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The effects of psychobiotics on the microbiota-gut-brain axis in early-life stress and neuropsychiatric disorders

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ABSTRACT

Psychobiotics are considered among potential avenues for modulating the bidirectional communication between the gastrointestinal tract and central nervous system, defined as the microbiota-gut-brain axis (MGBA). Even though causality has not yet been established, intestinal dysbiosis has emerged as a hallmark of several diseases, including neuropsychiatric disorders (NPDs). The fact that the microbiota and central nervous system are co-developing during the first years of life has provided a paradigm suggesting a potential role of psychobiotics for earlier interventions. Studies in animal models of early-life stress (ELS) have shown that they can counteract the pervasive effects of stress during this crucial developmental period, and rescue behavioral symptoms related to anxiety and depression later in life. In humans, evidence from clinical studies on the efficacy of psychobiotics at improving mental outcomes in most NPDs remain limited, except for major depressive disorder for which more studies are available. Consequently, the beneficial effect of psychobiotics on depression-related outcomes in adults are becoming clearer. While the specific mechanisms at play remain elusive, the effect of psychobiotics are generally considered to involve the hypothalamic-pituitary-adrenal axis, intestinal permeability, and inflammation. It is anticipated that future clinical studies will explore the potential role of psychobiotics at mitigating the risk developing NPDs in vulnerable individuals or in the context of childhood adversity. However, such studies remain challenging at present in terms of design and target populations; the profound impact of stress on the proper development of the MGBA during the first year of life is becoming increasingly recognized, but the trajectories post-ELS in humans and the mechanisms by which stress affects the susceptibility to various NPDs are still ill-defined. As psychobiotics are likely to exert both shared and specific mechanisms, a better definition of target subpopulations would allow to tailor psychobiotics selection by aligning mechanistic properties with known pathophysiological mechanisms or risk factors. Here we review the available evidence from clinical and preclinical studies supporting a role for psychobiotics at ameliorating depression-related outcomes, highlighting the knowledge gaps and challenges associated with conducting longitudinal studies to address outstanding key questions in the field.

1. Introduction

The microbiota-gut-brain axis (MGBA) refers to the bidirectional communication between the intestinal microbiome and the brain. This complex interplay, although not fully understood mechanistically, involves multiple physiological systems, such as the gastrointestinal system and its microbiota, the central, autonomic and enteric nervous systems, the immune system and the neuroendocrine system.(Mohajeri et al., 2018) The importance of the microbiome and the potential role of probiotics for mental health and neuropsychiatric disorders (NPDs) builds upon animal studies describing behavioral alterations observed in gnotobiotic and immunodeficient states (Bähr, 1970; Vidal, 1996; Diaz Heijtz et al., 2011; Neufeld et al., 2011; Smith et al., 2014; Quinnies et al., 2015; Luczynski et al., 2016) and showing that these behaviors could be transferred between animals and humans using fecal transplants (Bercik et al., 2011; Kelly et al., 2016; Zheng et al., 2016; De Palma et al., 2017) or rescued by timely microbial colonization via fecal transplants or probiotic administration.(Sudo et al., 2004; Smith et al.,

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2014) Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host", (Hill et al., 2014) and those that influence bacteria–brain relationships and confer mental health benefits are referred to as psychobiotics. (Dinan et al., 2013; Sarkar et al., 2016) Currently, psychobiotics are considered among the strategies aimed at alleviating or mitigating the effects of intestinal dysbiosis on mental health. It is now well recognized that dysbiosis accompanies several disease states,(Wilkins et al., 2019) including neuropsychiatric and neurodegenerative diseases.(Naseribafrouei et al., 2014; Jiang et al., 2015; Iannone et al., 2019; Jurek et al., 2020; Long-Smith et al., 2020) However, the potential causative role of dysbiosis in NPDs remains limited to correlations established from cross-sectional studies; more clinical studies including both microbiome profiling and functional outcomes are required to formally answer this question.(Cryan and Dinan, 2019; Wilkins et al., 2019; Simpson et al., 2020) Nevertheless, the profound and lasting impact of the perturbations of microbiota establishment in early life, such as stress- or antibiotic-induced dysbiosis, on mental health later in life argues for an implication of the microbiome in shaping the development of the central nervous system during this critical period.

ELS is a well-documented risk factor for the development of substance abuse disorders or NPDs later in life, and most notably major depressive disorder (MDD) and mood disorders, anxiety, post-traumatic stress disorder, or bipolar disorder.(Heim et al., 2019; Targum and Nemeroff, 2019; Merrick et al., 2020) The correlation between infancy or childhood adversity and an increased incidence or earlier onset of depression is supported by numerous epidemiological studies.(Kessler et al., 2010; Mandelli et al., 2015; LeMoult et al., 2019; Targum and Nemeroff, 2019) However, childhood adversity is not the only factor influencing the risk of developing NPDs; not everyone who experiences ELS develops NPDs later in life owing to complex mechanisms associated with resilience and coping which will not be discussed herein. At the physiological level, ELS was shown to induce persistent structural and functional changes to central nervous system (CNS) structures and circuits including the prefrontal cortex, hippocampus, amygdala, and other cortical/subcortical areas of brain, with increasing evidence that specific types of ELS result in specific neuroanatomical alterations and specific neuroinflammatory profiles.(Baumeister et al., 2016; Syed and Nemeroff, 2017) This is in accordance with the concept that specific types of ELS would predispose to different NPDs,(Mandelli et al., 2015) possibly by causing specific alterations in either microbiota composition or CNS structures. In rodents, ELS is often modeled by maternal separation (MS), which was defined as a means to study the susceptibility to depression. Interestingly, MS was also recognized for its effects on gastrointestinal functions and has been described as a model of gut-brain axis dysfunction suitable for the study of disorders such as irritable bowel syndrome (IBS).(O'Mahony et al., 2011) Indeed, IBS is associated with psychiatric comorbidities such as anxiety and major depression with a co-occurrence rate ranging between 44 and 84%, and both IBS and MDD have been postulated to share pathophysiological mechanisms involving a dysregulation of the MGBA.

Depression has a multifactorial and complex etiology, involving an interaction between physiological, genetic and environmental risk factors.(Flux and Lowry, 2020) One of the most documented of the core neurophysiological alterations in MDD is the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis activity, especially in response to stressors, which has been identified in 40–60% of individuals with depression.(Flux and Lowry, 2020) In patients with MDD, HPA axis dysfunctions include elevated levels of corticotropin-releasing hormone, increased secretion of cortisol, elevated neuronal activity and increased volumes of the pituitary and adrenal glands, as well as defective negative feedback loops owing to glucocorticoid resistance. (Misiak et al., 2020) The intestinal microbiome was shown to modulate the HPA axis outputs in animal studies, at baseline or in response to various stressors throughout lifespan.(Farzi et al., 2018; Misiak et al., 2020) Potential mechanisms of actions implicate alterations in the intestinal barrier integrity which, by increasing intestinal permeability to microbial antigens, may trigger subclinical neuroinflammation. The microbiome can also interact with autonomic afferents, notably with the enteric nervous system via the vagus nerve, possibly by the secretion of neurotransmitters or other neuroactive molecules.(van de Wouw et al., 2018; Caspani et al., 2019; Rea et al., 2020) Furthermore, intestinal microbes can regulate the secretion of hormones and peptides by the endocrine and epithelial cells in the ileum and colon.(Misiak et al., 2020) These bacterially-derived molecules can also modulate bacterial growth in the intestine, thereby affecting microbiota composition. This is in keeping with the microbiome alterations seen in MDD patients, which seemingly correlate with functional mechanistic insights. For example, some studies have shown a reduction in alpha diversity MDD patients with an increase in Bacteroidetes levels and a reduction in Firmicutes levels, which was postulated to account for the lower levels of short-chain fatty acids, increased intestinal permeability and low-grade inflammation associated with depression.(Huang et al., 2018; Bastiaanssen et al., 2020) At the genus level, the abundance of *Faecalibacterium*, *Dialister*, and *Prevotella* appear typically decreased while *Oscillibacter* and *Alistipes* genera, increased.(Naseribafrouei et al., 2014; Jiang et al., 2015; Kelly et al., 2016; Valles-Colomer et al., 2019; Bastiaanssen et al., 2020) Further studies are needed to confirm whether these changes are consistent between studies, have a causative effect or result from the disease or from other variable confounders such as antidepressant/antipsychotic medications, diet or exercise.(Bastiaanssen et al., 2020) In addition, the microbiome was recently shown to regulate microglia maturation, possibly via the serotonin pathway or secretion of metabolites such as short-chain fatty acids.(Wang et al., 2018) Furthermore, neonatal conventionalization or colonization of germ-free mice with four probiotic *Bifidobacteria* was shown to increase microglia reactivity and normalize synapse density in the cerebellum.(Erny et al., 2015; Luck et al., 2020) Microglia is activated in response to ELS in animal models,(Majcher-Maślanka et al., 2019) and targeting microglial activation has been proposed as a potential therapeutic avenue in MDD. (Zhang et al., 2018)

Mechanistic insight on the abovementioned roles of the microbiome (and psychobiotics) mostly originates from animal studies. Nevertheless, the efficacy of psychobiotics for NPDs is being increasingly studied in clinical trials, although more studies at this time have assessed outcomes related to anxiety- and depressive-like symptoms. Several systematic reviews and meta-analyses have examined the efficacy of psychobiotics on symptoms of stress, anxiety and depression in healthy populations experiencing stress or in populations with comorbid diseases.(Romijn and Rucklidge, 2015; Liu et al., 2018; Ng et al., 2018; Reis et al., 2018; Vaghef-Mehrabany et al., 2020) Overall, conclusions on the promising positive effects on various mental health outcomes in these populations warranted the conduct of additional studies in individuals with a clinical diagnosis.(Huang et al., 2018; Pirbaglou et al., 2016; McKean et al., 2017; Wallace and Milev, 2017; Liu et al., 2019; Smith et al., 2019; Yang et al., 2019) Clinical research on the effects of psychobiotics for MDD is more advanced than for other NPDs; the scarcity of human trials assessing the effects of potential psychobiotics in schizophrenia, autism patients, and obsessive-compulsive disorder patients precludes broad conclusions on psychobiotics for these indications at present.(Brenner et al., 2017; Fusar-Poli et al., 2019; Ng et al., 2019a; Ng et al., 2019b) Importantly, a substantial level of variability among studies was noted for all conditions tested, including MDD, with regards to psychobiotic formulation, dosage, outcome assessment tools used, and disease subtypes.(Vaghef-Mehrabany et al., 2020)

Here, we provide an overview of the multiple systematic reviews and meta-analyses of clinical studies on psychobiotics on depression-related outcomes in various populations. From these reviews, we summarize the clinical studies that assessed the role of psychobiotics in adult, clinically diagnosed MDD patients. Furthermore, as ELS was associated with the etiology of depressive disorders and antidepressant response, we summarize the key animal studies on the effects of psychobiotics in the MS

model of ELS, supporting the view that they could be a valuable addition to the strategies aimed at mitigating the lasting effects of ELS on the nervous system with earlier interventions during infancy and childhood. Insights from animal models suggest the existence of potential benefits of psychobiotics for mitigating the lasting effects of ELS during this critical period for both microbiota establishment and CNS development. Furthermore, when considered collectively, preclinical and clinical research on the topic suggests that assessment of childhood adversity could be a worthwhile addition to outcome assessment tools (e. g. the Childhood Trauma Questionnaire (CTQ)),(Karakula-Juchnowicz et al., 2019) in future clinical trials of psychobiotics or microbiome profiling in MDD patients.

2. Psychobiotics and ELS in animal models

An integrative view of the multifactorial developmental trajectory after ELS has emerged in parallel with findings from neurobiology research; multiple stresses throughout life ("hits") and additional predisposing factors (such as genetic/epigenetic regulation) appear to be necessary to trigger NPD onset.(Agorastos et al., 2019; Codagnone et al., 2019a) Generally viewed as one of the first "hits", ELS appears to sensitize individuals to subsequent stressors during adulthood.(Agorastos et al., 2019; Misiak et al., 2020) This first "hit" occurs at a critical co-developmental stage for both the central nervous system and the microbiome, hence ELS is considered as a major influential force on the MGBA during development.(Cowan et al., 2020) ELS was shown to alter microbiota composition in several species including rhesus monkeys (Bailey and Coe, 1999; Bailey et al., 2004), piglets (Schokker et al., 2014; Pena Cortes et al., 2018), and rodents.(De Palma et al., 2015)

Research findings from MS studies in conventionally housed rodents clearly demonstrate that psychobiotics can mitigate the pervasive effects of ELS on the developing nervous system, which suggests a possible role for psychobiotics in early-life interventions aimed at protecting the microbiome during ELS. The beneficial role of a psychobiotic combination of *Lacticaseibacillus* (*L.*) *rhamnosus* Rosell®-11 and *Lactobacillus* (*L.*) *helveticus* Rosell®-52 (Lacidofil®) on MS in rats is demonstrated in 5 studies. Lacidofil® administered orally and rectally to Sprague-Dawley rat pups subjected to MS for 3 h/day from post-natal day (PND) 4 to 19 rescued the deleterious effects of MS.(Gareau et al., 2007) Assessments were performed on PND20 and at 60–70 days of age. Lacidofil® counteracted the colonic dysbiosis induced by MS, as demonstrated by the restored levels of *Lactobacilli* in supplemented animals. In keeping with the fact that MS was initially described as a model of IBS, the barrier function integrity is affected by MS in rat pups. This defect was corrected by the administration of Lacidofil®. This formulation also mitigated the development of MS-enhanced HPA axis activity indicated by the lower serum corticosterone levels in psychobiotic-supplemented MS animals compared to those who received vehicle during MS. Furthermore, the timely administration of Lacidofil® during the ELS period also reduced the stress-response dysregulation caused by MS in adulthood; this psychobiotic formulation improved the performance of adult MS rats in a water avoidance stress (WAS) test. When administered to dams via drinking water from PND2-PND14, during which MS was performed for 3 h/day, the psychobiotic formulation was detectable in the stomachs of the pups in the treated group, but not in the vehicle control group, indicating it was transferred to the pups via breastmilk.(Cowan et al., 2016) In this study, Lacidofil® was shown to prevent the accelerated emotional development and transition to an adult-like memory system typically induced by MS, shown by the restoration of the normal trajectory of fear-retention and extinction. This phenotype was associated with a precocious maturation of the neural circuits underpinning fear expression, as measured by levels of phosphorylated MAPK expression in the prefrontal cortex.(Cowan et al., 2019) However, no effect on anxiety-like behavior were observed in the elevated plus maze (EPM) test, possibly because the assessment was performed on young rats at PND17.(Cowan et al., 2016)

Another group using the same MS protocol (3 h/day, from PND2-PND14) observed the same effect of Lacidofil® on the restoration of the normal trajectory of fear retention and extinction when assessed at PND20.(Peng et al., 2019) At this time point, Lacidofil® significantly improved anxiety-like behavior of MS pups in the EPM and light-dark box tests.(Peng et al., 2019) This effect was accompanied by a normalization of the heightened neuroendocrine stress response to restraint induced by MS, shown by the significant reduction in adrenocorticotropic hormone (ACTH) and corticosterone plasma levels compared to untreated MS infant rats. Furthermore, neuronal activation, assessed by number of c-Fos^{+} neurons and brain-derived neurotrophic factor (BDNF) protein levels, was reduced in the basolateral nucleus of amygdala in Lacidofil®-treated MS animals compared to MS controls.(Peng et al., 2019) The accelerated acquisition of adult-like fear retention and extinction patterns caused by MS was shown to be transferred to the next generation by MS-exposed fathers, possibly through epigenetic modifications. Importantly, Lacidofil® prevented the transmission of the deleterious effects of MS to the next generation (F1) when administered as a prophylactic to the paternal line (F0) during their infancy; the offspring of Lacidofil®-treated MS-fathers displayed age-appropriate fear retention and extinction phenotypes.(Callaghan et al., 2016) Lacidofil® was also effective at restoring proper fear retention and extinction phenotypes when administered as treatment to the F1 of MS-fathers.(Callaghan et al., 2016) Lacidofil® also restored normative pubertal timing in MS rats of both sexes despite the gender differences in the effect of MS, which accelerated puberty in females and delayed it in males.(Cowan and Richardson, 2019) Interestingly, a small study reported a reduction in depressive symptoms after Lacidofil® in medical students under stress, which provides a rationale for studying this formulation in clinical trials in the context of depression and stress in human.(Theodora et al., 2019)

A few single strains formulations have also shown positive effects in various MS models. *Bifidobacterium infantis* 35624 improved the performance of MS rats in the forced swim test and normalized noradrenaline levels in the brainstem and peripheral interleukin (IL)-6 levels.(Desbonnet et al., 2010) *Lactiplantibacillus plantarum* (*L. plantarum*) PS128 was shown to increase locomotor activity in the open field (OF) test, and to reduce the depression-like behaviors of MS mice in the forced swimming and sucrose preference tests, but did not affect anxiety-like behavior in these mice.(Liu et al., 2016) This was accompanied by a reduction in MS-induced circulating corticosterone levels, a reduction in pro-inflammatory cytokine IL-6, an increase in anti-inflammatory cytokine IL-10, as well as an increase in dopamine levels in the prefrontal cortex of MS mice.(Liu et al., 2016) It is noteworthy that this strain appears to exert different effects in conventionally reared rodents (naïve mice), which is reminiscent of the variability in the effects seen between healthy/non-clinical and clinically diagnosed human populations shown by some psychobiotic strains. For example, *L. plantarum* PS128 reduced anxiety-like behavior and increased serotonin levels in the medial prefrontal cortex in naive mice but not MS mice.(Liu et al., 2016) *Bifidobacterium pseudocatenulatum* CECT 7765 was shown to improve the dysregulated stress response of the HPA axis and intestinal inflammation in MS mice pups; shown by a reduced corticosterone production in response to acute restraint, a reduction in intestinal interferon gamma, a reduction in dopamine and adrenaline concentrations in the hypothalamus and small intestine, and a reversal of intestinal dysbiosis.(Moya-Perez et al., 2017) In adulthood, this strain reduced MS-induced anxiety in the EPM test and normalized neurotransmitter levels in the hypothalamus.(Moya-Perez et al., 2017) *Limosilactobacillus fermentum* (*L. fermentum*) CECT 5716 increased the exploratory behavior in the EPM test and prevented the increase in corticosterone induced by MS or WAS.(Vanhaecke et al., 2017) This strain also improved the intestinal barrier dysfunction induced by both MS and WAS in rats by reducing permeability in the small intestine and maintaining tight-junction integrity.(Vanhaecke et al., 2017)

L. rhamnosus GG, in combination with a prebiotic but not alone,

normalized MS-induced anxiety assessed by the OF test in rats and ameliorated spatial memory in the Morris Water Maze.(McVey Neufeld et al., 2019) However, the MS-induced increase in corticosterone levels was not corrected by *L. rhamnosus* GG, and the return to normal levels after testing was delayed. This strain alone reversed the stress-induced decrease in glucocorticoid receptor mRNA expression in the hippocampus and prevented the increase in the type 2 subunit of the GABA-A receptor (Gamma-Aminobutyric Acid Type A Receptor Subunit Alpha2; Gabra2) at the mRNA level induced by MS, but only in combination with a prebiotic.(McVey Neufeld et al., 2019) *Bifidobacterium (B.) bifidum* G9-1 prevented MS-induced hypercorticosteronemia, enhanced intestinal permeability and dysbiosis in MS rat pups, and reduced MS-induced corticosterone levels and fecal frequency in response to restraint stress in adulthood.(Fukui et al., 2018) *Lacticaseibacillus* (*L.*) *paracasei* PS23, either live or heat-killed, was shown to reduce the anxiety- and depression-like behavior and increased serum corticosterone levels induced by MS in mice, which was associated with immunomodulatory properties in light of the increased levels of IL-10 compared to untreated MS mice.(Liao et al., 2019) Interestingly, both live and heat-killed PS23 also reversed anxiety- and depression-like behaviors induced by chronic corticosterone administration in mice.(Wei et al., 2019) Collectively, insights from the MS model highlight a role for psychobiotics in regulating the HPA axis, inflammation and neurogenesis, and suggest that timely administration could be viewed as an integral part of comprehensive or holistic strategies aimed at mitigating the effects of ELS on mental health later in life. In humans, depression, anxiety, and stress are the most studied indications for psychobiotics in association with NPDs or in healthy individuals. As clinical studies assessing the efficacy of psychobiotics for anxiety- and depression-like symptoms in adults have been accumulating, several systematic reviews and meta-analyses were conducted to confirm the translational potential of psychobiotics' beneficial effects from animal models to human populations.

3. Clinical trials on psychobiotics for MDD

In total, we identified 21 systematic reviews and meta-analyses of RCTs on the effects of psychobiotics for symptoms of stress, anxiety, and depression between January 2015 and September 2020, which reflects the importance of this area of research and need for conclusions about the effect of psychobiotics on mental health outcomes. The effect of psychobiotics on other NPDs were also reviewed systematically, but with a small number of clinical trials conducted in populations clinically diagnosed with diseases such as autism spectrum disorder, bipolar disorder, post-traumatic stress disorder or schizophrenia, conclusions about the effect of psychobiotics in these pathologies, although promising, remain equivocal at present.(Brenner et al., 2017; Fusar-Poli et al., 2019; Ng et al., 2019a, 2019b) Of these 21 reviews, 4 were focused exclusively on anxiety symptoms (excluding depression) (Liu et al., 2018; Reis et al., 2018; Yang et al., 2019; Vitellio et al., 2020) and one included only studies where anxiety or depression symptoms were assessed in parallel to subjective stress levels, which resulted in the exclusion of trials conducted in MDD patients.(Zhang et al., 2020) With a limited number of trials conducted in populations with clinically diagnosed MDD, earlier reviews on the effects of psychobiotics on depressive symptoms included mostly trials conducted in non-clinical populations, generally healthy individuals experiencing stress or comorbid symptoms of low mood, anxiety or depression secondary to another condition such as IBS (Simrén et al., 2010; Pinto-Sanchez et al., 2017) or chronic fatigue syndrome (Rao et al., 2009). Overall, conclusions about the effect of psychobiotics on depression-related outcomes were positive in healthy populations, with some discrepancies; 5 reviews concluded to an insignificant or marginal effect of psychobiotics on mood in the general population or in individuals presenting subclinical symptoms.(Romijn and Rucklidge, 2015; Ng et al., 2018; Goh et al., 2019; Liu et al., 2019; Chao et al., 2020)

Nevertheless, most reviews highlighted the need for more trials assessing the efficacy of psychobiotics in clinically diagnosed MDD patients,(Romijn and Rucklidge, 2015; Huang et al., 2016; Pirbaglou et al., 2016; Wang et al., 2016; McKean et al., 2017; Wallace and Milev, 2017; Ng et al., 2018; Goh et al., 2019; Liu et al., 2019; Chao et al., 2020) while subgroup analyses including between 1 and 6 studies in clinically diagnosed MDD patients generally suggested or supported a positive effect of psychobiotics on depressive symptoms.(Goh et al., 2019; Liu et al., 2019; Nikolova et al., 2019; Smith et al., 2019; Amirani et al., 2020; Barbosa and Vieira-Coelho, 2020; Chao et al., 2020; Sanada et al., 2020; Vaghef-Mehrabany et al., 2020) Indeed, as the number of efficacy studies on psychobiotics in populations with a clinical MDD diagnosis increased, the conclusions about the apparent anti-depressant effect of psychobiotics in psychiatric samples became more promising despite some discrepancies between trials in terms of outcome assessment tools or psychobiotic formulations.(Barbosa and Vieira-Coelho, 2020, Chao et al., 2020, Sanada et al., 2020, Vaghef-Mehrabany et al., 2020) These factors are important to consider together with the clinical characteristics of the sample population (e. g. treatment responsiveness, inclusion criteria, etc.); not all psychobiotics are expected to affect the same aspects of the depression symptomatology, and not all assessment tools contain the same proportion of questions directed towards specific types of symptoms (emotional, physical, cognitive or behavioral).(Fried, 2020; Newson et al., 2020)

While studies at present do not yet permit to draw any conclusion on the superiority of a tool over another in specific contexts or subpopulations or with specific strains, a convincing beneficial effect of psychobiotics on depression is generally emerging from available studies focused on clinically diagnosed MDD patients (8 studies), which are summarized below. Of note, while this may not have affected the general conclusions of the most recent systematic reviews, none of these have included the 8 clinical studies described herein (6 randomized trials and 2 pilot studies) in their meta-analyses. As shown in Table 1, most of these trials enrolled MDD patients under an antidepressant treatment,(Akkasheh et al., 2016; Bambling et al., 2017; Ghorbani et al., 2018; Miyaoka et al., 2018; Kazemi et al., 2019a; Rudzki et al., 2019) except for one trial in participants not currently on conventional medications (Majeed et al., 2018) and a pilot study conducted in recently diagnosed, treatment-naïve individuals.(Wallace et al., 2020)

Almost all studies used multi-strain combinations of 2 or more strains, while three studies used single-strain psychobiotics. Majeed et al. (2018) assessed the effect of *Bacillus coagulans* MTCC 5856 in MDD patients with IBS not taking antidepressants.(Majeed et al., 2018) They showed a significant improvement in primary efficacy outcomes compared to placebo at 60 and 90 days, assessed by the 17-item Hamilton depression rating scale (HAMD-17), Montgomery-Åsberg Depression Rating Scale (MADRS), Center for Epidemiologic Studies Depression Scale (CES-D), and the IBS Quality of Life questionnaires. This strain was also shown to alleviate gastrointestinal symptoms and improve quality of life in a population with diarrhea-predominant IBS (Majeed et al., 2016), and to ameliorate inflammation in a rodent model of dextran sodium sulfate-induced colitis.(Shinde et al., 2020)

In an exploratory, open-label, single blind study, Miyaoka et al. (2018) showed that *Clostridium* (*C.*) *butyricum* MIYAIRI 588, used as adjuvant to selective serotonin reuptake inhibitors (SSRIs) or serotonin–norepinephrine reuptake inhibitors (SNRIs), improved the Beck Depression Inventory (BDI) and HAMD-17 scores in treatment-resistant MDD patients.(Miyaoka et al., 2018) They found that this strain reduced symptoms of depression assessed by both questionnaires (HAMD and BDI) in 70% of the participants, with 35% achieving remission. *C. butyricum* MIYAIRI 588 was also shown to increase lifespan and resistance to various stressors, such as pathogens or UV, in *Caenorhabditis elegans*,(Kato et al., 2018) and to modulate antibiotic-induced microbiota composition changes in mice.(Hagihara et al., 2018) Furthermore, vegetative *C. butyricum* MIYAIRI 588 cells acted as an adjuvant to cholera-toxin immunization by stimulating mucosal immunity in mice.(Murayama et al., 1995)

Table 1
Characteristics of the probiotic clinical trials conducted in clinically diagnosed MDD patients.

Study reference	Study Design	Population, study arms, n	Dosage Regimen	Formulation (Trade name, Manufacturer)	Primary outcome	Other outcomes
Akkasheh et al. (2016)	Randomized, Placebo-controlled Double-blind	40 MDD patients taking citalopram Placebo, n = 20 Probiotic, *n* = 20	1 capsule/ day, for 8 weeks	Capsules containing 2×10^9 CFU/g of each: *L. acidophilus*, *L. casei*, *B. bifidum* (Tak Gene Zist Inc.)	Significant reduction in BDI score after 8 weeks	Significant decreases in: Serum insulin levels, homeostasis model assessment of insulin resistance and serum hs-CRP concentrations. Significant increase in plasma total glutathione levels.
Bambling et al. (2017)	Pilot, open-label study	12 patients with treatment resistant MDD, taking SSRIs Probiotic, *n* = 12	0.5 dose BID, before meals, 8 weeks.	Capsules containing 2×10^{10} CFU of a combination of lyophilized probiotics (*L. acidophilus*, *B. bifidum*, *S. thermophilus*) Magnesium orotate 1600 mg. (NRGbiotic™, Medlab)	Significant reduction in average BDI and OQ45 scores at 8 weeks. Out of the 12 participants, 4 did not respond to probiotic treatment. At the 16-week follow-up (8 weeks after ceasing probiotic) all participants had relapsed.	N.R.
Ghorbani et al. (2018)	Randomized, Placebo-controlled Double-blind	40 MDD patients taking fluoxetine for 4 weeks before study start Placebo, *n* = 20 Probiotic, n = 20	2 × 500 mg capsules/day, 6 weeks	Blend of 7 strains: *L. casei* 3×10^8 CFU/g, *L. acidophilus* 2×10^8 CFU/g, *L. bulgaricus* 2×10^9 CFU/g, *L. rhamnosus* 3×10^8 CFU/g, *B. breve* 2×10^8 CFU/g, *B. longum* 1×10^9 CFU/g, *S. thermophilus* 3×10^8 CFU/g, FOS 100 mg/capsule. (Familact H®, Zist Takhmir Co.)	Significant reduction in HAMD-17 score at the end of the study	N.R.
Majeed et al. (2018)	Randomized, Placebo-controlled Double-blind	40 MDD patients with IBS, not taking antidepressants Placebo, *n* = 20 Probiotic, n = 20	1 cap/day, 30 min before a meal, for 90 days	*B. coagulans* MTCC 5856 (2×10^9 spores/capsule) (Lactospore®, Sabinsa)	Significant improvement in primary efficacy outcomes compared to placebo at 60 and 90 days, assessed by the HAMD-17, MADRS, CES-D, and IBS-QoL questionnaires.	Significant improvement in: CGI-I rating scale (60 and 90 d) CGI-S rating Scale (90 d) Dementia Total frequency scoring (90 d) GI-DQ (60 and 90 d) mESS (60 and 90 d) Serum myeloperoxidase (90 d)
Miyaoka et al. (2018)	Open-label, Randomized, Placebo-controlled, Single-blind (rater)	40 patients meeting criteria for treatment resistant depression, taking SSRIs or SNRIs for at least 1 month. Placebo, n = 20 Probiotic, n = 20	20 mg orally BID (week 1) and 20 mg orally TID (weeks 2–8).	*C. butyricum* MIYAIRI 588 60 mg (MIYARISAN Pharmaceuticals Co., Ltd.)	Significant reduction in HAMD-17 score at the end of the trial versus placebo. Positive response to treatment was seen in 70% of the participants, with a remission rate of 35% (score lower than 7).	Significant reduction in BDI and BAI scores at the end of the trial versus placebo.
Kazemi et al. (2019b)	Randomized, placebo-controlled Double-blind	110 MDD patients taking SSRIs Placebo, *n* = 36 Prebiotic (GOS), n = 36 Probiotic, *n* = 38	1 sachet daily, 8 weeks.	Sachets containing $\geq 10 \times 10^9$ CFU/5 g of a combination of L. *helveticus* Rosell®-52 and *B. longum* Rosell®-175 (CEREBIOME®, Lallemand Health Solutions Inc.)	Significant reduction in the BDI score at the end of the study compared to placebo. No significant differences were observed in the prebiotic group.	Significant reduction in the KYN/tryptophan ratio in the Probiotics group versus placebo. Increased appetite. Increased serum BDNF levels.
Rudzki et al. (2019)	Randomized, Placebo-controlled Double-blind	79 MDD patients taking SSRIs, Placebo, *n* = 39 Probiotic, *n* = 40	1 capsule BID, 8 weeks.	*L. plantarum* 299v (10×10^9 CFU/capsule) (Sanprobi IBS®; probiotic capsules manufacturer - Lallemand Health Solutions Inc.; strain owner - Probi AB).	There was no difference between groups in HAMD-17, SCL-90, or PSS-10.	Significant improvements in cognitive functions were observed: Work Speed in Attention and Perceptivity Test, CVLT total recall of trials 1–5. Significant decrease in KYN levels and significant increase in the 3HKYN:KYN ratio versus placebo.
Wallace et al. (2020)	Pilot, open-label study	12 MDD patients, treatment-naïve Probiotic, n = 12	1 sachet daily, 8 weeks.	Combination of *L. helveticus Rosell*®-52 and *B. longum* Rosell®-175 (3×10^9 CFU/day) (CEREBIOME®, Lallemand Health Solutions Inc.)	Significant improvements in mood (MADRS, QIDS-SR16), anhedonia (SHAPS), anxiety (GAD-7, STAI), and subjective sleep quality (PSQI).	N.R.

BDI, Beck Depression Inventory; BDNF, Brain-derived neurotrophic factor; BID, twice a day ("*bis in die*"); *B. coagulans*, *Bacillus coagulans*; *B. bifidum*, *Bifidobacterium bifidum*; *B. breve*, *Bifidobacterium breve*; *B. longum*, *Bifidobacterium longum*; CES-D, Center for Epidemiologic Studies Depression Scale; CFU, Colony forming units; CGI—I, Clinical Global Impression Improvement rating Scale; CGI—S, Clinical Global Impression Severity rating Scale; *C. butyricum*, *clostridium butyricum*; CVLT, California Verbal Learning Test; FOS, Fructooligosaccharides; GAD-7, Generalized Anxiety Disorder 7-item scale; GI-DQ, Gastrointestinal discomfort questionnaire; GOS; Galactooligosaccharides; HAMD, Hamilton depression rating scale; hs-CRP, high-sensitivity C-reactive protein test; 3HKYN:KYN, 3-hydroxykynurenine:

kynurenine; IBS-QoL, Irritable Bowel Syndrome Quality of Life questionnaire; *L. acidophilus*, *Lactobacillus acidophilus*; *L. bulgaricus*, *Lactobacillus bulgaricus*; *L. casei*, *Lacticaseibacillus casei*; *L. helveticus*, *Lactobacillus helveticus*; *L. plantarum, Lactiplantibacillus plantarum*; *L. rhamnosus*, *Lacticaseibacillus rhamnosus*; MADRS, Montgomery-Åsberg Depression Rating Scale; MDD, Major Depressive Disorder; mESS: Modified Epworth Sleepiness Scale; N.R., none reported; OQ45, Outcome Questionnaire 45; PSQI: Pittsburgh Sleep Quality Index; PSS, perceived stress scale; QIDS-SR16: Quick Inventory of Depressive Symptomatology 16-item self-report; SCL-90: Symptom Checklist; SHAPS: Snaith Hamilton Pleasure Scale; SNRI, Serotonin–norepinephrine reuptake inhibitor; SSRI, Selective serotonin reuptake inhibitor; STAI: State-Trait Anxiety Inventory; *S. thermophilus*, *Streptococcus thermophilus*; TID, three time a day (“*ter in die*”).

Rudzki et al. (2019) showed that despite not improving on depression outcomes, MDD patients who received *Lactiplantibacillus* (*L.*) *plantarum* 299v for 8 weeks demonstrated significant improvements in cognitive functions in the Work Speed in Attention and Perceptivity Test and CVLT total recall of trials 1–5.(Rudzki et al., 2019) This beneficial effect was accompanied by a significant increase in the 3-hydroxykynurenine (3-HKYN): kynurenine (KYN) ratio versus placebo. The significance of this finding is unclear as 3-HKYN is considered neurotoxic and KYN, neuroprotective. While several reasons could explain this result, more studies are required to validate the mechanisms behind the improvement in cognitive functions by *L. plantarum* 299v. Of note, in a previous study on healthy young adults under examination stress, administration of this strain for 14 days prior to the examination significantly reduced the pre-test cortisol levels on day 10 compared to the placebo group.(Andersson et al., 2016) Furthermore, this strain was shown to improve symptoms of IBS,(Ducrotté et al., 2012) and exerted beneficial effects on intestinal barrier integrity in various animal models.(Mangell et al., 2002; Mangell et al., 2006; Hulst et al., 2015; Wang et al., 2019)

Among multi-strain psychobiotics studied in MDD populations, few were also studied at the mechanistic level in animals. Kazemi et al. (2019b) reported a significant reduction in the BDI score in patients with mild-to-moderate MDD who received a combination of *L. helveticus* Rosell®-52 and *Bifidobacterium* (*B.*) *longum* Rosell®-175 for 8 weeks as an adjuvant to SSRIs.(Kazemi et al., 2019b) This beneficial effect on depression outcome was accompanied by a reduction in the KYN: tryptophan ratio after adjusting for isoleucine levels, which suggests an increased availability of tryptophan for the synthesis of serotonin centrally, and away from the inflammatory pathways. The levels of circulating pro-inflammatory cytokines were unchanged, but urinary cortisol levels were slightly reduced in the psychobiotic group in a potentially clinically important but not statistically significant manner.(Kazemi et al., 2019b) Post-hoc analyses of this population revealed that the psychobiotic improved appetite and increased circulating BDNF levels in MDD patients.(Heidazardeh-Rad et al., 2020; Kazemi et al., 2020) The effect of the *L. helveticus* Rosell®-52 and *B. longum* Rosell®-175 psychobiotic formulation was also assessed in self-referred individuals with symptoms of depression, scoring 14 or more on the depression subscale of the Depression, Anxiety and Stress Scale (DASS)-42, but not currently taking antidepressant treatment since at least 2 months.(Romijn et al., 2017) In this population likely to be heterogeneous in terms of past medication history, treatment responsiveness, and disease chronicity or severity, there was no effect on depression symptoms over placebo. Interestingly, an exploratory post-hoc analysis of the data revealed a potential role for vitamin D levels; the participants in the psychobiotic group with higher levels of vitamin D showed a greater improvement in mood and functioning assessed by the Improved Clinical Global Impressions Severity scale (iCGI-S), the Quick Inventory of Depressive Symptomatology 16-Item Self-Report (QIDS-SR16), and the Global Assessment of Functioning (GAF) scores than those with lower levels at baseline, while this association was not observed in the placebo group. It was hypothesized that the persistence of the disease in this population who had been exposed to antidepressants without amelioration could have overrepresented patients with treatment-resistant MDD even if SSRIs exerted a positive effect on average.

In a follow-up open-label pilot trial enrolling treatment-naïve MDD patients, the *L. helveticus* Rosell®-52 and *B. longum* Rosell®-175 combination showed a positive effect on depression-related outcomes over 8 weeks (mood: MADRS, QIDS-SR16, anhedonia: Snaith Hamilton Pleasure Scale, anxiety: Generalized Anxiety Disorder 7-item scale, State-Trait Anxiety Inventory; subjective sleep quality: Pittsburgh Sleep Quality Index), in the absence of concomitant antidepressant treatment. This raises the possibility that this formulation could be appropriate non-solely as an adjuvant in treatment-responsive MDD patients, but also on its own to alleviate depression in its early stages. However, this promising pilot trial requires further confirmation in a randomized controlled trial.(Wallace et al., 2020) The *L. helveticus* Rosell®-52 and *B. longum* Rosell®-175 psychobiotic formulation also alleviated stress-induced intestinal disturbances,(Diop et al., 2008) as well as symptoms of depression in healthy individuals experiencing stress using the Hospital anxiety and depression scale (HADS), and the global severity index of the Hopkins symptoms checklist (HSCL-90), which was associated with a decrease in free urinary cortisol levels in the supplemented group.(Messaoudi et al., 2011a) Furthermore, a subgroup analysis in participants with low cortisol levels at baseline revealed a significant improvement of psychological distress measures on the HADS-Depression, HSCL-90 and coping checklist.(Messaoudi et al., 2011b)

Pretreatment with the *L. helveticus* Rosell®-52 and *B. longum* Rosell®-175 psychobiotic formulation before surgically-induced myocardial infarction (MI) in rats prevented the typical post-MI depressive-like behavioral impairments in the social interaction, forced swimming and passive avoidance step-down tests compared to the sham controls and restored intestinal barrier integrity.(Arseneault-Bréard et al., 2012) This psychobiotic also reduced MI-induced apoptosis in the amygdala and dentate gyrus as assessed by the Bax/Bcl-2 ratio and caspase-3 levels compared with placebo,(Girard et al., 2009) and these beneficial effects were abolished by vagotomy.(Malick et al., 2015) The positive behavioral effects of the formulation were also observed when the psychobiotic was administered at the time of reperfusion in rats fed a low polyunsaturated fatty acids diet. In this model, the *L. helveticus* Rosell®-52 and *B. longum* Rosell®-175 psychobiotic formulation reduced the expression of the apoptosis marker caspase-3 and decreased the number of TUNEL-positive cells in the dentate gyrus and medial amygdala.(Gilbert et al., 2013) In mice subjected to chronic psychological stress by WAS, the same psychobiotic formulation prevented the negative effect of WAS on the stress response, hippocampal neurogenesis and hypothalamic synaptic plasticity-related gene expression changes. The *L. helveticus* Rosell®-52 and *B. longum* Rosell®-175 psychobiotic formulation attenuated HPA axis and autonomic nervous system activities as shown by the reduced corticosterone and norepinephrine levels, and reduced c-Fos and upregulated the glucocorticoid receptor and BDNF mRNA levels in different areas of the brain. Moreover, the formulation also reduced visceral pain and restored intestinal tight junction barrier integrity in WAS-subjected mice.(Ait-Belgnaoui et al., 2014; Ait-Belgnaoui et al., 2018) In rats, the *L. helveticus* Rosell®-52 and *B. longum* Rosell®-175 psychobiotic formulation also displayed anxiolytic-like activity assessed by the conditioned defensive burying test,(Messaoudi et al., 2011a) and counteracted the negative effect of LPS on neuroinflammatory and memory processes.(Mohammadi et al., 2019) Furthermore, in rats of the Flinders Sensitive Line, the psychobiotic formulation was found to regulate 1-Carbon and catecholamine metabolisms without affecting behavior in this genetic model depression. Specifically, the formulation reduced the flow of methyl groups via betaine, increased liver S-adenosyl-methionine, and decreased plasma dopamine and norepinephrine levels. Of note, dopamine and norepinephrine were unchanged in the brain suggesting that the uptake of these catecholamines or the extraneuronal pathways of metabolism were modified.(Tillmann et al., 2018)

Akkasheh et al. (2016), using another multi-strain formulation containing *Lactobacillus (L.) acidophilus*, *Lacticaseibacillus (L.) casei*, and *B. bifidum*, showed that an 8-week intervention in clinically diagnosed MDD patients (DSM-IV and ≥ 15 on HAMD-17, referred from Hospital) significantly reduced BDI total score compared with the placebo.(Akkasheh et al., 2016) The treated group showed a significant decrease in serum insulin levels (−2.3 ± 4.1 vs. 2.6 ± 9.3 μIU/mL, $P = 0.03$), homeostasis model assessment of insulin resistance (−0.6 ± 1.2 vs. 0.6 ± 2.1, $P = 0.03$), and serum c-reactive protein concentrations (−1138.7 ± 2274.9 vs. 188.4 ± 1455.5 ng/mL, $P = 0.03$), as well as a significant increase in plasma total glutathione levels (1.8 ± 83.1 vs. -106.8 ± 190.7 mmol/L, $P = 0.02$). Ghorbani et al. (2018) reported a significant improvement in HAMD-17 scores in MDD patients who consumed a formulation containing 7 strains (*L. casei*, *L. acidophilus, L. bulgaricus*, *L. rhamnosus*, *B. breve*, *B. longum* and *Streptococcus* (*S.*) *thermophilus*) for 8 weeks compared to placebo.(Ghorbani et al., 2018) A similar formulation of these 7 strains at slightly different dosage and provided along with yogurt containing *L. bulgaricus* and *S. thermophilus* for 6 weeks produced a significant improvement in DASS scores (18.9 ± 3.2 vs. 9.4 ± 4.0, $P = 0.006$) in healthy petrochemical workers. In this population known to experience high levels of stress, the 7-strain formulation also improved General Health Questionnaire scores (measuring somatic symptoms, anxiety and insomnia, social dysfunction, and severe depression), but did not affect outcome measures of HPA axis activity (i.e. KYN, tryptophan, neuropeptide Y, cortisol, or ACTH levels).(Mohammadi et al., 2016)

4. Discussion

Overall, the positive effects of some psychobiotics on symptoms of depression or anxiety appear consistent between human and animal studies. Importantly, while systematic reviews are usually aiming at a general overview of psychobiotics as a whole, there may be strain-specific effects; that is, not all psychobiotics are expected to share the same modes of action or result in significant outcomes using different assessment tools, which suggests that subtypes of patients may show different responses to different formulations.(Vaghef-Mehrabany et al., 2020) Considering that depression severity assessment tools were shown to vary significantly in their psychometric properties, with some tools focusing largely on specific types of symptoms and overlooking others (i.e. emotional, physical, behavioral, or cognitive domains),(Fried, 2020; Newson et al., 2020) the choice of assessment tool(s) in clinical trials should be evaluated carefully, taking into account the spectrum of depression symptoms, trial setting (inpatient or outpatient), participants' limitations towards self-assessments (i.e. open-label design or participant's age or literacy), and overall burden for participants or assessors.

As research on the classification of NPD patients' subtypes is evolving rapidly, as well as research on the characterization of specific changes in the microbiome associated with specific NPDs, there is much left to discover on the mechanisms by which psychobiotics act. This is exemplified by the mixed results between healthy or non-clinically diagnosed individuals experiencing depressive symptoms, with or without anxiety, when comparing all psychobiotics together. Nevertheless, some formulations or strains that showed beneficial effects on depressive symptoms in clinically diagnosed MDD patients have also shown positive effects on depression- and anxiety-like symptoms or low mood in non-depressed individuals. This suggests that while some psychobiotics show efficacy as treatment once the symptoms are established, the antidepressant- and anxiolytic-like effects observed in healthy individuals experiencing stressful events or situations may reflect a protective effect against stress before the establishment of a depressive state.

Stress and adversity being directly linked with the incidence of NPDs in vulnerable individuals, especially for those who experienced stress and adversity during early life, the potential preventive role of psychobiotics warrants further studies. While no clinical trials have yet examined the preventive effect of an early-life psychobiotic intervention on the development of depression in humans, cognitive outcomes were assessed in two studies where psychobiotics were provided to mothers of children at-risk of allergic diseases, with conflicting results. One pilot study showed a reduction in the number of Asperger's disease and attention deficit hyperactivity disorder cases at age 13 in a cohort who took *L. rhamnosus* GG or placebo during the first 6 months of life.(Partty et al., 2015) Another study with a similar design but including more participants, who were also children at risk of allergic disease, did not show any benefit on neurocognitive outcomes assessed at 11 years old following supplementation with *L. rhamnosus* HN001 or *B. animalis* subsp. *lactis* HN019 supplementation from 35 weeks gestational age until 2 years old.(Slykerman et al., 2018) Despite the obvious gaps in knowledge on the potential protective role of psychobiotics on the incidence of NPDs from human trials, the extensive research conducted in animal models of ELS appears to support this notion. As ELS was also associated with the response to antidepressants in MDD patients,(Williams et al., 2016) future studies on psychobiotics could include outcome assessment tools measuring exposure to ELS or childhood adversity (e. g. CTQ) to gather insight into the complex interplay between psychobiotics, the MGBA, ELS and MDD.

5. Conclusions

In summary, the effects of psychobiotics on brain biochemistry and behavior appear to be mediated in part by the regulation of the HPA axis activity and by the vagus nerve, but also possibly by the maintenance of the intestinal barrier integrity, the regulation of the inflammatory status, or the modulation of epigenetic mechanisms via a yet unknown mechanism, possibly involving gender-related differences.(Codagnone et al., 2019a) In addition, the possible effects of psychobiotics on microglia activation warrant further studies in the context of MS; microglia is activated during MS in rodents,(Roque et al., 2016) and a recent study showed that *Bifidobacteria* strains could normalize microglia reactivity in germ-free mice.(Luck et al., 2020) In parallel, psychosocial studies on the effect of ELS on NPDs have yielded conflicting results regarding the changes in the activation or inhibition of the HPA axis and stress responsiveness in different pathologies, as well as depending on the type of ELS experienced (i.e. physical abuse or emotional neglect).(Syed and Nemeroff, 2017) The high number of factors, either known or yet unknown, influencing the interplay between the microbiome and mental health trajectories during early-life,(Cowan et al., 2020) and especially after ELS in humans,(Agorastos et al., 2019; Codagnone et al., 2019b; Provensi et al., 2019) present countless challenges in the context of an interventional clinical trial. However, insights from animal models suggest that psychobiotics may have the potential to modulate the mental health trajectory post-ELS in some individuals, perhaps as part of a comprehensive strategy including nutritional and psychosocial support. Future studies will help unravel potential novel mechanisms and contribute to our understanding of the interactions between psychobiotics and the MGBA. In terms of clinical trials, several elements of trial design should be considered in the future, and perhaps more importantly the heterogeneity in outcome measures used to assess symptom severity or effect of treatment, with the aim of evaluating several domains across the depression symptomatology by collecting results from multiple questionnaires. Importantly, one should consider inclusion/exclusion criteria involving a medical assessment of depression severity (rather than self-reported or subclinical symptoms), an evaluation of treatment history and responsiveness, as well as the addition of outcomes with potential mechanistic value, such as an evaluation of ELS or childhood trauma, and microbiome profiling. Overall, on the basis of the positive effect on disease outcomes seen in MDD patients with several formulations and via an heterogenous array of assessment tools, psychobiotics hold the promise of benefitting both individuals at-risk or diagnosed with neuropsychiatric or neurodegenerative disorders sharing similar

pathophysiological mechanisms,(Kelly et al., 2020; Long-Smith et al., 2020; Misiak et al., 2020) including, among others, HPA axis or vagus nerve dysfunctions, epigenetics, intestinal barrier permeability, sub-clinical inflammation, or microglia activation.

Declaration of Competing Interest

A.T., L.L., M.M., B.F., and T.A.T. are employed by Lallemand Health Solutions Inc., a company that researches, manufactures, markets and sells probiotics to business clients but not to consumers.

References

Agorastos, A., Pervanidou, P., Chrousos, G.P., Baker, D.G., 2019. Developmental trajectories of early life stress and trauma: a narrative review on neurobiological aspects beyond stress system dysregulation. Front. Psychiatry 10, 118.

Ait-Belgnaoui, A., Colom, A., Braniste, V., Ramalho, L., Marrot, A., Cartier, C., Houdeau, E., Theodorou, V., Tompkins, T., 2014. Probiotic gut effect prevents the chronic psychological stress-induced brain activity abnormality in mice. Neurogastroenterol. Motil. 26 (4), 510–520.

Ait-Belgnaoui, A., Payard, I., Rolland, C., Harkat, C., Braniste, V., Théodorou, V., Tompkins, T.A., 2018. Bifidobacterium longum and Lactobacillus helveticus synergistically suppress stress-related visceral hypersensitivity through hypothalamic-pituitary-adrenal Axis modulation. J. Neurogastroenterol. Motil. 24 (1), 138–146.

Akkasheh, G., Kashani-Poor, Z., Tajabadi-Ebrahimi, M., Jafari, P., Akbari, H., Taghizadeh, M., Memarzadeh, M.R., Asemi, Z., Esmaillzadeh, A., 2016. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: a randomized, double-blind, placebo-controlled trial. Nutrition 32 (3), 315–320.

Amirani, E., Milajerdi, A., Mirzaei, H., Jamilian, H., Mansournia, M.A., Hallajzadeh, J., Ghaderi, A., 2020. The effects of probiotic supplementation on mental health, biomarkers of inflammation and oxidative stress in patients with psychiatric disorders: a systematic review and meta-analysis of randomized controlled trials. Complement. Ther. Med. 49, 102361.

Andersson, H., Tullberg, C., Ahrné, S., Hamberg, K., Lazou Ahrén, I., Molin, G., Sonesson, M., Håkansson, Å., 2016. Oral administration of *Lactobacillus plantarum* 299v reduces cortisol levels in human saliva during examination induced stress: a randomized, double-blind controlled trial. Int. J. Microbiol. 2016, 8469018.

Arseneault-Bréard, J., Rondeau, I., Gilbert, K., Girard, S.A., Tompkins, T.A., Godbout, R., Rousseau, G., 2012. Combination of Lactobacillus helveticus R0052 and Bifidobacterium longum R0175 reduces post-myocardial infarction depression symptoms and restores intestinal permeability in a rat model. Br. J. Nutr. 107 (12), 1793–1799.

Bähr, K.H., 1970. Observations of the behavior of gnotobiotic piglets. Dtsch. Tierarztl. Wochenschr. 77 (6), 138–140.

Bailey, M.T., Coe, C.L., 1999. Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. Dev. Psychobiol. 35 (2), 146–155.

Bailey, M.T., Lubach, G.R., Coe, C.L., 2004. Prenatal stress alters bacterial colonization of the gut in infant monkeys. J. Pediatr. Gastroenterol. Nutr. 38 (4), 414–421.

Bambling, M., Edwards, S.C., Hall, S., Vitetta, L., 2017. A combination of probiotics and magnesium orotate attenuate depression in a small SSRI resistant cohort: an intestinal anti-inflammatory response is suggested. Inflammopharmacology 25 (2), 271–274.

Barbosa, R.S.D., Vieira-Coelho, M.A., 2020. Probiotics and prebiotics: focus on psychiatric disorders - a systematic review. Nutr. Rev. 78 (6), 437–450.

Bastiaanssen, T.F.S., Cussotto, S., Claesson, M.J., Clarke, G., Dinan, T.G., Cryan, J.F., 2020. Gutted! Unraveling the role of the microbiome in major depressive disorder. Harv. Rev. Psychiatry 28 (1), 26–39.

Baumeister, D., Akhtar, R., Ciufolini, S., Pariante, C.M., Mondelli, V., 2016. Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-α. Mol. Psychiatry 21 (5), 642–649.

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, P., Macri, J., McCoy, K.D., Verdu, E.F., Collins, S.M., 2011. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology 141 (2), 599–609, 609.e591-593.

Brenner, L.A., Stearns-Yoder, K.A., Hoffberg, A.S., Penzenik, M.E., Starosta, A.J., Hernández, T.D., Hadidi, D.A., Lowry, C.A., 2017. Growing literature but limited evidence: a systematic review regarding prebiotic and probiotic interventions for those with traumatic brain injury and/or posttraumatic stress disorder. Brain Behav. Immun. 65, 57–67.

Callaghan, B.L., Cowan, C.S., Richardson, R., 2016. Treating generational stress: effect of paternal stress on development of memory and extinction in offspring is reversed by probiotic treatment. Psychol. Sci. 27 (9), 1171–1180.

Caspani, G., Kennedy, S., Foster, J.A., Swann, J., 2019. Gut microbial metabolites in depression: understanding the biochemical mechanisms. Microb. Cell 6 (10), 454–481.

Chao, L., Liu, C., Sutthawongwadee, S., Li, Y., Lv, W., Chen, W., Yu, L., Zhou, J., Guo, A., Li, Z., Guo, S., 2020. Effects of probiotics on depressive or anxiety variables in healthy participants under stress conditions or with a depressive or anxiety diagnosis: a meta-analysis of randomized controlled trials. Front. Neurol. 11, 421.

Codagnone, M.G., Spichak, S., O'Mahony, S.M., O'Leary, O.F., Clarke, G., Stanton, C., Dinan, T.G., Cryan, J.F., 2019a. Programming bugs: microbiota and the developmental origins of brain health and disease. Biol. Psychiatry 85 (2), 150–163.

Codagnone, M.G., Stanton, C., O'Mahony, S.M., Dinan, T.G., Cryan, J.F., 2019b. Microbiota and neurodevelopmental trajectories: role of maternal and early-life nutrition. Ann. Nutr. Metab. 74 (Suppl. 2), 16–27 (2).

Cowan, C.S.M., Richardson, R., 2019. Early-life stress leads to sex-dependent changes in pubertal timing in rats that are reversed by a probiotic formulation. Dev. Psychobiol. 61 (5), 679–687.

Cowan, C.S., Callaghan, B.L., Richardson, R., 2016. The effects of a probiotic formulation (*Lactobacillus rhamnosus* and L. helveticus) on developmental trajectories of emotional learning in stressed infant rats. Transl. Psychiatry 6 (5), e823.

Cowan, C.S.M., Stylianakis, A.A., Richardson, R., 2019. Early-life stress, microbiota, and brain development: probiotics reverse the effects of maternal separation on neural circuits underpinning fear expression and extinction in infant rats. Dev. Cogn. Neurosci. 37, 100627.

Cowan, C.S.M., Dinan, T.G., Cryan, J.F., 2020. Annual research review: critical windows - the microbiota-gut-brain axis in neurocognitive development. J. Child Psychol. Psychiatry 61 (3), 353–371.

Cryan, J.F., Dinan, T.G., 2019. Talking about a microbiome revolution. Nat. Microbiol. 4 (4), 552–553.

De Palma, G., Blennerhassett, P., Lu, J., Deng, Y., Park, A., Green, W., Denou, E., Silva, M., Santacruz, A., Sanz, Y., 2015. Microbiota and host determinants of behavioural phenotype in maternally separated mice. Nat. Commun. 6 (1), 1–13.

De Palma, G., Lynch, M.D., Lu, J., Dang, V.T., Deng, Y., Jury, J., Umeh, G., Miranda, P. M., Pigrau Pastor, M., Sidani, S., Pinto-Sanchez, M.I., Philip, V., McLean, P.G., Hagelsieb, M.G., Surette, M.G., Bergonzelli, G.E., Verdu, E.F., Britz-McKibbin, P., Neufeld, J.D., Collins, S.M., Bercik, P., 2017. Transplantation of fecal microbiota from patients with irritable bowel syndrome alters gut function and behavior in recipient mice. Sci. Transl. Med. 9 (379).

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J.F., Dinan, T.G., 2010. Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. Neuroscience 170 (4), 1179–1188.

Diaz Heijtz, R., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., Hibberd, M. L., Forssberg, H., Pettersson, S., 2011. Normal gut microbiota modulates brain development and behavior. Proc. Natl. Acad. Sci. U. S. A. 108 (7), 3047–3052.

Dinan, T.G., Stanton, C., Cryan, J.F., 2013. Psychobiotics: a novel class of psychotropic. Biol. Psychiatry 74 (10), 720–726.

Diop, L., Guillou, S., Durand, H., 2008. Probiotic food supplement reduces stress-induced gastrointestinal symptoms in volunteers: a double-blind, placebo-controlled, randomized trial. Nutr. Res. 28 (1), 1–5.

Ducrotté, P., Sawant, P., Jayanthi, V., 2012. Clinical trial: Lactobacillus plantarum 299v (DSM 9843) improves symptoms of irritable bowel syndrome. World J. Gastroenterol. 18 (30), 4012–4018.

Erny, D., Hrabě de Angelis, A.L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., Keren-Shaul, H., Mahlakoiv, T., Jakobshagen, K., Buch, T., Schwierzeck, V., Utermöhlen, O., Chun, E., Garrett, W.S., McCoy, K.D., Diefenbach, A., Staeheli, P., Stecher, B., Amit, I., Prinz, M., 2015. Host microbiota constantly control maturation and function of microglia in the CNS. Nat. Neurosci. 18 (7), 965–977.

Farzi, A., Fröhlich, E.E., Holzer, P., 2018. Gut microbiota and the neuroendocrine system. Neurotherapeutics 15 (1), 5–22.

Flux, M.C., Lowry, C.A., 2020. Finding intestinal fortitude: integrating the microbiome into a holistic view of depression mechanisms, treatment, and resilience. Neurobiol. Dis. 135, 104578.

Fried, E.I., 2020. Corrigendum to "The 52 symptoms of major depression: lack of content overlap among seven common depression scales", [Journal of Affective Disorders, 208, 191-197]. J. Affect. Disord. 260, 744.

Fukui, H., Oshima, T., Tanaka, Y., Oikawa, Y., Makizaki, Y., Ohno, H., Tomita, T., Watari, J., Miwa, H., 2018. Effect of probiotic Bifidobacterium bifidum G9-1 on the relationship between gut microbiota profile and stress sensitivity in maternally separated rats. Sci. Rep. 8 (1), 12384.

Fusar-Poli, L., Surace, T., Vanella, A., Meo, V., Patania, F., Furnari, R., Signorelli, M.S., Aguglia, E., 2019. The effect of adjunctive nutraceuticals in bipolar disorder: a systematic review of randomized placebo-controlled trials. J. Affect. Disord. 252, 334–349.

Gareau, M.G., Jury, J., MacQueen, G., Sherman, P.M., Perdue, M.H., 2007. Probiotic treatment of rat pups normalises corticosterone release and ameliorates colonic dysfunction induced by maternal separation. Gut 56 (11), 1522–1528.

Ghorbani, Z., Nazari, S., Etesam, F., Nourimajd, S., Ahmadpanah, M., Razeghi, S., 2018. The effect of synbiotic as an adjuvant therapy to fluoxetine in moderate depression: a randomized multicenter trial. Arch. Neurosci. 5 (e60507).

Gilbert, K., Arseneault-Bréard, J., Flores Monaco, F., Beaudoin, A., Bah, T.M., Tompkins, T.A., Godbout, R., Rousseau, G., 2013. Attenuation of post-myocardial infarction depression in rats by n-3 fatty acids or probiotics starting after the onset of reperfusion. Br. J. Nutr. 109 (1), 50–56.

Girard, S.A., Bah, T.M., Kaloustian, S., Lada-Moldovan, L., Rondeau, I., Tompkins, T.A., Godbout, R., Rousseau, G., 2009. Lactobacillus helveticus and Bifidobacterium longum taken in combination reduce the apoptosis propensity in the limbic system after myocardial infarction in a rat model. Br. J. Nutr. 102 (10), 1420–1425.

Goh, K.K., Liu, Y.W., Kuo, P.H., Chung, Y.E., Lu, M.L., Chen, C.H., 2019. Effect of probiotics on depressive symptoms: a meta-analysis of human studies. Psychiatry Res. 282, 112568.

Hagihara, M., Yamashita, R., Matsumoto, A., Mori, T., Kuroki, Y., Kudo, H., Oka, K., Takahashi, M., Nonogaki, T., Yamagishi, Y., Mikamo, H., 2018. The impact of Clostridium butyricum MIYAIRI 588 on the murine gut microbiome and colonic tissue. Anaerobe 54, 8–18.

Heidazardeh-Rad, N., Gokmen-Ozel, H., Kazemi, A., Almasi, N., Djafarian, K., 2020. Effects of a psychobiotic supplement on serum brain derived neurotrophic factor levels in depressive patients: a post-hoc analysis of a randomizaed clinical trial. J. Neurogastroenterol. Motil. **26** (October 2020) (In Press).

Heim, C.M., Entringer, S., Buss, C., 2019. Translating basic research knowledge on the biological embedding of early-life stress into novel approaches for the developmental programming of lifelong health. Psychoneuroendocrinology 105, 123–137.

Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S., Calder, P.C., Sanders, M.E., 2014. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol. 11 (8), 506–514.

Huang, R., Wang, K., Hu, J., 2016. Effect of probiotics on depression: a systematic review and meta-analysis of randomized controlled trials. Nutrients 8 (8).

Huang, Y., Shi, X., Li, Z., Shen, Y., Shi, X., Wang, L., Li, G., Yuan, Y., Wang, J., Zhang, Y., Zhao, L., Zhang, M., Kang, Y., Liang, Y., 2018. Possible association of Firmicutes in the gut microbiota of patients with major depressive disorder. Neuropsychiatr. Dis. Treat. 14, 3329–3337.

Hulst, M., Gross, G., Liu, Y., Hoekman, A., Niewold, T., van der Meulen, J., Smits, M., 2015. Oral administration of Lactobacillus plantarum 299v modulates gene expression in the ileum of pigs: prediction of crosstalk between intestinal immune cells and sub-mucosal adipocytes. Genes Nutr. 10 (3), 10.

Iannone, L.F., Preda, A., Blottière, H.M., Clarke, G., Albani, D., Belcastro, V., Carotenuto, M., Cattaneo, A., Citraro, R., Ferraris, C., Ronchi, F., Luongo, G., Santocchi, E., Guiducci, L., Baldelli, P., Iannetti, P., Pedersen, S., Petretto, A., Provasi, S., Selmer, K., Spalice, A., Tagliabue, A., Verrotti, A., Segata, N., Zimmermann, J., Minetti, C., Mainardi, P., Giordano, C., Sisodiya, S., Zara, F., Russo, E., Striano, P., 2019. Microbiota-gut brain axis involvement in neuropsychiatric disorders. Expert. Rev. Neurother. 19 (10), 1037–1050.

Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., Wang, W., Tang, W., Tan, Z., Shi, J., Li, L., Ruan, B., 2015. Altered fecal microbiota composition in patients with major depressive disorder. Brain Behav. Immun. 48, 186–194.

Jurek, L., Sevil, M., Jay, A., Schröder, C., Baghdadli, A., Héry-Arnaud, G., Geoffray, M. M., 2020 May 8. Is there a dysbiosis in individuals with a neurodevelopmental disorder compared to controls over the course of development? A systematic review. Eur. Child Adolesc. Psychiatry. https://doi.org/10.1007/s00787-020-01544-1.

Karakula-Juchnowicz, H., Rog, J., Juchnowicz, D., Łoniewski, I., Skonieczna-Żydecka, K., Krukow, P., Futyma-Jedrzejewska, M., Kaczmarczyk, M., 2019. The study evaluating the effect of probiotic supplementation on the mental status, inflammation, and intestinal barrier in major depressive disorder patients using gluten-free or gluten-containing diet (SANGUT study): a 12-week, randomized, double-blind, and placebo-controlled clinical study protocol. Nutr. J. 18 (1), 50.

Kato, M., Hamazaki, Y., Sun, S., Nishikawa, Y., Kage-Nakadai, E., 2018. Clostridium butyricum MIYAIRI 588 increases the lifespan and multiple-stress resistance of *Caenorhabditis elegans*. Nutrients 10 (12).

Kazemi, A., Noorbala, A.A., Azam, K., Djafarian, K., 2019a. Effect of probiotic and probiotic supplementation on circulating pro-inflammatory cytokines and urinary cortisol levels in patients with major depressive disorder: a double-blind, placebo-controlled randomized clinical trial. J. Funct. Foods 52, 596–602.

Kazemi, A., Noorbala, A.A., Azam, K., Eskandari, M.H., Djafarian, K., 2019b. Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: a randomized clinical trial. Clin. Nutr. 38 (2), 522–528.

Kazemi, A., Noorbala, A.A., Djafarian, K., 2020. Effect of probiotic and prebiotic versus placebo on appetite in patients with major depressive disorder: post hoc analysis of a randomised clinical trial. J. Hum. Nutr. Diet. 33 (1), 56–65.

Kelly, J.R., Borre, Y., Patterson, O.B.C.E., El Aidy, S., Deane, J., Kennedy, P.J., Beers, S., Scott, K., Moloney, G., Hoban, A.E., Scott, L., Fitzgerald, P., Ross, P., Stanton, C., Clarke, G., Cryan, J.F., Dinan, T.G., 2016. Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. J. Psychiatr. Res. 82, 109–118.

Kelly, J.R., Minuto, C., Cryan, J.F., Clarke, G., Dinan, T.G., 2020 Apr 23. The role of the gut microbiome in the development of schizophrenia. Schizophr. Res. https://doi.org/10.1016/j.schres.2020.02.010.

Kessler, R.C., McLaughlin, K.A., Green, J.G., Gruber, M.J., Sampson, N.A., Zaslavsky, A. M., Aguilar-Gaxiola, S., Alhamzawi, A.O., Alonso, J., Angermeyer, M., Benjet, C., Bromet, E., Chatterji, S., de Girolamo, G., Demyttenaere, K., Fayyad, J., Florescu, S., Gal, G., Gureje, O., Haro, J.M., Hu, C.Y., Karam, E.G., Kawakami, N., Lee, S., Lépine, J.P., Ormel, J., Posada-Villa, J., Sagar, R., Tsang, A., Ustün, T.B., Vassilev, S., Viana, M.C., Williams, D.R., 2010. Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. Br. J. Psychiatry 197 (5), 378–385.

LeMoult, J., Humphreys, K.L., Tracy, A., Hoffmeister, J.-A., Ip, E., Gotlib, I.H., 2019. Meta-analysis: exposure to early life stress and risk for depression in childhood and adolescence. J. Am. Acad. Child Adolesc. Psychiatry 59 (7), 842–855.

Liao, J.F., Hsu, C.C., Chou, G.T., Hsu, J.S., Liong, M.T., Tsai, Y.C., 2019. Lactobacillus paracasei PS23 reduced early-life stress abnormalities in maternal separation mouse model. Benefic. Microbes 10 (4), 425–436.

Liu, Y.W., Liu, W.H., Wu, C.C., Juan, Y.C., Wu, Y.C., Tsai, H.P., Wang, S., Tsai, Y.C., 2016. Psychotropic effects of Lactobacillus plantarum PS128 in early life-stressed and naive adult mice. Brain Res. 1631, 1–12.

Liu, B., He, Y., Wang, M., Liu, J., Ju, Y., Zhang, Y., Liu, T., Li, L., Li, Q., 2018. Efficacy of probiotics on anxiety-a meta-analysis of randomized controlled trials. Depress. Anxiety 35 (10), 935–945.

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

Long-Smith, C., O'Riordan, K.J., Clarke, G., Stanton, C., Dinan, T.G., Cryan, J.F., 2020. Microbiota-gut-brain axis: new therapeutic opportunities. Annu. Rev. Pharmacol. Toxicol. 60, 477–502.

Luck, B., Engevik, M.A., Ganesh, B.P., Lackey, E.P., Lin, T., Balderas, M., Major, A., Runge, J., Luna, R.A., Sillitoe, R.V., Versalovic, J., 2020. Bifidobacteria shape host neural circuits during postnatal development by promoting synapse formation and microglial function. Sci. Rep. 10 (1), 7737.

Luczynski, P., McVey Neufeld, K.A., Oriach, C.S., Clarke, G., Dinan, T.G., Cryan, J.F., 2016. Growing up in a bubble: using germ-free animals to assess the influence of the gut microbiota on brain and behavior. Int. J. Neuropsychopharmacol. 19 (8).

Majcher-Maślanka, I., Solarz, A., Chocyk, A., 2019. Maternal separation disturbs postnatal development of the medial prefrontal cortex and affects the number of neurons and glial cells in adolescent rats. Neuroscience 423, 131–147.

Majeed, M., Nagabhushanam, K., Natarajan, S., Sivakumar, A., Ali, F., Pande, A., Majeed, S., Karri, S.K., 2016. Bacillus coagulans MTCC 5856 supplementation in the management of diarrhea predominant irritable bowel syndrome: a double blind randomized placebo controlled pilot clinical study. Nutr. J. 15, 21.

Majeed, M., Nagabhushanam, K., Arumugam, S., Majeed, S., Ali, F., 2018. Bacillus coagulans MTCC 5856 for the management of major depression with irritable bowel syndrome: a randomised, double-blind, placebo controlled, multi-centre, pilot clinical study. Food Nutr. Res. 62.

Malick, M., Gilbert, K., Daniel, J., Arseneault-Breard, J., Tompkins, T.A., Godbout, R., Rousseau, G., 2015. Vagotomy prevents the effect of probiotics on caspase activity in a model of postmyocardial infarction depression. Neurogastroenterol. Motil. 27 (5), 663–671.

Mandelli, L., Petrelli, C., Serretti, A., 2015. The role of specific early trauma in adult depression: a meta-analysis of published literature. Childhood trauma and adult depression. Eur. Psychiatry 30 (6), 665–680.

Mangell, P., Nejdfors, P., Wang, M., Ahrné, S., Weström, B., Thorlacius, H., Jeppsson, B., 2002. *Lactobacillus plantarum* 299v inhibits Escherichia coli-induced intestinal permeability. Dig. Dis. Sci. 47 (3), 511–516.

Mangell, P., Lennernäs, P., Wang, M., Olsson, C., Ahrné, S., Molin, G., Thorlacius, H., Jeppsson, B., 2006. Adhesive capability of *Lactobacillus plantarum* 299v is important for preventing bacterial translocation in endotoxemic rats. Apmis 114 (9), 611–618.

McKean, J., Naug, H., Nikbakht, E., Amiet, B., Colson, N., 2017. Probiotics and subclinical psychological symptoms in healthy participants: a systematic review and meta-analysis. J. Altern. Complement. Med. 23 (4), 249–258.

McVey Neufeld, K.A., O'Mahony, S.M., Hoban, A.E., Waworuntu, R.V., Berg, B.M., Dinan, T.G., Cryan, J.F., 2019. Neurobehavioural effects of *Lactobacillus rhamnosus* GG alone and in combination with prebiotics polydextrose and galactooligosaccharide in male rats exposed to early-life stress. Nutr. Neurosci. 22 (6), 425–434.

Merrick, M.T., Ford, D.C., Haegerich, T.M., Simon, T., 2020. Adverse childhood experiences increase risk for prescription opioid misuse. J. Prim. Prev. 41 (2), 139–152.

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., Bisson, J.F., Rougeot, C., Pichelin, M., Cazaubiel, M., Cazaubiel, J.M., 2011a. Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. Br. J. Nutr. 105 (5), 755–764.

Messaoudi, M., Violle, N., Bisson, J.F., Desor, D., Javelot, H., Rougeot, C., 2011b. Beneficial psychological effects of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in healthy human volunteers. Gut Microbes 2 (4), 256–261.

Misiak, B., Łoniewski, I., Marlicz, W., Frydecka, D., Szulc, A., Rudzki, L., Samochowiec, J., 2020. The HPA axis dysregulation in severe mental illness: can we shift the blame to gut microbiota? Prog. Neuro-Psychopharmacol. Biol. Psychiatry 102, 109951.

Miyaoka, T., Kanayama, M., Wake, R., Hashioka, S., Hayashida, M., Nagahama, M., Okazaki, S., Yamashita, S., Miura, S., Miki, H., Matsuda, H., Koike, M., Izuhara, M., Araki, T., Tsuchie, K., Azis, I.A., Arauchi, R., Abdullah, R.A., Oh-Nishi, A., Horiguchi, J., 2018. Clostridium butyricum MIYAIRI 588 as adjunctive therapy for treatment-resistant major depressive disorder: a prospective open-label trial. Clin. Neuropharmacol. 41 (5), 151–155.

Mohajeri, M.H., La Fata, G., Steinert, R.E., Weber, P., 2018. Relationship between the gut microbiome and brain function. Nutr. Rev. 76 (7), 481–496.

Mohammadi, A.A., Jazayeri, S., Khosravi-Darani, K., Solati, Z., Mohammadpour, N., Asemi, Z., Adab, Z., Djalali, M., Tehrani-Doost, M., Hosseini, M., Eghtesadi, S., 2016. The effects of probiotics on mental health and hypothalamic-pituitary-adrenal axis: a randomized, double-blind, placebo-controlled trial in petrochemical workers. Nutr. Neurosci. 19 (9), 387–395.

Mohammadi, G., Dargahi, L., Naserpour, T., Mirzanejad, Y., Alizadeh, S.A., Peymani, A., Nassiri-Asl, M., 2019. Probiotic mixture of Lactobacillus helveticus R0052 and Bifidobacterium longum R0175 attenuates hippocampal apoptosis induced by lipopolysaccharide in rats. Int. Microbiol. 22 (3), 317–323.

Moya-Perez, A., Perez-Villalba, A., Benitez-Paez, A., Campillo, I., Sanz, Y., 2017. Bifidobacterium CECT 7765 modulates early stress-induced immune, neuroendocrine and behavioral alterations in mice. Brain Behav. Immun. 65, 43–56.

Murayama, T., Mita, N., Tanaka, M., Kitajo, T., Asano, T., Mizuochi, K., Kaneko, K., 1995. Effects of orally administered Clostridium butyricum MIYAIRI 588 on mucosal immunity in mice. Vet. Immunol. Immunopathol. 48 (3–4), 333–342.

Naseribafrouei, A., Hestad, K., Avershina, E., Sekelja, M., Linløkken, A., Wilson, R., Rudi, K., 2014. Correlation between the human fecal microbiota and depression. Neurogastroenterol. Motil. 26 (8), 1155–1162.

Neufeld, K.M., Kang, N., Bienenstock, J., Foster, J.A., 2011. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterol. Motil. 23 (3), 255–264 (e119).
Newson, J.J., Hunter, D., Thiagarajan, T.C., 2020. The heterogeneity of mental health assessment. Front. Psychiatry 11 (76).
Ng, Q.X., Peters, C., Ho, C.Y.X., Lim, D.Y., Yeo, W.S., 2018. A meta-analysis of the use of probiotics to alleviate depressive symptoms. J. Affect. Disord. 228, 13–19.
Ng, Q.X., Loke, W., Venkatanarayanan, N., Lim, D.Y., Soh, A.Y.S., Yeo, W.S., 2019a. A systematic review of the role of prebiotics and probiotics in autism spectrum disorders. Medicina (Kaunas) 55 (5).
Ng, Q.X., Soh, A.Y.S., Venkatanarayanan, N., Ho, C.Y.X., Lim, D.Y., Yeo, W.S., 2019b. A systematic review of the effect of probiotic supplementation on schizophrenia symptoms. Neuropsychobiology 78 (1), 1–6.
Nikolova, V., Zaidi, S.Y., Young, A.H., Cleare, A.J., Stone, J.M., 2019. Gut feeling: randomized controlled trials of probiotics for the treatment of clinical depression: systematic review and meta-analysis. Ther. Adv. Psychopharmacol. 9, 2045125319859963.
O'Mahony, S., Hyland, N., Dinan, T., Cryan, J., 2011. Maternal separation as a model of brain–gut axis dysfunction. Psychopharmacology $V 214 (1), 71–88.
Partty, A., Kalliomaki, M., Wacklin, P., Salminen, S., Isolauri, E., 2015. A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. Pediatr. Res. 77 (6), 823–828.
Pena Cortes, L.C., LeVeque, R.M., Funk, J.A., Marsh, T.L., Mulks, M.H., 2018. Development of the tonsil microbiome in pigs and effects of stress on the microbiome. Front. Vet. Sci. 5, 220.
Peng, H.H., Tsai, T.C., Huang, W.Y., Wu, H.M., Hsu, K.S., 2019. Probiotic treatment restores normal developmental trajectories of fear memory retention in maternally separated infant rats. Neuropharmacology 153, 53–62.
Pinto-Sanchez, M.I., Hall, G.B., Ghajar, K., Nardelli, A., Bolino, C., Lau, J.T., Martin, F.P., Cominetti, O., Welsh, C., Rieder, A., Traynor, J., Gregory, C., De Palma, G., Pigrau, M., Ford, A.C., Macri, J., Berger, B., Bergonzelli, G., Surette, M.G., Collins, S. M., Moayyedi, P., Bercik, P., 2017. Probiotic Bifidobacterium longum NCC3001 reduces depression scores and alters brain activity: a pilot study in patients with irritable bowel syndrome. Gastroenterology 153 (2), 448–459 e448.
Pirbaglou, M., Katz, J., de Souza, R.J., Stearns, J.C., Motamed, M., Ritvo, P., 2016. Probiotic supplementation can positively affect anxiety and depressive symptoms: a systematic review of randomized controlled trials. Nutr. Res. 36 (9), 889–898.
Provensi, G., Schmidt, S.D., Boehme, M., Bastiaanssen, T.F.S., Rani, B., Costa, A., Busca, K., Fouhy, F., Strain, C., Stanton, C., Blandina, P., Izquierdo, I., Cryan, J.F., Passani, M.B., 2019. Preventing adolescent stress-induced cognitive and microbiome changes by diet. Proc. Natl. Acad. Sci. 116 (19), 9644–9651.
Quinnies, K.M., Cox, K.H., Rissman, E.F., 2015. Immune deficiency influences juvenile social behavior and maternal behavior. Behav. Neurosci. 129 (3), 331–338.
Rao, A.V., Bested, A.C., Beaulne, T.M., Katzman, M.A., Iorio, C., Berardi, J.M., Logan, A. C., 2009. A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. Gut Pathog 1 (1), 6.
Rea, K., Dinan, T.G., Cryan, J.F., 2020. Gut microbiota: a perspective for psychiatrists. Neuropsychobiology 79 (1), 50–62.
Reis, D.J., Ilardi, S.S., Punt, S.E.W., 2018. The anxiolytic effect of probiotics: a systematic review and meta-analysis of the clinical and preclinical literature. PLoS One 13 (6), e0199041.
Romijn, A.R., Rucklidge, J.J., 2015. Systematic review of evidence to support the theory of psychobiotics. Nutr. Rev. 73 (10), 675–693.
Romijn, A.R., Rucklidge, J.J., Kuijer, R.G., Frampton, C., 2017. A double-blind, randomized, placebo-controlled trial of Lactobacillus helveticus and Bifidobacterium longum for the symptoms of depression. Aust N Z J Psychiatry 51 (8), 810–821. https://doi.org/10.1177/0004867416686694.
Roque, A., Ochoa-Zarzosa, A., Torner, L., 2016. Maternal separation activates microglial cells and induces an inflammatory response in the hippocampus of male rat pups, independently of hypothalamic and peripheral cytokine levels. Brain Behav. Immun. 55, 39–48.
Rudzki, L., Ostrowska, L., Pawlak, D., Małus, A., Pawlak, K., Waszkiewicz, N., Szulc, A., 2019. Probiotic Lactobacillus Plantarum 299v decreases kynurenine concentration and improves cognitive functions in patients with major depression: a double-blind, randomized, placebo controlled study. Psychoneuroendocrinology 100, 213–222.
Sanada, K., Nakajima, S., Kurokawa, S., Barceló-Soler, A., Ikuse, D., Hirata, A., Yoshizawa, A., Tomizawa, Y., Salas-Valero, M., Noda, Y., Mimura, M., Iwanami, A., Kishimoto, T., 2020. Gut microbiota and major depressive disorder: a systematic review and meta-analysis. J. Affect. Disord. 266, 1–13.
Sarkar, A., Lehto, S.M., Harty, S., Dinan, T.G., Cryan, J.F., Burnet, P.W.J., 2016. Psychobiotics and the manipulation of bacteria-gut-brain signals. Trends Neurosci. 39 (11), 763–781.
Schokker, D., Zhang, J., Zhang, L.L., Vastenhouw, S.A., Heilig, H.G., Smidt, H., Rebel, J. M., Smits, M.A., 2014. Early-life environmental variation affects intestinal microbiota and immune development in new-born piglets. PLoS One 9 (6), e100040.
Shinde, T., Perera, A.P., Vemuri, R., Gondalia, S.V., Beale, D.J., Karpe, A.V., Shastri, S., Basheer, W., Southam, B., Eri, R., Stanley, R., 2020 Feb 17. Synbiotic supplementation with prebiotic green banana resistant starch and probiotic *Bacillus coagulans* spores ameliorates gut inflammation in mouse model of inflammatory bowel diseases. Eur. J. Nutr. https://doi.org/10.1007/s00394-020-02200-9.
Simpson, C., Schwartz, O., Simmons, J., 2020. The human gut microbiota and depression: widely reviewed, yet poorly understood. J. Affect. Disord. 1 September 2020, 73–75.
Simrén, M., Ohman, L., Olsson, J., Svensson, U., Ohlson, K., Posserud, I., Strid, H., 2010. Clinical trial: the effects of a fermented milk containing three probiotic bacteria in patients with irritable bowel syndrome - a randomized, double-blind, controlled study. Aliment. Pharmacol. Ther. 31 (2), 218–227.
Slykerman, R.F., Kang, J., Van Zyl, N., Barthow, C., Wickens, K., Stanley, T., Coomarasamy, C., Purdie, G., Murphy, R., Crane, J., Mitchell, E.A., 2018. Effect of early probiotic supplementation on childhood cognition, behaviour and mood a randomised, placebo-controlled trial. Acta Paediatr. 107 (12), 2172–2178.
Smith, C.J., Emge, J.R., Berzins, K., Lung, L., Khamishon, R., Shah, P., Rodrigues, D.M., Sousa, A.J., Reardon, C., Sherman, P.M., Barrett, K.E., Gareau, M.G., 2014. Probiotics normalize the gut-brain-microbiota axis in immunodeficient mice. Am. J. Physiol. Gastrointest. Liver Physiol. 307 (8), G793–G802.
Smith, K.S., Greene, M.W., Babu, J.R., Frugé, A.D., 2019. Psychobiotics as treatment for anxiety, depression, and related symptoms: a systematic review. Nutr. Neurosci. 1–15.
Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.N., Kubo, C., Koga, Y., 2004. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J. Physiol. 558 (Pt 1), 263–275.
Syed, S.A., Nemeroff, C.B., 2017. Early life stress, mood, and anxiety disorders. Chronic Stress (Thousand Oaks) 1.
Targum, S.D., Nemeroff, C.B., 2019. The effect of early life stress on adult psychiatric disorders. Innov Clin Neurosci 16 (1–2), 35–37.
Theodora, R.H., Sarjana, W., Fitrikasari, A., Ss, D., Sari, S.P., 2019. Differences of BDI-II (beck depression inventory-II) score before and after probiotics administration. P J M H S 13 (4).
Tillmann, S., Awwad, H.M., Eskelund, A.R., Treccani, G., Geisel, J., Wegener, G., Obeid, R., 2018. Probiotics affect one-carbon metabolites and Catecholamines in a genetic rat model of depression. Mol. Nutr. Food Res. 62 (7), 1701070.
Vaghef-Mehrabany, E., Maleki, V., Behrooz, M., Ranjbar, F., Ebrahimi-Mameghani, M., 2020. Can psychobiotics "mood" ify gut? An update systematic review of randomized controlled trials in healthy and clinical subjects, on anti-depressant effects of probiotics, prebiotics, and synbiotics. Clin. Nutr. 39 (5), 1395–1410.
Valles-Colomer, M., Falony, G., Darzi, Y., Tigchelaar, E.F., Wang, J., Tito, R.Y., Schiweck, C., Kurilshikov, A., Joossens, M., Wijmenga, C., Claes, S., Van Oudenhove, L., Zhernakova, A., Vieira-Silva, S., Raes, J., 2019. The neuroactive potential of the human gut microbiota in quality of life and depression. Nat. Microbiol. 4 (4), 623–632.
van de Wouw, M., Boehme, M., Lyte, J.M., Wiley, N., Strain, C., O'Sullivan, O., Clarke, G., Stanton, C., Dinan, T.G., Cryan, J.F., 2018. Short-chain fatty acids: microbial metabolites that alleviate stress-induced brain-gut axis alterations. J. Physiol. 596 (20), 4923–4944.
Vanhaecke, T., Aubert, P., Grohard, P.A., Durand, T., Hulin, P., Paul-Gilloteaux, P., Fournier, A., Docagne, F., Ligneul, A., Fressange-Mazda, C., Naveilhan, P., Boudin, H., Le Ruyet, P., Neunlist, M., 2017. *L. fermentum* CECT 5716 prevents stress-induced intestinal barrier dysfunction in newborn rats. Neurogastroenterol. Motil. 29 (8).
Vidal, J., 1996. Differences of nu / + and nu / nu mice in some behaviors reflecting temperament traits. Physiol. Behav. 59 (2), 341–348.
Vitellio, P., Chira, A., De Angelis, M., Dumitrascu, D.L., Portincasa, P., 2020. Probiotics in psychosocial stress and anxiety. A systematic review. J. Gastrointestin Liver Dis. 29 (1), 77–83.
Wallace, C.J.K., Milev, R., 2017. The effects of probiotics on depressive symptoms in humans: a systematic review. Ann. General Psychiatry 16, 14.
Wallace, C.J.K., Foster, J.A., Soares, C.N., Milev, R.V., 2020. The effects of probiotics on symptoms of depression: protocol for a double-blind randomized placebo-controlled trial. Neuropsychobiology 79 (1), 108–116.
Wang, H., Lee, I.S., Braun, C., Enck, P., 2016. Effect of probiotics on central nervous system functions in animals and humans: a systematic review. J. Neurogastroenterol. Motil. 22 (4), 589–605.
Wang, Y., Wang, Z., Wang, Y., Li, F., Jia, J., Song, X., Qin, S., Wang, R., Jin, F., Kitazato, K., Wang, Y., 2018. The gut-microglia connection: implications for central nervous system diseases. Front. Immunol. 9 (2325).
Wang, Q., Sun, Q., Qi, R., Wang, J., Qiu, X., Liu, Z., Huang, J., 2019. Effects of Lactobacillus plantarum on the intestinal morphology, intestinal barrier function and microbiota composition of suckling piglets. J. Anim. Physiol. Anim. Nutr. (Berl.) 103 (6), 1908–1918.
Wei, C.-L., Wang, S., Yen, J.-T., Cheng, Y.-F., Liao, C.-L., Hsu, C.-C., Wu, C.-C., Tsai, Y.-C., 2019. Antidepressant-like activities of live and heat-killed Lactobacillus paracasei PS23 in chronic corticosterone-treated mice and possible mechanisms. Brain Res. 1711, 202–213.
Wilkins, L.J., Monga, M., Miller, A.W., 2019. Defining dysbiosis for a cluster of chronic diseases. Sci. Rep. 9 (1), 12918.
Williams, L.M., Debattista, C., Duchemin, A.M., Schatzberg, A.F., Nemeroff, C.B., 2016. Childhood trauma predicts antidepressant response in adults with major depression: data from the randomized international study to predict optimized treatment for depression. Transl. Psychiatry 6 (5), e799.
Yang, B., Wei, J., Ju, P., Chen, J., 2019. Effects of regulating intestinal microbiota on anxiety symptoms: a systematic review. Gen. Psychiatr. 32 (2), e100056.
Zhang, L., Zhang, J., You, Z., 2018. Switching of the microglial activation phenotype is a possible treatment for depression disorder. Front. Cell. Neurosci. 12 (306).
Zhang, N., Zhang, Y., Li, M., Wang, W., Liu, Z., Xi, C., Huang, X., Liu, J., Huang, J., Tian, D., Mu, J., Liao, X., Zhai, S., 2020. Efficacy of probiotics on stress in healthy volunteers: A systematic review and meta-analysis based on randomized controlled trials. Brain Behav. e01699.
Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., Zeng, L., Chen, J., Fan, S., Du, X., Zhang, X., Yang, D., Yang, Y., Meng, H., Li, W., Melgiri, N.D., Licinio, J., Wei, H., Xie, P., 2016. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. Mol. Psychiatry 21 (6), 786–796.