.46 ± 10.88 ug/dL in CG) and TNF-\(\alpha\) after treatment (PG: 0.17 ± 1.29 vs. 1.69 ± 1.80 pg/mL in CG) | L. plantarum P8 reduces some stress and anxiety symptoms via anti-inflammatory properties and enhances memory and cognitive abilities | ++++               |
Table 3. Cont.

| Author (Year) | Subjects | Design/ Country | Strain(s)/Dose/Duration | Questionnaires/ Techniques Used | Main Outcomes | Conclusions | Quality of Evidence |
|---------------|----------|----------------|-------------------------|---------------------------------|---------------|-------------|---------------------|
| Chong et al., (2019) [109] | Stressed adults; \( n = 111 \) | Randomized DBPC/Malaysia | Lactobacillus plantarum DR7; \( 10^9 \) CFU/d; 3 m | DASS-42 PSS-10 | Total score for stress (PSS-10): NS differences between groups | L. plantarum DR7 reduces symptoms of stress and anxiety, improves cognitive and memory functions, and reduces levels of plasma cortisol and pro-inflammatory cytokines. | ++++ |
| Takada et al., (2017) [101] | Fourth-grade medical students in exam period; \( n = 94; 41.4\% \) ♀ | Randomized DBPC/Japan | Lactobacillus casei Shirota YIT9029 in fermented milk (100 mL); \( 1 \times 10^9 \) CFU/mL, 11 w | STAI; OSA/EEG | No difference between groups regarding sleep scores (OSA). After sub analysis, YIT9029 significantly relieved sleepiness on rising and increased sleep length. Initiation and maintenance of sleep, dreaming, and recovery from fatigue did not change | YIT9029 may help to maintain sleep quality during a period of increasing stress. | +++ |
| Author (Year)                     | Subjects                                                                 | Design/Strain/Dose/Duration                                                                 | Questionnaires/Techniques Used | Main Outcomes                                                                                                                                                                                                 | Conclusions                                                                                                                                                                                                 | Quality of Evidence |
|----------------------------------|--------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| Slykerman et al., (2017) [110]    | Pregnant women in their 14–16 weeks gestation; n = 380 Maori: 12.9% Pacific: 2.1% Asian: 7.1% European: 77.6% Other: 0.26% | Randomized DBPC/New Zealand Lactobacillus rhamnosus HN001; 6 × 10^9 CFU/d; From 35 weeks gestation until six months if breastfeeding | Modified questionnaires of STAI6 and EPDS. No validated | Depression (EPDS): PG reported, retrospectively, significantly lower depression scores (HN001 mean=7.7 (SD = 5.4), placebo 9.0 (6.0); effect size −1.2, (95% CI −2.3, −0.1), p = 0.037) Anxiety (STAI6): PG reported, retrospectively, significantly lower anxiety scores (HN001 mean = 12.0 (SD = 4.0), placebo 13.0 (4.0); effect size −1.0 (−1.9, −0.2), p = 0.014) | HN001 may be useful for the prevention or treatment of symptoms of depression and anxiety postpartum. | ++                  |
| Kelly et al., (2016) [111]        | Healthy male volunteers; n = 39                                          | Randomized placebo-controlled, cross-over design/Ireland Lactobacillus rhamnosus (JB-1); 1 × 10^9 CFU/d, 4 w | SECPT; EEG; STAI; BDI; BAI | SECPT: NS differences between groups STAI: NS differences between groups BDI: NS differences between groups BAI: NS differences between groups EEG: NS differences between groups | JB-1 was not superior to placebo in modifying stress-related measures, HPA response, inflammation, or cognitive performance in healthy male participants | +++                 |
| Colica et al., (2017) [106]       | Healthy subjects; n = 33; 83.3% ♀                                         | RCT/Italy Group A: POS ¥ + no dietary change (n = 11); 1 bag/d Group B: hypocaloric diet (n = 11) Group C: POS ¥ + hypocaloric diet (n = 11) 3 w | HAM-A | Group A: ↓ in HAM-A total score for all study population (p = 0.01). Significant reduction in the number of anxious subjects after sub analysis (p = 0.03; Δ% = −39.3%) Group B: No differences for all study population Group C: ↓ in HAM-A total score for all study population (p = 0.04). All anxious subjects became nonanxious after sub analysis (p =0.01; Δ% = −100%) | A balanced diet, associated with this probiotic mixture, has a greater effect on the improvement of anxiety symptoms than probiotic alone | ++                  |
Table 3. Cont.

| Author (Year)                               | Subjects                                      | Design/ Country          | Strain (s)/Dose/Duration                                                                 | Questionnaires/ Techniques Used | Main Outcomes                                                                                   | Conclusions                                                                 | Quality of Evidence |
|---------------------------------------------|-----------------------------------------------|--------------------------|-----------------------------------------------------------------------------------------|--------------------------------|------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------|
| Takada et al., (2016) [103]                 | Fourth-grade medical students in exam period; n = 140; 45.7% ♀ | Randomized DBPC annually for three consecutive years/Japan | Lactobacillus casei Shirota YIT9029 (LcS) in fermented milk (100 mL); 1 × 10⁹ CFU/mL, 8 w | STAI                          | Significant increase of STAI score in both groups before exam compared to baseline (CG: 37.4 ± 1.1 and 48.7 ± 1.4, p < 0.01, PG: 38.7 ± 0.8 and 50.7 ± 1.2, p < 0.01). NS differences between groups NS differences in salivary cortisol levels between groups in each trial. Significant lower salivary cortisol levels in PG before exam when all data were pooled | Daily administration of LcS for eight weeks did not affect subjective anxiety | ++++
| Mohammadi et al., (2016) [105]              | Petrochemical Iranian workers; n = 70; 48.5% ♀ | Randomized DBPC/Iran     | Group A: probiotic yogurt § + placebo (n = 25) Group B: conventional yogurt + probiotic capsule (n = 25) Group C: conventional yogurt + placebo (n = 20) 6 w | DASS-14; GHQ-28            | Group A and B: significant improvement of general health after 6 w. (ΔGHQ-28: -4.5 ± 1.7 and -7.1 ± 1.7 in group A and B, respectively) Group A and B: significant improvement of anxiety and depression scores after 6 w. (ΔDASS-14: -10.3 ± 3.9 and -9.5 ± 4.3 in group A and B, respectively) No change in kynurenine, tryptophan, neuropeptide Y, cortisol or ACTH levels between groups. No influence on hypothalamic–pituitary–adrenal axis | The consumption of probiotic yogurt or a multispecies probiotic capsule had beneficial effects on mental health parameters in petrochemical workers. | +++
| Kato-Kataoka et al., (2016) [102]           | Fourth-grade medical students in exam period; n = 47; 36.8% ♀ | Randomized DBPC/Japan    | Lactobacillus casei Shirota YIT9029 (LcS) in fermented milk (100 mL); 1 × 10⁹ CFU/mL/mL, 8 w | STAI;HADS;SDS;PSQI          | Significant increase of STAI score in both groups before exam compared to baseline but no differences between groups NS differences in the HADSD-anxiety, HADS depression, SDS, and PSQI scores either within or between groups. NS in cortisol, L-tryptophan, and L-kynurenine levels between groups. Logarithmic level of fecal serotonin was significantly higher in the LcS group than in the placebo group at two weeks after the examination | Daily administration of LcS for eight weeks did not affect subjective anxiety | ++++
Table 3. Cont.

| Author (Year) | Subjects | Design/Country | Strain(s)/Dose/Duration | Questionnaires/Techniques Used | Main Outcomes | Conclusions | Quality of Evidence |
|---------------|----------|----------------|-------------------------|--------------------------------|---------------|-------------|-------------------|
| Allen et al., (2016) [104] | Healthy male volunteers under psychological and physiological stressor (SECPT); n = 22 | Placebo-controlled, repeated-measures, design/Ireland | *Bifidobacterium longum* 1714; 1 × 10^9 CFU/d; 1 m | PSS-10/EEG; cognitive tasks | Significant increase of anxiety score (STAI) post-stress with placebo. No significant increase under probiotic treatment | Consumption of *B. longum* 1714 is associated with reduced stress and improved memory | +++ |
| Messaoudi et al., (2011) [107] | Healthy Caucasian people; n = 55; 74.5% ♀ | Randomized DBPC/France | *L. helveticus* R0052 and *B. longum* R0175; 9 × 10^9 CFU/d; 1 m | HSCL-90; HADS, PSS, CCL | HSCL-90: PG significantly changed the global severity index due to improvement in somatization, depression, and anger-hostility HADS: The percentage changes in HADS and HADS-A scores were higher in the PG (p < 0.05). No differences in HADS-D between groups PSS: NS differences between groups CCL: NS changes between groups. Significant improve of Problem solving score in PG compared to baseline | *L. helveticus* R0052 and *B. longum* R0175 taken in combination display beneficial psychological effects in healthy human volunteers. | +++ |

Abbreviations. ACQ-R: Anxiety control questionnaire-revised; BAI: Beck anxiety inventory; BDI: Beck depression inventory; CANTAB: Cambridge Neuropsychological Test Automated Battery; CCL: coping checklist; CFQ: Chalder Fatigue Survey; CG: control group; DASS-42: Depression, Anxiety and Stress Scale; DBPC: Randomized Double-Blind, Placebo-Controlled; EEG: electroencephalography; EPDS: Edinburgh Postnatal Depression Scale; GHQ-28: General Health Questionnaire; HADS: Hospital Anxiety and Depression Scale; HAM-A: Hamilton anxiety rating scale; HSCL-90: Hopkins Symptom Checklist; IFN-γ: interferon-gamma; NMR: Negative mood regulation; OSA: Oguri–Shirakawa–Azumi sleep inventory; PANAS: positive and negative affect schedule; PG: probiotic/postbiotic group; POS: psychobiotics oral suspension; PSQI: Pittsburgh Sleep Quality Index; PSS-10: Cohen’s Perceived Stress Scale; PSWQ: Penn state worry questionnaire; RCT: randomized control trial; SDS: Zung Self-Rating Depression Scale; SCFA: short chain fatty acids; SECPT: socially evaluated cold pressor test; STAI: Spielberger State–Trait Anxiety Inventory; STAI6: State Trait Anxiety Inventory 6 item version; TNF-α: tumor necrosis factor alpha; d: days; w:weeks; m: months. NS: non-significant; *p < 0.05; **p < 0.01. Quality of evidence grades: high (+++), moderate (+++), low (++).. Symbols: ↓: decrease; ↑: increase; §: *S. thermophilus* CNCM I-1630; *L. bulgaricus* CNCM I-1632 and I-1519; *L. lactis* subsp. *lactis* CNCM I-1631; *L. acidophilus*; *S. thermophilus*; *L. plantarum*; *B. lactis* CNCM I-2494; *L. reuteri* DSM 17938 (1.5 × 10^10 each); §: *L. acidophilus* LAS and *B. lactis* BB12 (1 × 10^7 CFU); §: *L. casei* (3 × 10^9), *L. acidophilus* (3 × 10^7), *L. rhamnosus* (5 × 10^8), *L. bulgaricus* (5 × 10^7), *B. breve* (2 × 10^10), *B. longum* (1 × 10^9), *S. thermophilus* (3 × 10^8 CFU/g), and 100 mg fructo-oligosaccharide.
There is a growing interest in the immunomodulatory role of prebiotics and probiotics for the treatment of mood disorders. A recent meta-analysis of 34 controlled clinical trials evaluating the effects of prebiotics (all with bifidogenic properties) and probiotics (mostly lactobacilli and *Bifidobacterium*) on depression and anxiety concluded that prebiotics have no effect on psychologic disorders, while probiotics have antidepressant and anxiolytic effects [114]. The association of anxiety with EDs is accepted. Probiotic supplementation with lactobacilli and bifidobacteria, by stimulating a cross-feeding mechanism and increasing *Roseburia* abundance and butyrate production, would ameliorate the imbalance of gut structure in AN patients [115]. Future research on this field might offer a new alternative in the therapy of these disorders.

Finally, FMT is being evaluated in the management of the EDs. Preclinical studies in a mice model showed the positive effects in the reverse of compulsive behavior of germ-free mice previously reconstituted with the microbiota of restricting-type of AN patients [38]. Similarly, weight gain was achieved in an AN patient with recurrent underweight after FMT (*Firmicutes, Bacteroidetes, Verrucomicrobia, and Euryarchaeota*) [76], while the other case report showed no clinical improvement after FMT (*Akkermansia muciniphila, Methanobrevibacter smithii*) [75]. The total SCFA were increased [75,76] and serotonin decreased after FMT [75]. On the other hand, *Firmicutes, Rikenellaceae, Ruminococcaceae, Clostridiaceae, and Prevotellaceae* are families of bacteria negatively associated with weight gain and positively associated with gut microbiota ClpB KEGG function (K03695, Kyoto Encyclopedia of Genes and Genomes annotation) after fecal transplantation from humans to mice [116]. Therefore, future studies to examine the role of fecal microbiota transplantation in eating behavior and weight gain may be helpful in unraveling altered pathways in EDs.

3. Discussion

The composition of the gut microbiota is strongly modulated according to the characteristics of the diet [50,117]. Moreover, the gut microbiota plays a critical role in weight gain and energy intake from the aliments. For that, disruptions in the balance of human microbiota are associated with several diseases, such as diet-related mental illness [118] or generalized anxiety disorders. Therefore the scientific research focused on the gut microbiota profile in patients diagnosed with anxiety or ED leads to an innovative approach to understanding the etiopathogenesis of EDs and anxiety.

Several studies show imbalances in the intestinal microbiota of patients with EDs and anxiety. In GAD, for example, there is an increase of bacterial groups with inflammatory capacity such as *Ruminococcus gnavus, Fusobacteria, or Escherichia–Shigella*, positively associated with anxiety severity and a lower prevalence of SCFA-producing genera, whose lack triggers an intestinal barrier dysfunction [119]. Therefore, an increase of pathogenic bacteria able to degrade gastrointestinal mucins and produce exotoxins and inflammation could exacerbate anxious symptoms. In contrast, *Prevotella* correlated positively with anxiety reduction [47]. This is particularly interesting, because a significantly tighter connection between emotional well-being and a *Prevotella*-dominant condition [120] as well as an increased response to affective images in the limbic system has been suggested [121]. *Faecalibacterium*, related with anxiolytic and antidepressant-like effects, was also decreased in GAD population. *F. prausnitzii* increase SCFAs and IL-10 levels and reduce corticosterone and IL-6 levels in rats under a chronic unpredictable mild stress [122]. Therefore, their depletion can increase the anxiety states. On the other hand, the increase of *Enterobacteriaceae*, together with a weakened intestinal barrier, would enhance the translocation of the proinflammatory endotoxin LPS in blood [123]. LPS produces an inflammatory cascade that activates the kynurenic pathway; tryptophan will be degraded by indoleamine 2,3-dioxygenase (IDO), decreasing the serotonin levels [124].

Although it has not been clarified if gender influences the shaping of the microbial community in patients with GAD, if it seems that the medication does [42]. However, this exogenous factor has not been adequately considered by studies. Of the three studies comparing the microbiota of healthy controls with GAD patients, only Jiang et al., (2018)
examined a subgroup with treatment-naïve patients. Furthermore, in six of the 14 studies with probiotics or postbiotics, no data on the use or not of psychotropic drugs during follow-up were reported [99,100,105,106,108,109]. Psychotropic medication is a significant source of inter-study variation, since, in addition to change the microbial community, its absorption and efficacy can be affected by the patient’s own microbiota [125,126]. Therefore, this variable must be taken into consideration in future studies where the main aim is the reduction of symptoms in these patients through microbiome modulating solutions.

During this review, we have found numerous studies and reviews with microbiota modulators that tested the changes in anxiety levels. However, studies generally focused on patients with other pathologies, especially individuals with irritable bowel syndrome and major depression, as other authors also point out [114]. For this reason, as well as for the co-occurrence with EDs, we considered it necessary to collect those studies focused on healthy individuals with anxiety or those subjected to stressful conditions that can lead to anxious states.

One of the main conclusions of the present study is that it seems that the effect of probiotics in reducing anxiety seems all the more effective the higher the baseline anxiety level of the individual. Of the 14 studies, seven reported basal anxiety or stress based on the HADS-A [99], STAI [101,103,107], Hamilton Rating Scale for Anxiety-A (HAM-A), [106] or Perceived Stress Scale 10 (PSS-10) scales [108,109]. Of these seven studies, five achieved a positive outcome in reducing anxiety, despite using different treatments and conditions [99,106–109], while the same strain (L. casei Shirota) failed to decrease subjective anxiety levels in the other two remaining studies. This observation coincides with the sub-analysis of two groups with different baseline levels of distress, where no significant improvement was observed in the normative distress group after probiotic treatment [100]. Similarly, this occurs in depressive symptoms, where a meta-analysis with a total of 1349 patients showed that probiotic supplementation significantly improved the moods of individuals with mild–moderate depressive symptoms versus healthy individuals [127]. This trend is also observed in mice, but not rats, according to a meta-analysis, where a subgroup analysis revealed that probiotic administration significantly reduced anxiety-like behavior in diseased but not in naïve animals [94].

The studies in this review bring up some points that deserve to be considered for future studies in the anxiety field. First, we have not found a relationship between the main outcomes and the use of a multi-strain or a single-strain product. This fact corroborates once again that it is the intrinsic characteristics of the strains and their combinations and not the number of strains added in a product that determine its efficacy. A recent review based on 65 randomized clinical trials (RCTs) for eight different diseases remarks on our findings and shows how, in most cases, multi-strain mixtures are not significantly more effective than single-strain probiotics [128]. Secondly, we found a great diversity of questionnaires used to measure anxiety levels, questionnaires based on self-reports and not supervised by specialized personnel. In one study, authors used modified questionnaires of STA6 and Edinburgh Postnatal Depression Scale (EPDS), which was not validated [110]. This makes it difficult to obtain comparable results among studies. For this reason, the use of specific biomarkers and the choice of the right questionnaire for the right population is needed. One should be cautious about the title of the instrument and find a compromise between precision and respondent burden [129]. Finally, it is necessary to mention the few studies with postbiotics in the field, only one, despite the growing interest that this “-biotic” generates due its several advantages over probiotics, in terms of stability or use in several food matrices.

Interestingly, two studies measured plasma pro-inflammatory cytokines such as IFN-γ and TNF-α after probiotic treatment, obtaining reduced levels as anxiety decreased. Moreover, these two cytokines were significantly correlated with psychological traits of Depression Anxiety Stress Scale 42 (DASS–42) (anxiety and stress), indicating that inflammation is probably a major cause of stress and anxiety [108,109]. In fact, inflammation affects anxiety-related brain regions such as amygdala, insula, and anterior cingulate
Therefore, the use of specific probiotic strains with anti-inflammatory effects could be a powerful coadjuvant tool in the treatment or prevention of anxiety, which, as mentioned, is usually linked to other pathologies, such as EDs.

In addition to their anti-inflammatory effects, probiotics can also act on anxiety states in various ways, although we insist again that these characteristics will vary depending on the probiotic strain chosen. First, probiotics can attenuate the increased and prolonged activation of the HPA axis in GAD patients. In murine models, *B. breve* CCFM1025 significantly reduced the hyperactive HPA response, possibly via regulating the expression of glucocorticoid receptors (Nr3c1) [131]. These results are remarkable, because persistent HPA hyperactivity has been associated with higher rates of relapse, and glucocorticoid receptor function is impaired in anxiety disorders [132]. Other probiotic strains can also regulate the HPA axis, improving the systemic and nervous antioxidant status [133], reducing cFos protein expression in different brain areas, and changing the brain plasticity due to BDNF production [134]. Second, some strains from *Bifidobacterium* genus such as *B. adolescentis* PRL2019 and *B. adolescentis* HD17T2H can stimulate the in vivo production of GABA [44]. This inhibitory neurotransmitter and its neurotransmitter system is the target of BZs to treat anxiety disorder [135]. In zebrafish, probiotics can also modulate the GABAergic pathway through the differential expression of genes such as *gabra1* that encode the GABA-A alpha 1 receptor and serotonin transporter A (slc6a4a) [136]. Some probiotics can also increase the serotonin pathway and maintain the levels of norepinephrine and dopamine, which are higher in stressed subjects [109]. Finally, oxidative stress and intestinal permeability are also therapeutic targets and fields of enormous interest in probiotics due to their relationship with anxiety [137,138]. The increase of antioxidant enzymatic and non-enzymatic defenses in rat brains generated neuroprotective effects and reduced compulsiveness/anxiety using a marble burying test and open field test [93]. Interestingly, the strain *B.longum* CECT 7347 that showed antioxidant capacity in the nematode *C. elegans* [139] and improved the structure of the intestinal epithelium in a murine model of enteropathy by gliadin [140] also decreased anxiety behaviors in zebrafish [95]. *Weissella paramesenteroides* WpK4 also exerted their beneficial roles in the gut–brain axis through the reinforcement of the intestinal barrier in murine models of colitis and chronic stress [141]. Therefore, the effect of probiotics on anxiety cannot be attributed to a single mechanism of action, nor do all probiotics have the same characteristics to exert their effect on anxiety. Physical and psychological characteristics of AN patients entail differences in their gut microbiota composition [58,59]. Different studies have shown the involvement of the intestinal microbiota in neuroendocrine and metabolic pathways, including SCFA and anorexigenic hormones like αMSH [52], as well its association with gut inflammation. Different microorganisms are associated with the features of the disorder: *Bifidobacterium spp.* and *Lactobacillus spp.* are involved in the regulation of anxiety; *Enterococcus spp.*, *Escherichia spp.*, *Streptococcus spp.*, *Candida spp.* and *Bacillus spp.* are related to mood regulation [53,54]. Furthermore, LPS produced by gram-negative bacteria are related to the regulation of food intake [55]. Although several studies are conducted in order to recognize the dysbiosis of AN subjects, well-designed studies are needed for a complete understanding of the disbalances of gut microbiota of these patients, in addition to more research aimed at knowing the molecular pathways responsible for the disorder.

Despite the studies reporting on the intestinal dysbiosis in AN patients, reports about the composition of gut microbiota of BN and BED patients are scarce. The main molecular pathway studied is the anorexigenic and anxiogenic caseinolytic protease b (ClpB) produced by *Escherichia coli* species. Studies in AN patients showed an increased abundance of *E. coli* linked to the production of the ClpB neuropeptide [63]. Although there are no human studies in BN and BED, preclinical models of EDs show the mediator effect of ClpB with the gut–brain axis [51,87,90]. Initiatives such as BEGIN [88] are necessary to understand the gut microbiota composition in these patients.

This review shows some of the desirable characteristics in future microbiome-based solutions for eating disorders: first, the decrease of the anorexigenic ClpB protein in an AN...
patients, whose plasma concentration is correlated with proportion of *Enterobacteriaceae* in faeces [142]. The screening of probiotic strains with antagonistic properties against ClpB protein producers could be helpful in the treatment of EDs. Second, the modulation of the ghrelinergic signaling to balance the feeding behavior could be another mechanism for the development of new therapeutic tools. In fact, different *Lactobacillus* and *Bifidobacterium* strains are able to modulate the ghrelin receptor (GHS-R1a) [143]. Finally, the increase of butyrate-producing gut commensals by cross-feeding from some species of *Bifidobacterium* could stimulate epithelial cell proliferation. Butyrate would reinforce the intestinal wall, resulting in a larger absorptive surface to improve nutrients absorption from the diet [144].

As is discussed above, anxiety can promote Eds, and they often appear together. Therefore, reducing anxiety levels through microbiome modulators could be an additional strategy to prevent the EDs.

Knowledge of the intestinal microbiota will allow progress in the study of the pathophysiological mechanisms of the EDs in order to establish possible therapeutic options as probiotics or fecal microbiota transplantation. The correction of dysbiosis may be associated with the physical and emotional well-being of ED subjects.

4. Materials and Methods

This systematic review has been performed following the PRISMA statement for systematic reviews and meta-analyses [145,146], which includes definition of the research question and bibliographic search; data collection, evaluation, comparison, and synthesis; and critical analysis and findings presentation, indicating the strengths and weakness of the studies evaluated (Figure 1). A meta-analysis to evaluate the role of microbiota in anxiety and/or EDs was not considered due to the experimental design and clinical differences observed among the studies selected as well as by the low sample size achieved in these studies, which would generate an important bias in the statistical results.

The bibliographic search strategy was conducted to identify all studies reporting microbiota alterations in patients diagnosed with anxiety and/or EDs (AN, BN, or BED), also highlighting the use of probiotics as therapeutic tools. The electronic databases consulted were PubMed (MeSH), Cochrane Central Register of Controlled Trials, and Scopus. The following descriptors were used (as MeSH terms or not) with Boolean operators (AND/OR) in multiple combinations (see Supplemental Material S1): Section 2.1. “((fecal microbiota) OR (gut microbiota)) AND (generalized anxiety disorder)”; Section 2.2. “(microbiota OR microbiome OR dysbiosis) AND (anorexia nervosa)”; Section 2.3. “(microbiota OR gastrointestinal microbiome OR dysbiosis) AND (bulimia nervosa OR binge-eating disorder)”; Section 2.4. “Probiotics AND (anxiety OR anorexia nervosa OR bulimia nervosa OR binge-eating disorder)” and “(Psychobiotic OR probiotic OR prebiotic OR symbiotic OR fecal transplantation OR postbiotic) AND (generalized anxiety disorder OR anxiety)”.

Inclusion criteria were papers written in English and Spanish (with no geographical restrictions) published from January 1, 2009 to December 31, 2020; the presence of the selected terms in the title or as keywords; original research performed in humans; selected experimental designs including clinical trials, case–control, longitudinal cohort, cross-sectional, and case report studies. Sample size ≥ 10. The quality of controlled studies referring to randomized, nonrandomized, and pre-post treatment was critically appraised following the Cochrane Collaboration’s Risk of Bias Tool [147]. Exclusion criteria were infant population to 10 years old and no association with other psychiatric diseases. Interventions using only prebiotics or immunotherapy were also excluded for Section 2.4.
The selection of original manuscripts started by screening titles and abstracts for inclusion, creating a reference list of relevant papers for the topics explored in this review. Two investigators (T.L. and N.-T.E.) conducted each stage of the studies selection, deleted duplicate inputs, and reviewed studies as excluded or requiring further assessment. All data were extracted by one investigator (T.L.) and cross-checked by a second investigator (N.-T.E.). In case of discrepancies in the selected studies, we opted for reconciliation through team discussion. The information evaluated from each study was first author, experimental design, number of participants, control groups, main outcomes/findings, conclusions, and strengths and limitations (including biases). The eligibility criteria followed the PICOS (patient, intervention, comparators, outcome, and study design) approach. Population: Men or women diagnosed with anxiety or ED (anorexia, bulimia, or binge-eating disorder); intervention: if applicable, any doses, strains, or species of probiotics administered; comparators: if applicable, placebo or no probiotics; outcome: the primary outcome was microbiota composition in anxiety or ED patients. All authors performed a critical appraisal for the studies selected following the inclusion criteria, also analyzing the methodology and key results.
In the literature evaluated following PRISMA methodology, we observed heterogeneous results due to the different populations (patients with different ED) compared; the distinct health conditions derived to the clinical complexity of ED diagnosis; the reduced number of randomized trials in ED patients using probiotics; and the small sample size observed in these studies. Finally, the studies indicated in Figure 1 for each section of this review were identified through databases searching, selecting after meeting the inclusion criteria, and the application of the exclusion criteria the following: Section 2.1 (3), Section 2.2 (14), Section 2.3 (13), and Section 2.4 (20).

Finally, the articles were evaluated using the GRADE (Grades of Recommendation, Assessment, Development, and Evaluation) approach, which describes four levels of quality of evidence: high, moderate, low, and very [148]. Quality of evidence was obtained (Table 4) after judging the designs of the studies, the risk of bias, inconsistency, indirectness, imprecision, number of subjects, and publication bias.
Table 4. Summary of GRADE evidence profile.

| No. of Studies | Design          | Risk of Bias          | Inconsistency | Indirectness | Imprecision | Ctrl | Relative (95%CI) | Absolute | Quality | Importance |
|----------------|-----------------|-----------------------|---------------|--------------|-------------|------|-----------------|----------|---------|------------|
| 1              | Cross-sectional study | No serious risk of bias | No serious inconsistency | No serious indirectness | No serious imprecision | 101 obese subjects | - | OR 0.79–4.4 | 1.96 ± 4.44 non binge-eaters 0.51 ± 1.03 BED patients 0.04 ± 0.09 non binge-eaters 0.01 ± 0.02 BED patients | Low ++/++++ | Important |

Leyrolle (2020) [82]: Composition of the obese BED patients fecal microbiome (no follow-up)

1              | Case control study | Serious | No serious inconsistency | No serious indirectness | No serious imprecision | 66 cases | 29 | - | 1.7–162.7 and 1.4–333 pM | Low ++/++++ | Important |

Breton (2016) [86]: ClpB presence in human plasma (no follow-up)

1              | Case control study | No serious risk of bias | No serious inconsistency | No serious indirectness | No serious imprecision | 1592 cases | 6368 | OR 0.9 AN-OR 2.6 BED | 71.2% AN (74.3% controls) 89.6% BED (77.6% controls) | Moderate +++/++++ | Important |

Raevuori (2016) [89]: Use of antimicrobials as an indicator for infection prior to the onset of the eating disorder (Five years follow-up)

1              | Cross-sectional study | No serious risk of bias | No serious inconsistency | No serious indirectness | No serious imprecision | 25AN 14 ANR 11 ANBP | 21 | - | AN: the counts of bacteria, Clostridium cocoidea, Clostridium leptom, Bacteroides fragilis, Streptococcus and Lactobacillus were significantly lower p< 0.00084 | Low ++/++++ | Important |

Morita 2015 [62]: Gut dybiosis in patients with AN (no follow-up)
| No. of Studies | Design               | Risk of Bias          | Inconsistency | Indirectness | Imprecision | No. of Patients | Effect                                                                                       | Quality Importance |
|---------------|----------------------|-----------------------|---------------|--------------|-------------|----------------|---------------------------------------------------------------------------------------------|-------------------|
| 1             | Case control study   | No serious risk of bias | No serious inconsistency | No serious indirectness | No serious imprecision | 15 AN, 15       | AN: increased level of Gram-negative bacteria \(p = 0.03\) AN: affected the composition of microbiota at every taxonomic level, \(p < 0.05\) | Low ++/++++ Important |
|               |                      |                       |               |              |             |                | Mack (2016) \[66\]: weight gain in AN (no follow-up)                                         |
| 1             | Case control study   | No serious risk of bias | No serious inconsistency | No serious indirectness | No serious imprecision | 55 AN before WG, 44 AN after WG, 55 NW | % of relative abundance AN 0.10 (0.05–0.14) NW 0.01 (0.04–0.04) Proportions of BCFA: ANT1 11.9 (8.1–17.1) ANT2 13.6 (9.7–18.3) NW 21.1 (15.3–26.7) | Low ++/++++ Important |
|               |                      |                       |               |              |             |                | Morkl (2017) \[69\]                                                                 |
| 1             | Cross Sectional study | No serious risk of bias | No serious inconsistency | No serious indirectness | No serious imprecision | 18 AN 20 athletes, 22 overweight (OW), and 20 obese (OB) women 26 | AT displayed higher level of species richness compared to AN patients \(p = 0.038\) and OB participants \(p = 0.012\). OB participants had significantly lower diversity than OW participants \(p = 0.047\) Comparative analysis revealed Coriobacteriaceae as the only enriched phylotype in AN compared to other entities (LDA score >3.5) | Low ++/++++ Important |
Table 4. Cont.

| No. of Studies | Design          | Risk of Bias        | Inconsistency | Indirectness | Imprecision | No. of Patients | Effect                                                                                          | Quality Assessment | Importance |
|---------------|----------------|---------------------|---------------|--------------|-------------|----------------|------------------------------------------------------------------------------------------------|---------------------|------------|
| 1             | Case control study | No serious risk of bias | No serious inconsistency | No serious indirectness | No serious imprecision | 9 AN20 obese 20 | - Highly sensitive of Bacteroides system (89.89%) and Firmicutes (88.89%) and M. Smithii (88.89%)  
- Reduction in the Bacteroidetes community in obese patients \( (p < 0.01) \).  
Higher Lactobacillus species concentration in obese \( (p = 0.0197) \) or AN \( (p = 0.0332) \). Higher M. smithii in AN \( (p = 0.0171) \) | Low ++/++++ | Important |
| 1             | Case control study | No serious risk of bias | No serious inconsistency | No serious indirectness | No serious imprecision | 33 AN 22 | - AN: lower alpha-diversity \( (p = 0.03) \) for Chao1 indexes but not \( (p = 0.203) \) for Shannon indexes.  
- Severity of FIDs was strongly correlated with several microbial genera \( (r = -0.581 \text{ belonging to Peptostreptococcaceae family}; r = 0.392 \text{ for Dialister}, r = 0.444 \text{ for Robinsonella and } r = 0.488 \text{ for Enterococcus}) \) | Low ++/++++ | Important |
|               |                 |                     |               |              |             |                |                                                                                                 |                     |           |

**Armougom (2009) [64]:** (no follow-up)

**Hanachi (2018) [67]:** (no follow-up)

**Kleiman (2017) [74], Pfeider (2013) [58], Gouba (2014) [59], Prochazkoya (2019) [75], De Clercq (2019) [76]:** (no follow-up): clinical cases

**Intestinal microbiota in single patients with AN**

- Very Low ++/++++ | Less Important
Table 4. Cont.

| No. of Studies | Design | Risk of Bias | Inconsistency | Indirectness | Imprecision | Group A: 11; Group B: 13; Group D: 15; Group E: 15 | 11 | Participants with the probiotics intervention as a whole showed a significant decrease in panic anxiety (Mdiff = 0.42, SD = 1.55), and negative affect (Mdiff = 1.79, SD = 6.49) | Low | +++/++++ | Moderate |
|----------------|--------|--------------|---------------|--------------|-------------|-------------------------------------------------|-----|-------------------------------------------------|------|---------|---------|
| Tran et al., (2019) [100]: effect of probiotics intervention on mental health like anxiety in students; 28 days of follow-up |

| No. of Studies | Design | Risk of Bias | Inconsistency | Indirectness | Imprecision | 15 | 18 | No significant difference in changes for HADS-A (p = 0.621), HADS-D (p = 0.506) or CFQ-11 (p = 0.773) | Moderate | +++/++++ | Low |
|----------------|--------|--------------|---------------|--------------|-------------|-----|----|-------------------------------------------------|---------|---------|------|
| Smith-Ryan et al., (2019) [99]: effects of a multi-strain probiotic plus prebiotic on anxiety in female healthcare workers; 6 weeks of follow-up |

| No. of Studies | Design | Risk of Bias | Inconsistency | Indirectness | Imprecision | 29 | 31 | Significantly reduction of STAI-trait scores in PG compared to CG (~1.9 vs +1.1) | Moderate | +++/++++ | Critical |
|----------------|--------|--------------|---------------|--------------|-------------|-----|----|-------------------------------------------------|---------|---------|--------|
| Nishida et al., (2019) [98]: effect of a heat treated probiotic strain on mental conditions in students; 6 months of follow-up |

| No. of Studies | Design | Risk of Bias | Inconsistency | Indirectness | Imprecision | 52 | 51 | Reduced scores of stress (mean difference 2.94; 95% CI 0.08 to 5.73; p = 0.048), anxiety (mean difference 2.82; 95% CI 0.35 to 5.30; p = 0.031) and total score (mean difference 8.04; 95% CI 0.73 to 15.30; p = 0.041) | High | +++/++++ | Critical |
|----------------|--------|--------------|---------------|--------------|-------------|-----|----|-------------------------------------------------|---------|---------|--------|
| Lew et al., (2019) [108]: effects of one probiotic strain in alleviation of stress in stressed adult; 3 months of follow-up |
Table 4. Cont.

| Quality Assessment | No. of Patients | Effect | Quality | Importance |
|--------------------|-----------------|--------|---------|------------|
| No. of Studies     | Design          | Risk of Bias | Inconsistency | Indirectness | Imprecision | Ctrl | Relative (95%CI) | Absolute |
|---------------------|-----------------|-------------|---------------|---------------|-------------|------|------------------|----------|
| Chong et al., (2019) [109]: effects of one probiotic strain in alleviation of anxiety in stressed adult; 3 months of follow-up |
| 1                   | RDBPC           | No serious risk of bias | No major inconsistencies | Low          | Low         | 56   | 55               | High     |
|                     |                 |             |               |              |             |      |                  | (+++/++++) Critical |
| No. of Studies | Design | Risk of Bias | Inconsistency | Indirectness | Imprecision | Ctrl | Relative (95%CI) | Absolute | Effect |
|---------------|--------|--------------|---------------|--------------|-------------|------|-----------------|----------|--------|
| 1             | RDBPC  | No serious risk of bias | No major inconsistencies | Low          | Low         | Group A: 25 | 20          | Significant decrease in DASS score after probiotic intake (from 18.9 ± 3.2 to 9.4 ± 4.0; change: −9.5 ± 4.3); | Moderate +++/++++ | Moderate |
|               |        |              |               |              |             | Group B: 25 |             | p = 0.006 |        |
|               |        |              |               |              |             |      |                |          |        |
| 1             | Repeated measures, placebo-controlled | No serious risk of bias | No major inconsistencies | Low          | Low         | 22          | No significant differences of STAI scores pre and post stressor; | Moderate +++/++++ | Critical |
|               |        |              |               |              |             |      |                |          |        |
| 1             | RDBPC  | No serious risk of bias | No major inconsistencies | Moderate     | Low         | 26          | Significant decrease in HADS-A score in probiotic group. Change between baseline and follow-up (%); median: 36.9% (20–50%; IQ-SQ); | Moderate +++/++++ | Moderate |

Quality of evidence grades: high (+++), moderate (++), low (+), very low (+).
Supplementary Materials: The following are available online at https://www.mdpi.com/1422-0067/22/5/2351/s1: Supplemental Material S1: Search Methodology.

Author Contributions: O.G.-A., V.A.-F., L.A.-T., and E.N.-T. designed and coordinated the review. E.N.-T., L.A.-T., and V.A.-F. conceptualized the topics, tables, and figures presented in the manuscript. All the authors drafted the initial manuscript and reviewed the different versions of the manuscripts, figures, and tables. M.S.-D. wrote Introduction section, E.N.-T. Sections 2.1 and 2.4, G.S. Section 2.2, and L.A.-T. drafted the Sections 2.3 and 2.4. V.A.-F. performed the remaining sections and defined the strategy of search and the general inclusion and exclusion criteria. He drafted the methodology section. All authors have read and agreed to the published version of the manuscript. O.G.-A. as supervisor reviewed critically all the versions of the manuscript and provided the funding acquisition.

Funding: This research was funded by grants from Red de Salud Materno-Infantil y del Desarrollo (SAMID) (RD12/0026/0003 and RD16/0022/0002), from Instituto de Salud Carlos III, and from FEDER and Instituto de Salud Carlos III (PI16/00566 and PI19/01853).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

α-MSH $\alpha$-Melanocyte stimulating hormone
AD Anti-depressant
BBB Blood-brain barrier
BDNF Brain-derived neurotrophic factor
BED Binge eating disorder
BEGIN Begin Eating Genetics Initiative
BN Bulimia nervosa
BMI Body mass index
BN Bulimia nervosa
BZ Benzodiazepine
ClpB Caseinolytic protease b
CLDN1 Claudin 1
CNS Central nervous system
DASS-42 Depression Anxiety Stress Scale 42
ED Eating disorder
EEG Electroencephalography
EPDS Edinburgh Postnatal Depression Scale
FMT Fecal microbiota transplantation
GABA Gamma aminobutyric acid
GAS Generalized anxiety disorders
GHQ General Health Questionnaire
HADS-A Hospital Anxiety and Depression Scale-anxiety
HAM-A Hamilton Rating Scale for Anxiety
HPA Hypothalamic-pituitary-adrenal
IDO Indoleamine 2,3-dioxygenase
IFN-γ Interferon-γ
IL Interleukin
KEGG Kyoto Encyclopedia of Genes and Genomes annotation
LPS Lipopolysaccharides
OCD Obsessive-compulsive disorder
OUT Operational taxonomic unit
PSQI Pittsburgh Sleep Quality Index
PSS Perceived Stress Scale
References

1. Alonso, J.; Angermeyer, M.C.; Bernert, S.; Bruffaerts, R.; Brugha, T.S.; Bryson, H.; de Girolamo, G.; Graef, R.; Demyttenaere, K.; Gasquet, I.; et al. 12-Month comorbidity patterns and associated factors in Europe: Results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. Acta Psychiatr. Scand. Suppl. 2004, 109, 28–37. [CrossRef]

2. Baxter, A.J.; Scott, K.M.; Vog, T.; Whiteford, H.A. Global prevalence of anxiety disorders: A systematic review and meta-regression. Psychol. Med. 2013, 43, 897–910. [CrossRef]

3. Preti, A.; Girolamo G de Vilagut, G.; Alonso, J.; Graaf R de Bruffaerts, R.; Demyttenaere, K.; et al. 12-Month comorbidity patterns and associated factors in Europe: Results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. Acta Psychiatr. Scand. Suppl. 2004, 109, 28–37. [CrossRef]

4. Salvador, N.; Hosseini-Far, A.; Jalali, R.; Vaisi-Raygani, A.; Rasoulopor, S.; Mohammadi, M.; Rasoulopor, S. Prevalence of Stress, Anxiety, Depression Among the General Population during the COVID-19 Pandemic: A Systematic Review and Meta-Analysis. Glob. Health 2020, 16, 57. [CrossRef] [PubMed]

5. Petrovska, L. High-throughput clone library analysis of the mucosa-associated microbiota reveals dysbiosis and differences between inflamed and non-inflamed regions of the intestine in inflammatory bowel disease. BMC Microbiol. 2011, 11. [CrossRef]

6. Maria Monteleone, A.; Monteleone, P.; Dalle Grave, R.; Negro, M.; Girolamo G de Vilagut, G.; Alonso, J.; Graaf R de Bruffaerts, R.; Demyttenaere, K.; et al. 12-Month comorbidity patterns and associated factors in Europe: Results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. Acta Psychiatr. Scand. Suppl. 2004, 109, 28–37. [CrossRef] [PubMed]

7. Ilyas, A.; Hubel, C.; Ismail, K.; Treasure, J.; Breen, G.; Kan, C. Anorexia nervosa and insulin sensitivity: A systematic review and meta-analysis. In European Neuropsychopharmacology; Elsevier: Amsterdam, The Netherlands, 2017; p. 5624.

8. Monteleone, P.; Scognamiglio, P.; Monteleone, A.M.; Perillo, D.; Maj, M. Cortisol awakening response with Harm Avoidance, Weight Gain, and Hypothalamic Effective Connectivity in Adolescent Anorexia Nervosa. JAMA Psychiatry 2018, 75, 1071–1080. [CrossRef] [PubMed]

9. Vaga, S.; Lee, S.; Ji, B.; Andreasson, A.; Talley, N.J.; Agréus, L.; Bidkhori, G.; Kovatcheva-Datchary, P.; Park, J.; Lee, D.; et al. Compositional and functional differences of the mucosal microbiota along the intestine of healthy individuals. Sci. Rep. 2020, 10, 1–12. [CrossRef]

10. Arumugam, M.; Raes, J.; Pelletier, E.; Le Paslier, D.; Yamada, T.; Mende, D.R.; Fernandes, G.R.; Tap, J.; Bruls, T.; Batto, J.M.; et al. Enterotypes of the human gut microbiome. Nature 2011, 473, 174–180. [CrossRef]

11. Bailer, U.F.; Price, J.C.; Meltzer, C.C.; Wagner, A.; Mathis, C.A.; Gamst, A.; Kaye, W.H. Dopaminergic activity and altered reward modulation in anorexia nervosa-insight from multimodal imaging. Int. J. Eat. Disord. 2017, 50, 593–596. [CrossRef] [PubMed]

12. Volkow, N.D.; Wang, G.J.; Maynard, L.; Jayne, M.; Fowler, J.S.; Zhu, W.; Logan, J.; Gatley, S.J.; Ding, Y.S.; Wong, C.; et al. Brain dopamine is associated with eating behaviors in humans. Int. J. Eat. Disord. 2003, 33, 136–142. [CrossRef]

13. Frank, G.K.W.; Shott, M.E.; Riederer, J.; Pryor, T.L. Altered structural and effective connectivity in anorexia and bulimia nervosa in circuits that regulate energy and reward homeostasis. Transl. Psychiatry 2016, 6, e932-10. [CrossRef] [PubMed]

14. Balodis, I.M.; Molina, N.D.; Kober, H.; Worhunsky, P.D.; White, M.A.; Sinha, R.; Grilo, C.M.; et al. Divergent neural substrates of inhibitory control in binge eating disorder relative to other manifestations of obesity. Obesity 2013, 21, 367–377. [CrossRef]

15. Maria Monteleone, A.; Monteleone, P.; Dalle Grave, R.; Negro, M.; Girolamo G de Vilagut, G.; Alonso, J.; Graaf R de Bruffaerts, R.; Demyttenaere, K.; Pinto-Meza, A.; Haro, J.M.; et al. 12-Month comorbidity patterns and associated factors in Europe: Results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. Acta Psychiatr. Scand. Suppl. 2004, 109, 28–37. [CrossRef] [PubMed]

16. Maria Monteleone, A.; Monteleone, P.; Dalle Grave, R.; Negro, M.; Girolamo G de Vilagut, G.; Alonso, J.; Graaf R de Bruffaerts, R.; Demyttenaere, K.; et al. 12-Month comorbidity patterns and associated factors in Europe: Results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. Acta Psychiatr. Scand. Suppl. 2004, 109, 28–37. [CrossRef] [PubMed]

17. Maria Monteleone, A.; Monteleone, P.; Dalle Grave, R.; Negro, M.; Girolamo G de Vilagut, G.; Alonso, J.; Graaf R de Bruffaerts, R.; Demyttenaere, K.; Pinto-Meza, A.; Haro, J.M.; et al. 12-Month comorbidity patterns and associated factors in Europe: Results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. Acta Psychiatr. Scand. Suppl. 2004, 109, 28–37. [CrossRef] [PubMed]

18. Maria Monteleone, A.; Monteleone, P.; Dalle Grave, R.; Negro, M.; Girolamo G de Vilagut, G.; Alonso, J.; Graaf R de Bruffaerts, R.; Demyttenaere, K.; et al. 12-Month comorbidity patterns and associated factors in Europe: Results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. Acta Psychiatr. Scand. Suppl. 2004, 109, 28–37. [CrossRef] [PubMed]

19. Maria Monteleone, A.; Monteleone, P.; Dalle Grave, R.; Negro, M.; Girolamo G de Vilagut, G.; Alonso, J.; Graaf R de Bruffaerts, R.; Demyttenaere, K.; et al. 12-Month comorbidity patterns and associated factors in Europe: Results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. Acta Psychiatr. Scand. Suppl. 2004, 109, 28–37. [CrossRef] [PubMed]
20. Ruusunen, A.; Rocks, T.; Jacka, F.; Loughman, A. The gut microbiome in anorexia nervosa: Relevance for nutritional rehabilitation. *Psychopharmacology* 2019, 236, 1545–1558. [CrossRef] [PubMed]

21. Zhang, J.; Ma, L.; Chang, L.; Pu, Y.; Qu, Y.; Hashimoto, K. A key role of the subdiaphragmatic vagus nerve in the depression-like phenotype and abnormal composition of gut microbiota in mice after lipopolysaccharide administration. *Transl. Psychiatry* 2020, 10. [CrossRef]

22. Luo, Y.; Zeng, B.; Zeng, L.; Du, X.; Li, B.; Huo, R.; Liu, L.; Wang, H.; Dong, M.; Pan, J.; et al. Gut microbiota regulates mouse behaviors through glucocorticoid receptor pathway genes in the hippocampus. *Transl. Psychiatry* 2018, 8. [CrossRef] [PubMed]

23. Swinbourne, J.; Hunt, C.; Abbott, M.; Russell, J.; St Clare, T.; Touyz, S. The comorbidity between eating disorders and anxiety disorders: Prevalence in an eating disorder sample and anxiety disorder sample. *Aust. New Zealand J. Psychiatry* 2012, 46, 118–131. [CrossRef] [PubMed]

24. Bandelow, B.; Michaelis, S. Epidemiology of anxiety disorders in the 21st century. *Dialogues Clin Neurosci*. 2015, 17, 327–335. [CrossRef]

25. Kaye, W.H.; Bulik, C.M.; Thornton, L.; Barbarich, N.; Masters, K. Comorbidity of anxiety disorders with anorexia and bulimia nervosa. *Am. J. Psychiatry* 2004, 161, 2215–2221. [CrossRef]

26. Micali, N.; Hilton, K.; Natatani, E.; Heyman, I.; Turner, C.; Mataix-Cols, D. Is childhood OCD a risk factor for eating disorders later in life? A longitudinal study. *Psychol. Med.* 2011, 41, 2507. [CrossRef] [PubMed]

27. De Weerth, C. Do bacteria shape our development? CROSstalk between intestinal microbiota and HPA axis. *Neurosci. Biobehav. Rev.* 2017, 83, 458–471. [CrossRef]

28. Gur, T.; Shay, L.; Palkar, A.; Fisher, S.; Varaljay, V.A.; Dowd, S.; Bailey, M.T. Prenatal Stress Affects Placental Cytokines and Neurotrophins, Commensal Microbes, and Anxiety-Like Behavior in Adult Female Offspring. *Brain Behav. Immun.* 2017, 64, 50–58. [CrossRef]

29. Jasarevic, E.; Howard, C.D.; Morrison, K.; Misic, A.; Weinkopff, T.; Scott, P.; Hunter, C.; Beiting, D.; Bale, T.L. The maternal vaginal microbiome partially mediates the effects of prenatal stress on offspring gut and hypothalamus. *Nat. Neurosci.* 2018, 21, 1061–1071. [CrossRef] [PubMed]

30. Zheng, G.; Victor Fon, G.; Meixner, W.; Creekmore, A.; Zong, Y.; KDame, M.; Colacino, J.; Dedhia, P.H.; Hong, S.; Wiley, J.W. Chronic stress and intestinal barrier dysfunction: Glucocorticoid receptor and transcription repressor HES1 regulate tight junction protein Claudin-1 promoter. *Sci. Rep.* 2017, 7, 4502. [CrossRef]

31. Da Silva, S.; Robbe-Masselot, C.; Ait-Belgnaoui, A.; Mancuso, A.; Mercade-Loubière, M.; Salvador-Martí, G.; Gillet, M.; Ferrier, L.; Loubière, P.; Dague, E.; et al. Stress disrupts intestinal mucus barrier in rats via mucin O-glycosylation shift: Prevention by a probiotic treatment. *Am. J. Physiol. Gastrointest Liver Physiol.* 2014, 307. [CrossRef]

32. Shigeshiro, M.; Tanabe, S.; Brain, T.S. Repeated Exposure to Water immersion Stress Reduces the MUC2 Gene Level in the Rat Colon Via Two Distinct Mechanisms. *Brain Behav. Immun.* 2012, 26, 1061–1065. [CrossRef]

33. Martin-Hernández, D.; Caso, J.; Bris, A.; Maus, S.; Madrigal, J.; García-Bueno, B.; MacDowell, K.; Alou, L.; Gómez-Lus, M.L.; Leza, J.C. Bacterial Translocation Affects Intracellular Neuroinflammatory Pathways in a Depression-Like Model in Rats. *Neuropharmacology* 2016, 103, 122–133. [CrossRef]

34. Wong, M.; Insera, A.; Lewis, M.; Mastronardi, C.A.; Leong, L.; Choo, L.; Kentsh, S.; Xie, P.; Morrison, M.; Wesselingh, S.L.; et al. Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition. *Mol Psychiatry*. 2016, 21, 797–805. [CrossRef]

35. Rutsch, A.; Kantsjö, J.B.; Ronchi, F. The Gut-Brain Axis: How Microbiota and Host Inflammomas Influence Brain Physiology and Pathology. *Front. Immunol.* 2020, 11. [CrossRef]

36. Gárate, I.; García-Bueno, B.; Luis, J.; Madrigal, M.; Caso, J.R.; Alou, L.; Gomez-Lus, M.L.; Micó, J.A.; Leza, J.C. Stress-Induced Neuroinflammation: Role of the Toll-like Receptor-4 Pathway. *Bioi. Psychiatry.* 2013, 73, 32–43. [CrossRef] [PubMed]

37. Mayerhofer, R.; Fröhlich, E.E.; Reichmann, F.; Farzi, A.; Kogelnik, N.; Fröhlich, E.; Sattler, W.; Holzer, P. Diverse action of lipoteichoic acid and lipopolysaccharide on neuroinflammation, blood-brain barrier disruption, and anxiety in mice. *Brain Behav. Immun.* 2017, 60, 174–187. [CrossRef] [PubMed]

38. Hata, T.; Miyata, N.; Takakura, S.; Yoshihara, K.; Asano, Y.; Kimura-Todani, T.; Yamashita, M.; Zhang, X.T.; Watanabe, N.; Mikami, K.; et al. The Gut Microbiome Derived from Anorexia Nervosa Patients Impairs Weight Gain and Behavioral Performance in Female Mice. *Endocrinology* 2019, 160, 2441–2452. [CrossRef]

39. Neufeld, K.M.; Kang, N.; Bienenstock, J.; Foster, J.A. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol. Motil.* 2011, 23. [CrossRef]

40. Morais, L.; Golubeva, A.; Moloney, G.; Moya-Perez, A.; Ventura-Silva, A.; Arboleya, S.; Bastaiansson, F.S.; O’Sullivan, O.; Rea, K.; Borre, Y. Enduring Behavioral Effects Induced by Birth by Caesarean Section in the Mouse. *Current Biology* 2019, 30, 3761–3774. [CrossRef] [PubMed]

41. Zhao, Z.; Wang, B.; Mu, L.; Wang, H.; Luo, J.; Yang, Y.; Tao, C. Long-Term Exposure to Ceftriaxone Sodium Induces Alteration of Gut Microbiota Accompanied by Abnormal Behaviors in Mice. *Front. Cell Infect. Microbiol.* 2020, 10. [CrossRef] [PubMed]

42. Jiang, H.; Zhang, X.; Yu, Z.; Zhang, Z.; Deng, M.; Zhao, J.H.; Ruan, B. Altered Gut Microbiota Profile in Patients with Generalized Anxiety Disorder. *J. Psychiatr. Res.* 2018, 104, 130–136. [CrossRef] [PubMed]
43. Lach, G.; Fülling, C.; Bastiaansen, T.; Fouhy, F.; O’Donovan, A.; Ventura-Silva, A.; Stanton, C.; Dinan, T.; Cryan, J. Enduring neurobehavioral effects induced by microbiota depletion during the adolescent period. *Transl. Psychiatry* 2020, 10, 382. [CrossRef] [PubMed]

44. Duranti, S.; Ruiz, L.; Lugli, G.A.; Tames, H.; Milani, C.; Mancabelli, L.; Mancino, W.; Longhi, G.; Carnevali, L.; Sgoifo, A.; et al. Bifidobacterium adolescentis as a key member of the human gut microbiota in the production of, G.A.B.A. *Sci. Rep.* 2020, 10, 14112. [CrossRef] [PubMed]

45. Foster, J.; Rinaman, L.; Cryan, J.F. Stress & the Gut-Brain Axis: Regulation by the Microbiome. *Neurobiol. Stress* 2017, 7, 124–136. [CrossRef] [PubMed]

46. Mason, B.; Li, Q.; Minhajuddin, A.; Czyzsz, A.; Coughlin, L.; Hussain, S.; Koh, A.; Triverdi, M. Reduced Anti-Inflammatory GUT microbiota are Associated with Depression and Anhedonia. *Affect. Disord.* 2020, 266, 394–401. [CrossRef]

47. Chen, Y.; Bai, J.; Yu, S.; Qi, X.; Bai, H.; Wang, H.; Peng, Z. Association between Fecal Microbiota and Generalized Anxiety Disorder: Severity and Early Treatment Response. *J. Affect. Disord.* 2019, 259, 56–66. [CrossRef]

48. Henke, M.T.; Kenny, D.J.; Cassilly, C.D.; Vlamakis, H.; Xavier, R.J.; Claridy, J. Ruminococcus gnavus, a member of the human intestine. *J. Affect. Disord.* 2014, 4, e458. [CrossRef] [PubMed]

49. Seitz, J.; Trinh, S.; Herpetz-Dahlmann, B. The Microbiome and Eating Disorders. *Psychiatr. Clin. North Am.* 2019, 42, 93–103. [CrossRef] [PubMed]

50. Barrett, E.; Ross, R.P.; O’Toole, P.W.; Fitzgerald, G.F.; Stanton, C. Effects of the enteric bacterial metabolic product propionic acid design and use of probiotics. *BioEssays* 2011, 33, 574–581. [CrossRef] [PubMed]

51. Palazzo, M.; Balsari, A.; Rossini, A.; Selleri, S.; Calcaterra, C.; Gariboldi, S.; Zanobbi, L.; Arnaboldi, F.; Shirai, Y.F.; Serrao, G.; et al. Activation of EnteroeンドCrIne Cells via TLRs Induces Hormone, Chemokine, and Defensin Secretion. *J. Immunol.* 2007, 178, 4296–4303. [CrossRef] [PubMed]

52. Roubalov, R.; Procházková, P.; Papežová, H.; Smítková, K.; Bilej, M.; Tlaskalová-Hogenová, H. Anorexia nervosa: Gut microbiota-immune-brain interactions. *Clin. Nutr.* 2020, 39, 676–684. [CrossRef]

53. Fischbach, M.A.; et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2009, 461, 620–624. [CrossRef] [PubMed]

54. Chen, Y.; Bai, J.; Yu, S.; Qi, X.; Bai, H.; Wang, H.; Peng, Z.; et al. Correlation between body mass index and gut concentrations of Lactobacillus reuteri, Bifidobacterium animalis, Methanobrevibacter smithii and Escherichia coli. *Int. J. Obes.* 2013, 37, 1460–1466. [CrossRef] [PubMed]

55. MacFabe, D.F.; Cain, N.E.; Boon, F.; Ossenkopp, K.P.; Cain, D.P. Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: Relevance to autism spectrum disorder. *Behav. Brain Res.* 2011, 217, 47–54. [CrossRef] [PubMed]

56. Duranti, S.; Ruiz, L.; Lugli, G.A.; Tames, H.; Milani, C.; Mancabelli, L.; Mancino, W.; Longhi, G.; Carnevali, L.; Sgoifo, A.; et al. Bifidobacterium adolescentis as a key member of the human gut microbiota in the production of, G.A.B.A. *Sci. Rep.* 2020, 10, 14112. [CrossRef] [PubMed]

57. Lyte, M. Probiotics function mechanistically as delivery vehicles for neuroactive compounds: Microbial endocrinology in the gut microbiome. *Trends Neurosci.* 2013, 36, 305–312. [CrossRef]

58. Pfleiderer, A.; Lagier, J.C.; Armougom, F.; Robert, C.; Vialettes, B.; Raoult, D. Culturomics identified 11 new bacterial species from a single anorexia nervosa stool sample. *Eur. J. Clin. Microbiol. Infect. Dis.* 2013, 32, 1471–1481. [CrossRef] [PubMed]

59. Armougom, F.; Henry, M.; Vialettes, B.; Raccah, D.; Raoult, D. Microbiota in anorexia nervosa: The triangle between bacterial species, metabolites and psychological tests. *PloS One* 2020, 15, e02351. [CrossRef]

60. Lach, G.; Fülling, C.; Bastiaansen, T.; Fouhy, F.; O’Donovan, A.; Ventura-Silva, A.; Stanton, C.; Dinan, T.; Cryan, J. Enduring neurobehavioral effects induced by microbiota depletion during the adolescent period. *Transl. Psychiatry* 2020, 10, 382. [CrossRef] [PubMed]

61. MacFabe, D.F.; Cain, N.E.; Boon, F.; Ossenkopp, K.P.; Cain, D.P. Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: Relevance to autism spectrum disorder. *Behav. Brain Res.* 2011, 217, 47–54. [CrossRef] [PubMed]

62. Gouba, N.; Raoult, D.; Drancourt, M. Gut microeukaryotes during anorexia nervosa: A case report. *Microbiol. Immunol.* 2009, 53, 166–169. [CrossRef] [PubMed]

63. MacFabe, D.F.; Cain, N.E.; Boon, F.; Ossenkopp, K.P.; Cain, D.P. Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: Relevance to autism spectrum disorder. *Behav. Brain Res.* 2011, 217, 47–54. [CrossRef] [PubMed].
91. Gomez, A.F.; Barthel, A.L.; Hofmann, S.G. Comparing the efficacy of benzodiazepines and serotonergic anti-depressants for adults with generalized anxiety disorder: A meta-analytic review. Expert. Opin. Pharm. 2018, 19, 883–894. [CrossRef]

92. Chan, T.T.; Li, W.; Wong, K.Y.W.; Chu, W.M.; Leung, K.C.; Chu, L.W. Association between high cumulative dose of benzodiazepine in Chinese patients and risk of dementia: A psychogeriatrics 2017, 17, 310–316. [CrossRef] [PubMed]

93. Luang-In, V.; Katisart, T.; Konsue, A.; Nudnamud-Thanoi, S.; Narbad, A.; Saengha, W.; Wangkahart, E.; Pumriiw, S.; Samappito, W.; Ma, N.L. Psychobiologic effects of multi-strain probiotics originated from Thai fermented foods in a rat model. Food Sci. Anim. Resour. 2020, 40, 1014–1032. [CrossRef]

94. Reis, D.J.; Iardi, S.S.; Punt, S.E.W. The anxiolytic effect of probiotics: A systematic review and meta-analysis of the clinical and preclinical literature. PloS ONE 2018, 13. [CrossRef] [PubMed]

95. Valcarce, D.G.; Martinez-Vázquez, J.M.; Riesco, M.F.; Robles, V. Probiotics reduce anxiety-related behavior in zebrafish. Helikon 2020, 6. [CrossRef]

96. Yang, J.; Wang, C.; Huang, K.; Zhang, M.; Wang, J.; Pan, X. Compound Lactobacillus sp. administration ameliorates stress and body growth through gut microbiota optimization on weaning piglets. Appl. Microbiol. Biotechnol. 2020, 104, 6749–6765. [CrossRef]

97. Swinbourne, J.M.; Touyz, S.W. The co-morbidity of eating disorders and anxiety disorders: A review. Eur. Eat. Disord. Rev. 2007, 15, 253–274. [CrossRef]

98. Nishida, K.; Sawada, D.; Kuwano, Y.; Tanaka, H.; Rokutan, K. Health Benefits of Lactobacillus gasseri CP2305 Tablets in Young Adults Exposed to Chronic Stress: A Randomized, Double-Blind, Placebo-Controlled Study. Nutrients 2019, 11, 1859. [CrossRef] [PubMed]

99. Smith-Ryan, A.E.; Mock, M.G.; Trexler, E.T.; Hirsch, K.R.; Blue, M.N.M. Influence of a multistrain probiotic on body composition and mood in female occupational shift workers. Appl. Physiol. Nutr. Metab. 2019, 44, 765–773. [CrossRef] [PubMed]

100. Tran, N.; Zhebrak, M.; Yacob, C.; Pelletier, J.; Hawley, D. The gut-brain relationship: Investigating the effect of multispecies probiotics on anxiety in a randomized placebo-controlled trial of healthy young adults. J. Affect Disord. 2019, 252, 271–277. [CrossRef] [PubMed]

101. Takada, M.; Nishida, K.; Gondo, Y.; Kikuchi-Hayakawa, H.; Ishikawa, H.; Suda, K.; Kawai, M.; Hoshi, R.; Kuwano, Y.; Miyazaki, K.; et al. Beneficial effects of Lactobacillus casei strain Shirota on academic stress-induced sleep disturbance in healthy adults: A double-blind, randomised, placebo-controlled trial. Benef. Microbes. 2017, 8, 153–162. [CrossRef] [PubMed]

102. Kato, A.; Nishida, K.; Kazunori, S.; Kawai, M.; Shizimu, K.; Kusumoto, A.; Hoshi, R.; Watanabe, O.; Igarashi, T.; Kusumoto, A.; et al. Fermented milk containing Lactobacillus casei strain Shirota prevents the onset of physical symptoms in medical students under academic examination stress. Benef. Microbes 2015, 7, 153–156. [CrossRef] [PubMed]

103. Takada, M.; Nishida, K.; Kataoka-Akao, A.; Gondo, Y.; Ishikawa, H.; Suda, K.; Kawai, M.; Hoshi, R.; Watanabe, O.; Igarashi, T.; et al. Probiotic Lactobacillus casei strain Shirota relieves stress-associated symptoms by modulating the gut-brain interaction in human and animal models. Neurogastroenterol. Motil. 2016, 28, 1027–1036. [CrossRef]

104. Allen, A.; Hutch, W.; Borre, Y.; Kennedy, PJ.; Temko, A.; Boylan, G.; Murphy, E.; Cryan, J.F.; Dinan, T.G.; Clarke, G. Bifidobacterium longum 1714 as a psychobiotic: Modulation of stress, electrophysiology and neurorecognition in healthy volunteers. Transl. Psychiatry 2016, 6, e639. [CrossRef]

105. Mohammad, A.A.; Jayzarei, S.; Khoosravi-Darani, K.; Bolari, Z.; Mohammadpour, N.; Asemi, Z.; Adab, Z.; Djalali, M.; Tahrani-Doost, M.; Hosseini, M.; et al. The effects of probiotics on mental health and hypothalamic-pituitary-adrenal axis: A randomized, double-blind, placebo-controlled trial in petrochemical workers. Nutr. Neurosci. 2016, 187–395. [CrossRef] [PubMed]

106. Colica, C.; Avolio, E.; Bollaro, P.; De Miranda, R.C.; Salimei, P.S.; De Lorenzo, A.; Di Renzo, L. Evidences of a new psychobiotic formulation on body composition and anxiety. Mediat. Inflamm. 2017, 5650627. [CrossRef]

107. Messaoudi, M.; Lalonde, R.; Violle, N.; Javelot, H.; Desor, D.; Nejdi, A.; Bisson, J.F.; Rougeot, C.; Pichelin, M.; Cazaubiel, M. Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. Br. J. Nutri. 2011. [CrossRef]

108. Lew, L.C.; Hor, Y.Y.; Yusoff, N.A.; Choi, S.-B.; Yusoff, M.S.B.; Roslan, N.S.; Ahmad, A.; Mohammad, J.A.M.; Abdullah, M.F.H.; Zakaria, N.; et al. Probiotic Lactobacillus plantarum P8 alleviated stress and anxiety while enhancing memory and cognition in stressed adults: A randomised, double-blind, placebo-controlled study. Clin. Nutr. 2019, 38, 2053–2064. [CrossRef]

109. Chong, H.X.; Yusoff, N.A.A.; Hor, Y.Y.; Lew, L.C.; Jaafar, M.H.; Choi, S.B.; Yusoff, M.S.B.; Wahid, N.; Abdullah, M.F.H.; Zakaria, N.; et al. Lactobacillus plantarum DR7 alleviate stress and anxiety in adults: A randomised, double-blind, placebo-controlled study. Benef. Microbes 2019, 10, 355–373. [CrossRef] [PubMed]

110. Slykerman, R.; Hood, F.; Wickens, K.; Thompson, J.M.D.; Barthow, C.; Murphy, R.; Kang, J.; Rowden, J.; Stone, P.; Crane, J.; et al. Effect of Lactobacillus Rhamnosus HN001 in Pregnancy on Postpartum Symptoms of Depression and Anxiety: A Randomised Double-Blind Placebo-Controlled Trial. EbioMedicine 2021, 24, 159–165. [CrossRef]

111. Kelly, A.G.; Allen, A.P.; Temko, A.; Hutch, W.; Kennedy, P.J.; Farid, N.; Murphy, E.; Boylan, G.; Bienenstock, J.; Cryan, J.F. Title Lost in translation? The Potential Psychobiotic Lactobacillus Rhamnosus (B-1) Fails to Modulate Stress or Cognitive Performance in Healthy Male Subjects. Brain Behav. Immun. 2017, 61, 50–59. [CrossRef] [PubMed]

112. Ma, T.; Jin, H.; Kwok, L.Y.; Sun, Z.; Liong, M.T.; Zhang, H. Probiotic consumption relieved human stress and anxiety symptoms possibly via modulating the neuroactive potential of the gut microbiota. Neurobiol. Stress 2021, 14. [CrossRef]
113. Ley, R.E.; Bä Ckhed, F.; Turnbaugh, P.; Lozupone, C.A.; Knight, R.D.; Gordon, J.I. Obesity Alters Gut Microbial Ecology. Proc. Natl. Acad. Sci. USA 2005, 102, 11070–11075. [CrossRef]

114. Liu, R.T.; Walsh, R.F.L.; Sheehan, A.E. Prebiotics and probiotics for depression and anxiety: A systematic review and meta-analysis of controlled clinical trials. Neurosci. Biobehav. Rev. 2019, 102, 13–23. [CrossRef] [PubMed]

115. Tamanai-Shacoori, Z.; Smida, I.; Bousarghin, L.; Loreal, O.; Meuric, V.; Fong, S.B.; Bonnare-B-Mallet, M.; Jolivet-Gougeon, A. Roseburia spp.: A marker of health? Future Microbiol. 2017, 12, 157–170. [CrossRef] [PubMed]

116. Amorriaga-Rodriguez, M.; Mayneris-Perxachs, J.; Burokas, A.; Perez-Brocal, V.; Moya, A.; Portero-Otin, M.; Ricart, W.; Maldonado, R.; Fernandez-Real, J.M. Gut bacterial CpG-like gene function is associated with decreased body weight and a characteristic microbiota profile. Microbiome 2020, 8, 59. [CrossRef] [PubMed]

117. Gentile, C.L.; Weir, T.L. The gut microbiota at the intersection of diet and human health. Science 2018, 362, 776–780. [CrossRef] [PubMed]

118. Fekstein, S.O.; Hökfelt, T. On the origin of eating disorders: Altered signaling between gut microbiota, adaptive immunity and the brain melanocortin system regulating feeding behavior. Curr. Opin. Pharmacol. 2019, 48, 82–91. [CrossRef]

119. Feng, Y.; Yang, W.; Wang, P.; Huang, Y.; Fang, F. Short-Chain Fatty Acids Manifest Stimulative and Protective Effects on Intestinal Barrier Function Through the Inhibition of NLRP3 Inflammasome and Autophagy. Cell Physiol. Biochem. 2018, 49, 190–205. [CrossRef]

120. Lee, S.H.; Yoon, S.H.; Jung, Y.; Kim, N.; Min, U.; Chun, J.; Choi, I. Emotional well-being and gut microbiome profiles by enterotype. Sci. Rep. 2020, 10, 1–9. [CrossRef] [PubMed]

121. Tillisch, K.; Mayer, E.A.; Gupta, A.; Gill, Z.; Brazelle, R.; Le Neveu, B.; van Hylckama Vlieg, J.E.T.; Guyonnet, D.; Derrien, M.; Labus, J.S. Brain Structure and Response to Emotional Stimuli as Related to Gut Microbial Profiles in Healthy Women. Psychosom. Med. 2017, 79, 905–913. [CrossRef]

122. Hao, Z.; Wang, W.; Guo, R.; Liu, H. Faecalibacterium prausnitzii (ATCC 27766) has preventive and therapeutic effects on chronic stress-induced depression-like and anxiety-like behavior in rats. Psychoneuroendoocrinology 2019, 104, 132–142. [CrossRef] [PubMed]

123. Stevens, B.R.; Goel, R.; Seungbum, K.; Richards, E.M.; Holbert, R.C.; Pepine, C.J.; Raizada, M.K. Increased human intestinal barrier permeability plasma biomarkers zonulin and FABP2 correlated with plasma LPS and altered gut microbiome in anxiety or depression. Gut 2018, 67, 1555–1557. [CrossRef] [PubMed]

124. Reus, G.Z.; Jansen, K.; Titus, S.; Carvalho, A.F.; Gabbay, V.; Quevedo, J. Kynurenine pathway dysfunction in the pathophysiology and treatment of depression: Evidence from animal and human studies. J. Psychiatr. Res. 2015, 68, 316–328. [CrossRef] [PubMed]

125. Zimmermann, M.; Zimmermann-Kogadeeva, M.; Wegmann, R.; Goodman, A.L. Separating host and microbiome contributions to drug pharmacokinetics and toxicity. Science 2019, 363. [CrossRef]

126. Vich Vila, A.; Collij, V.; Sanna, S.; Sinha, T.; Immann, F.; Bourgonje, A.R.; Majicig, Z.; Jonkers, T.M.A.E.; Masclee, A.A.M.; Fu, J.; et al. Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. Future Microbiol. 2020, 15, 97–111. [CrossRef]

127. Ng, Q.X.; Peters, C.; Ho, C.Y.X.; Lim, D.Y.; Yeo, W.S. A meta-analysis of the use of probiotics to alleviate depressive symptoms. J. Affect. Disord. 2018, 228, 13–19. [CrossRef]

128. McFarland, L.V. Efficacy of Single-Strain Probiotics Versus Multi-Strain Mixtures: Systematic Review of Strain and Disease Specificity. Dig. Dis. Sci. 2020. [CrossRef] [PubMed]

129. Rose, M.; Devine, J. Assessment of patient-reported symptoms of anxiety. Dialogues Clin. Neurosci. 2014, 16, 197–211. [CrossRef]

130. Felger, J.C. Imaging the role of inflammation in mood and anxiety-related disorders. Curr. Neuropharmacol. 2017, 15, 533. [CrossRef]

131. Tian, P.; O’Riordan, K.J.; Lee Y kun Wang, G.; Zhao, J.; Zhang, H.; Cryan, J.F.; Chen, W. Towards a psychobiological therapy for depression: Bifidobacterium breve CCFCM1025 reverses chronic stress-induced depressive symptoms and gut microbial abnormalities in mice. Neurobiol. Stress 2020, 12. [CrossRef]

132. Juruena, M.F.; Eror, F.; Cleare, A.J.; Young, A.H. The role of early life stress in HPA axis and anxiety. Adv. Exp. Med. Biol. 2020, 1191, 141–153. [CrossRef]

133. Mosrshedi, M.; Valenlia, K.B.; Hosseinifard, E.S.; Shahabi, P.; Abbasi, M.M.; Ghorbani, M.; Barzegar, A.; Sadigh-Eteghad, S.; Saghafi-Asl, M. Beneficial psychological effects of novel psychobiotics in diabetic rats: The interaction among the gut, blood, and brain. J. Nutr. Biochem. 2018, 57, 145–152. [CrossRef] [PubMed]

134. Ait-Belgnaoui, A.; Colom, A.; Braniste, V.; Ramalho, L.; Marron, A.; Cartier, C.; Houdeau, E.; Theodorou, V.; Tompkins, T. Probiotic gut effect prevents the chronic psychological stress-induced brain activity abnormality in mice. Neurogastroenterol. Motil. 2014, 26, 510–520. [CrossRef] [PubMed]

135. Nuss, P. Anxiety disorders and GABA neurotransmission: A disturbance of modulation. Neuropsychiatr. Dis. Treat. 2015, 11, 165–175. [CrossRef]

136. Davis, D.J.; Doerr, H.M.; Grzelak, A.K.; Busi, S.B.; Jasaroevic, E.; Ericsson, A.C.; Bryda, E.C. Lactobacillus plantarum attenuates anxiety-related behavior and protects against stress-induced dysbiosis in adult zebrafish. Sci. Rep. 2016, 6, 1–11. [CrossRef] [PubMed]

137. Fedoce A das, G.; Ferreira, F.; Bota, R.G.; Bonet-Costa, V.; Sun, P.Y.; Davies, K.J.A. The role of oxidative stress in anxiety disorder: Cause or consequence? Free Radic. Res. 2018, 52, 737–750. [CrossRef]
138. Kelly, J.R.; Kennedy, P.J.; Cryan, J.F.; Dinan, T.G.; Clarke, G.; Hyland, N.P. Breaking down the barriers: The gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Front. Cell. Neurosci.* 2015, 9. [CrossRef]

139. Valcarce, D.G.; Genovés, S.; Riesco, M.F.; Martorell, P.; Herráez, M.P.; Ramón, D.; Robles, V. Probiotic administration improves sperm quality in asthenozoospermic human donors. *Benef. Microbes.* 2017, 8, 193–206. [CrossRef]

140. Laparra, J.M.; Olivares, M.; Gallina, O.; Sanz, Y. Bifidobacterium longum CECT 7347 modulates immune responses in a gliadin-induced enteropathy animal model. *PLoS ONE* 2012, 7. [CrossRef]

141. Sandes, S.; Figueiredo, N.; Pedroso, S.; Sant’Anna, F.; Acucio, L.; Abatemarco Junior, M.; Barros, P.; Oliveira, F.; Cardoso, V.; Generoso, S.; et al. Weissella paramesenteroides WpK4 plays an immunobiotic role in gut-brain axis, reducing gut permeability, anxiety-like and depressive-like behaviors in murine models of colitis and chronic stress. *Food Res Int.* 2020, 137. [CrossRef]

142. Dominique, M.; Legrand, R.; Galmiche, M.; Azhar, S.; Deroissart, C.; Guérin, C.; do Rego, J.L.; Leon, F.; Nobis, S.; Lambert, G.; et al. Changes in microbiota and bacterial protein caseinolytic peptidase b during food restriction in mice: Relevance for the onset and perpetuation of Anorexia Nervosa. *Nutrients* 2019, 11, 2514. [CrossRef] [PubMed]

143. Fuentes, C.T.; Schellekens, H.; Hoevenaars, N.; Ross, P.; Roy, B.; Stanton, C.; Timothy, G.D.; John, F.C. Identification of Novel Probiotics to Modify Appetite and Satiety Directly Targeting the Ghrelin Receptor. *FASEB J.* 2016, 30, 717.2. [CrossRef]

144. Sakata, T.; Adachi, M.; Hashida, M.; Sato, N.; Kojima, T. Effect of n-butyric acid on epithelial cell proliferation of pig colonic mucosa in short-term culture. *Dtsch Tierarztl Wochenschr.* 1995, 102, 163–164.

145. Shamseer, L.; Moher, D.; Clarke, M.; Ghersi, D.; Liberati, A.; Petticrew, M. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015: Elaboration and Explanation. *BMJ* 2015, 2, 350g7647. [CrossRef]

146. Liberati, A.; Altman, D.; Tetzlaff, J.; Mulrow, C.; Gøtzsche, P.C.; Ioannidis, J.P.; Clarke, M.; Devereaux, P.J.; Kleijnen, J.; Moher, D. The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies that Evaluate Health Care Interventions: Explanation and Elaboration. *BMJ* 2009, 21, 339:b2700. [CrossRef]

147. Higgins, J.P.T.; Altman, D.G.; Getzschke, J.; Jüni, P.; Moher, D.; Oxman, A.D.; Savovic, J.; Schulz, K.F.; Weeks, L.; Sterne, J.A.; et al. The Cochrane Collaboration’s tool for assessing risk of bias in randomised trials. *BMJ* 2011, 343. [CrossRef] [PubMed]

148. Guyatt, G.; Oxman, A.D.; Akl, E.A.; Kunz, R.; Vist, G.; Brozek, J.; Norris, S.; Falck-Ytter, Y.; Glasziou, P.; DeBeer, H. GRADE Guidelines: 1. Introductionandgrade Evidence Profiles and Summary of Findings Tables. *J. Clin. Epidemiol.* 2011, 64, 383–394. [CrossRef] [PubMed]