From isoniazid to psychobiotics: the gut microbiome as a new antidepressant target

ABSTRACT

An awareness of the importance of the gut–brain axis in psychiatric disorders such as depression is increasing. The gut microbiome is a key component of this axis. Gut bacteria can communicate with the brain through a variety of pathways including the hypothalamic–pituitary–adrenal axis, immune modulation, tryptophan metabolism and the production of various neuroactive compounds. Patients with depression, and other mood and anxiety disorders, show distinct compositional changes in their gut bacteria profile, raising the question about a possible aetiological role for the microbiome in these disorders. Evidence is emerging that the gut microbiome may represent a new potential antidepressant target and the term 'psychobiotic' has been coined to describe bacteria which confer mental health benefits. Gut bacteria are easily accessible and can be altered in a variety of ways including through the use of probiotics, prebiotics and dietary change. Psychobiotics containing various *Lactobacillus* and *Bifidobacterium* species have demonstrated the ability to improve mood, reduce anxiety and enhance cognitive function in both healthy populations and patient groups. This article provides an overview of the identification and development of antidepressant psychobiotics, from the preclinical evidence in the laboratory to the more recent encouraging results from human trials.

Many people do not know that the first antidepressant discovered was actually an antibiotic. Isoniazid was an antibacterial drug developed in the USA in the 1950s for treating tuberculosis. Unexpected side effects of euphoria, psychostimulation, increased appetite and improved sleep prompted an interest in the medication as a potential antidepressant. Subsequent clinical trials confirmed antidepressant efficacy, which was attributed to isoniazid's ability to inhibit monoamine oxidase enzymes and therefore increase levels of monoamines such as noradrenaline, serotonin and dopamine in the brain. The resultant 'monoamine hypothesis of depression' proved fruitful and heralded the development of further antidepressant medications, including the tricyclic antidepressants and selective serotonin-reuptake inhibitors, both of which act to increase central monoamine levels.

At the time it was not considered that the antimicrobial action of isoniazid might be responsible for, or at least contribute to, its antidepressant action. Over six decades later, in the era of a new understanding of the microbiome–gut–brain axis, such a possibility can reasonably be deliberated on. The gut–brain axis is a bidirectional communication system involving neural, endocrine and immune pathways which allow the CNS and gastrointestinal tract to interact with, and respond to, each other rapidly and effectively. Thus, gut homeostasis and function have the ability to reciprocally impact emotional states and behaviour. It is becoming increasingly clear that the gut bacteria are a major player in this complex system. Trillions of bacteria reside in the gastrointestinal tract, vastly outnumbering a person's own human cells. It is now appreciated that the immense collective genetic material of these gut bacteria, comprising the 'gut microbiome', has the ability to shape neurodevelopment and impact psychological functioning to a remarkable extent. While monoamine oxidase inhibition undoubtedly contributed to the antidepressant action of isoniazid, it is highly plausible that its antimicrobial action also played a role in alleviating depressive symptoms.

Development of psychobiotics: from the laboratory to the clinic

It is notoriously difficult to develop new psychotherapeutics. This is, in part, a result of limited knowledge about the neurobiological basis for mental illness and the absence of targetable biomarkers. Many landmark psychotropic medications, such as lithium for mania and chlorpromazine as the first antipsychotic, were discovered serendipitously. The development of 'psychobiotics' has followed a more logical and step-wise course. While the term 'probiotic' refers to a live organism that, when ingested in adequate amounts, exerts a health benefit, a psychobiotic is one which is specifically beneficial for mental health. Most microbiome research over the last decade has focussed on understanding the mechanisms of microbiome–gut–brain interaction through in-vitro and animal studies (*Figure 1*). While ongoing mechanistic studies are needed in the laboratory, a major effort is currently underway to progress the exciting preclinical findings to human studies and clinical trials with psychobiotics.

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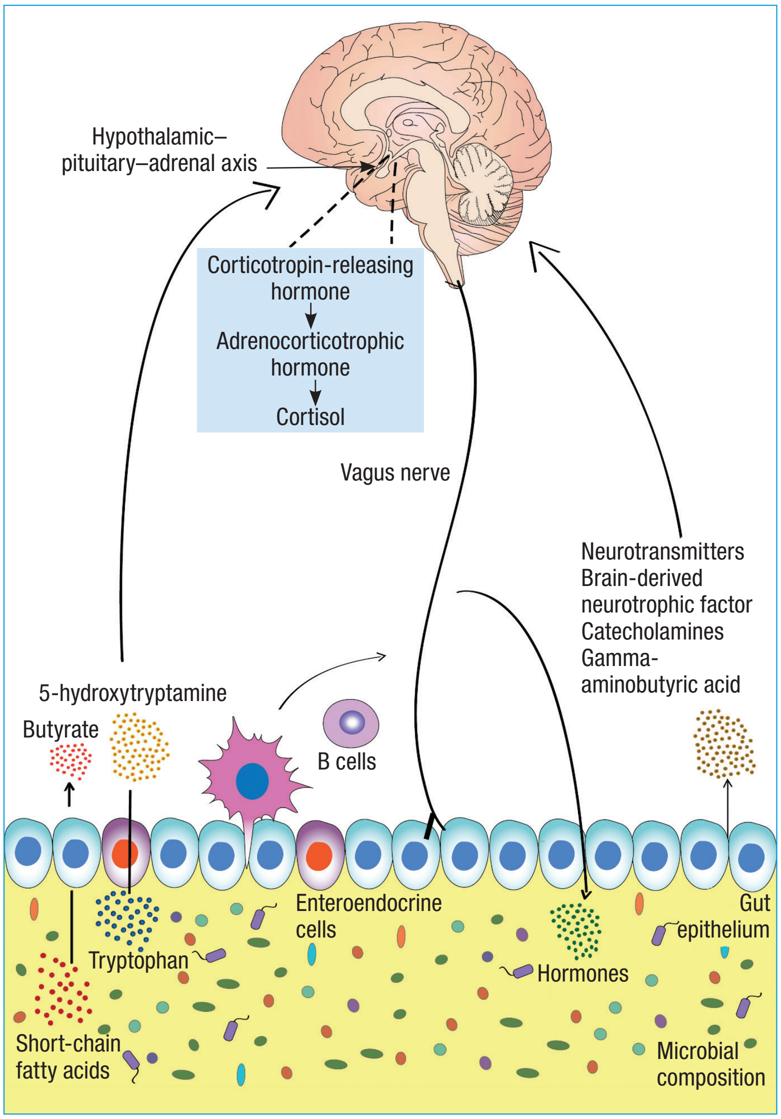
Figure 1. Pathways of microbiome–gut–brain axis communication.



Psychobiotics in the laboratory

The psychobiotic narrative first gained traction in 2004 when a landmark study (Sudo et al, 2004) demonstrated that the gut microbiome could dramatically influence the development and function of the hypothalamic–pituitary–adrenal axis, the body's primary stress response system which culminates in the production of cortisol, and has consistently been shown to be dysfunctional in major depressive disorder. This Japanese research team demonstrated that germ-free mice (mice born and housed in sterile conditions, thus lacking a microbiome) exhibited an exaggerated stress response with greater cortisol release in comparison to their control counterparts. Strikingly, the abnormal response was partially reversed by colonization with faeces from the control mice and completely reversed by feeding with a specific bacterial strain, *Bifidobacterium infantis*. However, the reversal only occurred if recolonization took place at an early stage, indicating a critical time period for normal, microbiome-dependent hypothalamic–pituitary–adrenal axis development. That the gut microbiome could influence the stress response was a seminal discovery which generated much speculation about the antidepressant potential of probiotic bacteria such as *Bifidobacterium*. However, it was early days and much remained to be elucidated about the mechanisms of microbiome–gut–brain interaction. A dysfunctional hypothalamic–pituitary–adrenal axis is only one component of the multifaceted aetiology of depression and questions remained about whether the microbiome could influence other pathways of interest in depression research such as immune regulation and serotonin metabolism.

The next decade saw great efforts undertaken to increase understanding of the relationship between the gut microbiome and immune system. It was well recognized that depression was associated with a state of low-grade inflammation characterized by elevated levels of pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) (O'Brien et al, 2004) but the source of these excess inflammatory markers was unknown.

It now appears that the gut microbiome plays a role in generating this pro-inflammatory state. Under normal homeostatic conditions gut microbes are safely confined to the gut and prevented from extra-intestinal access by the tightly-adherent gut epithelial barrier. However, gut permeability can be increased by various factors, such as chronic stress, a well-established precipitant of depression. The resultant 'leaky gut' can lead to translocation of gut bacteria, and bacterial components such as lipopolysaccharides, into the bloodstream, thus stimulating a low-grade immune response such as that seen in depression (Maes et al, 2008). Such a theory has been supported by studies that demonstrate the ability of various probiotics to improve gut barrier function (Ait-Belgnaoui et al, 2012) and normalize the immune disturbance seen in animal models of depression (Desbonnet et al, 2010).

The potential of the gut microbiome as an antidepressant target was further reinforced by evidence that it could influence neurotransmitter pathways. In the first instance, gut bacteria can directly produce many common human neurotransmitters including gamma-aminobutyric acid (GABA), noradrenaline, serotonin, acetylcholine and dopamine (Roshchina, 2010). Serotonin, the most well-studied neurotransmitter in relation to depressive illness, appears to be particularly susceptible to influence by the gut microbiome. A key study in 2009 revealed that the plasma serotonin levels of germ-free mice were almost three times less than those of conventional mice (Wikoff et al, 2009). It was subsequently demonstrated that this differential serotonin level was secondary to the remarkable ability of gut microbes to directly promote the synthesis of serotonin from its amino acid precursor, tryptophan, in intestinal enterochromaffin cells (Yano et al, 2015). Furthermore, the gut microbiome was also shown to influence serotoninergic levels in the hippocampus, an area of the brain which plays an important role in stress, anxiety and depression (Clarke et al, 2013).

Table 2. Probiotic interventional studies in patients with depression

Reference	Study participants: number, characteristics	Study design, duration	Probiotic intervention	Mood outcomes	Other outcomes
Akkasheh et al (2016)	40; adult patients with a diagnosis of major depressive disorder	Randomized, placebo-controlled, double-blind trial; 8 weeks	*Lactobacillus acidophilus*, *L. casei* and *Bifidobacterium bifidum* (2×10^9 colony-forming units/g)	Probiotic group demonstrated significant improvement in depression scores on Beck Depression Inventory	Probiotic group also showed reduced serum insulin, reduced C-reactive protein and increased plasma total glutathione levels
Romijn et al (2017)	79; adult patients recruited via an online screening resource. Subjects had self-reported depressive symptoms. Psychotropic medications were an exclusion criterion	Randomized, placebo-controlled, double-blind trial; 8 weeks	*L. helveticus* and *B. longum* (3×10^9 colony-forming units/1.5 g)	No effect of probiotic on any psychological outcome including Montgomery–Åsberg Depression Rating Scale	No effect of probiotic on serum inflammatory markers, brain-derived growth factor or vitamin D
Pinto-Sanchez et al (2017)	44; adult participants had irritable bowel syndrome and mild to moderate anxiety and/or depression scores based on self-report questionnaires. Psychotropic medications were an exclusion criterion	Randomized, placebo-controlled, double-blind trial; 6 weeks	*B. longum* NCC3001 (1×10^{10} colony-forming units/g)	Probiotic group demonstrated significant reduction in depression scores on Hospital Anxiety and Depression Scale	No effect on anxiety scores or irritable bowel syndrome symptoms. Probiotic reduced responses to negative emotional stimuli in multiple brain areas, including amygdala and fronto-limbic regions. No effect of probiotic on serum inflammatory markers, neurotransmitters (5-hydroxytryptamine, substance P and calcitonin gene-related peptide) or brain-derived growth factor levels. No effect on faecal microbiome profile

Another possible mechanism of action of psychobiotic bacteria may lie in their ability to produce short-chain fatty acids from the fermentation of non-digestible carbohydrates and proteins in the colon. The main short-chain fatty acids include propionate, acetate and butyrate and these bacterial metabolites appear to exert far-reaching effects in the body, including a role in immune signalling and regulation of plasma lipid levels. Butyrate, in particular, is of major interest given its ability to regulate gene transcription and has been shown to have an antidepressant effect in mice (Schroeder et al, 2007).

Psychobiotics in the clinic

The major challenge in drug development lies in translating what seems promising in the laboratory into the clinical setting. The complexity of human disease means that only a very small fraction of new therapeutics progress through to the drug development process from preclinical evaluation to successful clinical trials. The challenge of psychobiotic identification and translation is no different. Despite promising preclinical findings, results in human studies have been modest. However, the evidence base is growing and clinical research on the antidepressant properties of psychobiotics is hopeful.

Most human studies investigating the potential of probiotics to improve mood have been conducted in healthy subjects and results have been variable (*Table 1* online at www.bjhm.co.uk). A combination of *Lactobacillus helveticus* and *B. longum*, administered to 66 healthy adults, resulted in slight improvements in mood (Messaoudi et al, 2011), as did a polybiotic combination of various *Actobacillus*, *Lactobacillus*, *Bifidobacterium* and *Streptococcus* strains (Mohammadi et al, 2016). *L. casei*-Shirota improved mood in healthy adults with low baseline mood scores (Benton et al, 2007) and, although it did not improve mood in patients with chronic fatigue syndrome, it did reduce anxiety scores (Rao et al, 2009). Results in older adults have been less optimistic. Three studies (Shinkai et al, 2013; Chung et al, 2014; Östlund-Lagerström et al, 2016), two of which had up to 300 participants, investigated single probiotics of various *Lactobacillus* strains in healthy adults over 65 years of age. They reported no benefits in terms of mood although an improvement in cognition was noted with *L. helveticus* IDCC3801 (Chung et al, 2014).

Three randomized controlled probiotic trials have been undertaken in patients with depression and two have reported positive findings (*Table 2*). An Iranian team described improvements in Beck Depression Inventory scores in adults with a diagnosis of major depressive disorder following 8 weeks of consumption of a polybiotic containing *L. acidophilus*, *L. casei* and *B. bifidum* (Akkasheh et al, 2016). Interestingly, patients also showed significant decreases in serum insulin and C-reactive protein concentrations along with increased plasma glutathione levels, thus demonstrating a beneficial probiotic effect on metabolic, immune and antioxidant parameters alongside the antidepressant action. A major limitation of the study was that no information was provided on the concomitant use of antidepressant medication and so it was unclear whether the probiotic was being used as an adjunctive or sole treatment. Another *Lactobacillus*/*Bifidobacterium* polybiotic containing alternative species of the genera (*L. helveticus* and *B. longum*) found no benefit in terms of improving mood or moderating inflammatory or other biomarkers in patients with depression (Romijn et al, 2017), highlighting the differential antidepressant potential of species within the same bacterial genus. A third clinical trial demonstrated an antidepressant effect of *B. longum* NCC3001 consumed over 10 weeks by patients with comorbid irritable bowel syndrome (Pinto-Sanchez et al, 2017). However, the presence of irritable bowel syndrome represented an obvious confounding factor and depressive symptoms were merely self-reported on screening questionnaires with no diagnostic interview performed to establish a clinical diagnosis of major depressive disorder.

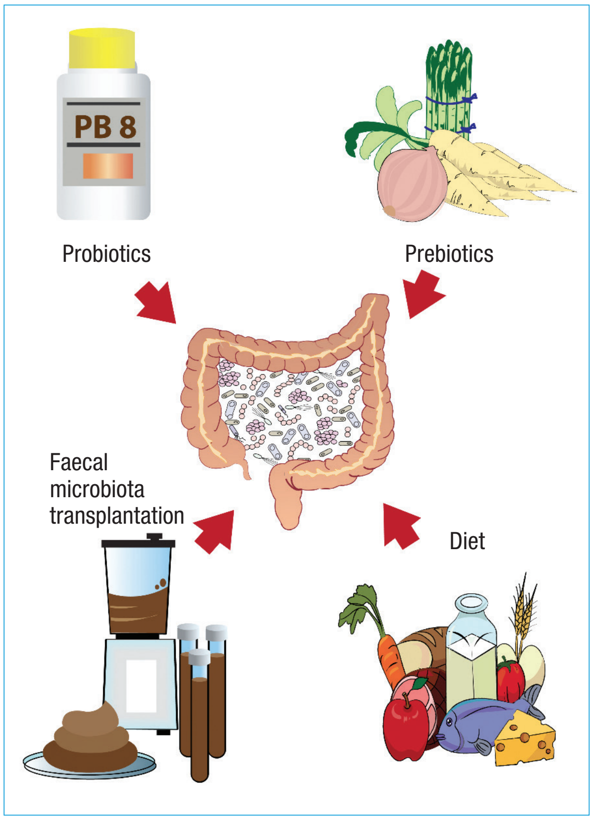
These human data as a whole provide tentative but definite optimism for the future of psychobiotics in the treatment of depression, most likely as an adjunctive strategy alongside traditional pharmacological and psychological therapies. Most benefit seems to be derived by those with low mood and depressive symptoms at baseline, a conclusion confirmed by recent meta-analysis (Ng et al, 2018). Effects in healthy subjects with normal baseline moods appear to be more limited although the use of psychobiotics as a preventative strategy for depression in those at higher risk must be considered.

Alternative methods of microbiome manipulation

Of course, psychobiotics are only one means of altering the microbiome (*Figure 2*), and perhaps, even, the least effective. While the term psychobiotics originally referred to beneficial live organisms such as bacteria, the definition has been expanded in recent years to include ‘prebiotics’. Prebiotics are non-digestible carbohydrates which are selectively fermented by bacteria in the large intestine, and can therefore be used to target beneficial host bacteria by specifically supporting and enhancing their growth (Bindels et al, 2015). Prebiotics include substances such as inulin, fructo-oligosaccharides, galacto-oligosaccharides, resistant starch and other soluble dietary fibres (although not all dietary fibres are prebiotic, i.e. not all modify selective gut microbiota). Natural sources of prebiotics include fruits and vegetables such as asparagus, leek, banana and chicory, as well as grains such as oats and wheat, foodstuffs which have become increasingly lacking in Western-style diets.

Evidence for the potential of prebiotics to improve psychological health is accumulating. A fructo-oligosaccharide+galacto-oligosaccharide combination demonstrated significant antidepressant and anti-anxiety effects in mice exposed to chronic stress (Burokas et al, 2017) and in healthy human volunteers, galacto-

Figure 2. Methods of altering microbiome composition.



oligosaccharide supplementation for 3 weeks resulted in suppression of the neuroendocrine stress response and an increase in the processing of positive *vs* negative attentional vigilance (Schmidt et al, 2015).

Faecal microbiota transplantation

Another means of modifying the microbiome is through the use of faecal microbiota transplantation, a process which involves transferring the faecal matter from one individual to another, thereby colonizing the recipient with the donor’s microbiota (Khoruts and Sadowsky, 2016). It has been used to explore the potential transference of disease phenotypes to healthy animals by microbiota transplantation from specific human conditions or from animal models of disease. Faecal microbiota transplantation from patients with major depressive disorder to microbiota-depleted rats resulted in the recipient rodents developing a depressive phenotype, both behaviourally and biochemically (Kelly et al, 2016). Similar findings were seen following depression-related faecal microbiota transplantation to germ-free mice (Zheng et al, 2016). Such studies strongly support an aetiological role for the gut microbiome in depressive illness and have garnered interest in the role of faecal microbiota transplantation as a therapeutic intervention in psychiatric disorders.

Most evidence for the therapeutic use of faecal microbiota transplantation in humans has been in the treatment of refractory *Clostridioides difficile* infection but there is also

KEY POINTS

- The gut–brain axis is of major interest in mood and anxiety disorders.
- The gut microbiome is a key player in gut–brain communication and can influence a wide range of physiological processes relevant to depression, including the hypothalamic–pituitary–adrenal axis-mediated stress response, immune modulation and tryptophan metabolism.
- The gut microbiome can be altered through the use of probiotics, prebiotics, antibiotics, faecal microbiota transplantation and dietary change.
- Certain bacteria, predominantly of the *Lactobacillus* and *Bifidobacterium* species, reduce anxiety and improve mood, an ability which has successfully translated from animal to human studies.
- Much work is needed to further elucidate the mechanisms by which the gut microbiome influences brain function and thus identify those bacteria with psychobiotic activity.

evidence emerging that it may be beneficial in functional gastrointestinal disorders such as irritable bowel syndrome, and in metabolic syndrome (Mullish et al, 2018). A recent Japanese study observed psychiatric symptoms in 17 patients who underwent faecal microbiota transplantation for the treatment of irritable bowel syndrome, functional diarrhoea or functional constipation. Patients with elevated depression scores at baseline experienced a significant improvement in mood which correlated with an increase in microbiota diversity (Kurokawa et al, 2018). Although this was an open-label, uncontrolled, observational study, it does raise the possibility that faecal microbiota transplantation may be of benefit in depression. There has, to date, been only one faecal microbiota transplantation interventional study in a neuropsychiatric population. Kang et al (2017) administered faecal microbiota transplantation from healthy donors to 18 children diagnosed with autism spectrum disorder over a 10-week period. They reported an increase in overall bacterial diversity and significant improvements in both gastrointestinal and autistic behavioural symptoms, which were maintained at assessment 8 weeks after treatment had ended. The use of faecal microbiota transplantation for psychiatric illness is in its infancy but may offer exciting new therapeutic opportunities.

Dietary modification

The final, and probably most important, method of introducing microbiota change is by targeting the diet. There is strong evidence that adherence to a Mediterranean diet, as well as lower intakes of food items such as processed meats and trans fats, confer protection against depression (Lassale et al, 2018). The mechanisms by which specific dietary factors promote resilience to depressive illness are not fully understood, but it is likely that the gut microbiome plays a significant role. A change in diet can dramatically and rapidly alter the microbiome composition (for review see Singh et al, 2017) and thus alter levels of health-promoting bacteria and beneficial bacterial metabolites such as short-chain fatty acids. The potential for significantly reducing the incidence of depression and other neuropsychiatric diseases through large-scale dietary interventions is widely recognized and has led to the development of new initiatives such as The International Society for Nutritional Psychiatry Research to promote the growth of this field.

Conclusions

Antidepressant therapy has come a long way from isoniazid and the monoamine hypothesis. However, perhaps the journey will ultimately prove to have been a circular rather than a linear one. That the first antidepressant arose from an antibiotic is surely a notable coincidence in the current psychobiotic climate and forces one to reconsider and question widely-accepted concepts with fresh eyes. The traditional view of functional gastrointestinal disorders such as irritable bowel syndrome is that these are anxiety- or stress-driven conditions, allowing for the concept of a top-down, brain–gut influence. New insights on the gut microbiome have turned these assumptions on their head and it may well be the human gut that is driving the rapidly-escalating incidence rates of depression and anxiety.

Nonetheless, the subject of psychobiotics is still relatively new. Much work needs to be done to further delineate microbiome–gut–brain interaction mechanisms and establish the extent of influence of this system on physiological and psychological processes. Further human studies are needed to improve characterization of normal and 'dysbiotic', or unbalanced, microbiota configurations and to investigate patterns related to specific disease processes. Solid conclusions from probiotic studies are limited by the significant heterogeneity across trials, in particular in relation to the strain of probiotic, dosage levels and duration of treatment. Further studies are imperative to shed light on these variables and optimize psychobiotic treatment strategies as this exciting field moves forward. **BJHM**

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Table 1. Probiotic interventional studies in healthy human volunteers

Reference	Study participants: number, characteristics	Study design; duration	Probiotic intervention	Mood outcomes	Other outcomes
Benton et al (2007)	132; healthy adults	Randomized, placebo-controlled, double-blind trial; 3 weeks	*Lactobacillus casei* Shirota (6.5×10^9 colony-forming units/g)	No overall effect on anxiety or mood subsets on Profile of Mood States Scale. However, significant improvement in mood in those who had lowest baseline mood levels	
Messaoudi et al (2011)	66; healthy adults	Randomized, placebo-controlled, double-blind trial; 30 days	*L. helveticus* R0052 and *Bifidobacterium longum* R0175 (3×10^9 colony-forming units/g)	Significant reduction in overall Hospital Anxiety and Depression Scale score. (No effect on Hospital Anxiety and Depression Scale – depression subscale.) Moderate effect on per cent change on global severity index of Hopkins Symptom Checklist-90, mainly as a result of improvements across three subscales – somatization, depression and anger-hostility	Moderate, but not statistically significant, effect on Hospital Anxiety and Depression Scale – anxiety subscale. No effect on Perceived Stress Scale or urinary free cortisol levels
Steenbergen et al (2015)	40; healthy adults	Randomized, placebo-controlled, triple-blind trial; 4 weeks	*B. bifidum* W23, *B. lactis* W52, *L. acidophilus* W37, *L. brevis* W63, *L. casei* W56, *L.* salivarius W24, and *L.* lactis (W19 and W58) (2.5×10^9 colony-forming units/g)	Significant reductions in Leiden Index of Depression Sensitivity-Revised post-intervention, indicating reduced cognitive reactivity to sad mood. However, no changes in Beck Anxiety Inventory or Beck Depression Inventory	
Tillisch et al (2013)	23; healthy women	Randomized, placebo-controlled, double-blind trial; 4 weeks	*B. animalis* subsp *Lactis* (1.25×10^{10} colony-forming units/cup) *Streptococcus thermophilus*, *L. bulgaricus*, and *L. lactis* subsp *Lactis* (1.2×10^9 colony-forming units/cup)	No change in Hospital Anxiety and Depression Scale	On functional magnetic resonance imaging: alterations in activity of brain regions that control central processing of emotion and sensation
Kelly et al (2017)	29; healthy men	Randomized, placebo-controlled, cross-over trial; 8 weeks	*L. rhamnosus* (1×10^9 colony-forming units/g)	No effect on mood as measured on Beck Depression Inventory	No effect on anxiety or stress as measured by Beck Anxiety Inventory, Perceived Stress Scale, State Trait Anxiety Inventory. No effect on sleep, hypothalamic–pituitary–adrenal axis response to acute stress, electroencephalogram measures or cytokine levels
Mohammadi et al (2016)	75; healthy adults	Randomized, placebo-controlled, double-blind trial; 6 weeks	Probiotic yoghurt containing *L. acidophilus* and *B. lactis* (1×10^7 colony-forming units/g) OR probiotic capsule containing *Actobacillus casei* (3×10^3), *L. acidophilus* (3×10^7), *L. rhamnosus* (7×10^9), *L. bulgaricus* (5×10^8), *B. breve* (2×10^{10}), *B. longum* (1×10^9) and *S. thermophilus* (3×10^8 colony-forming units/g)	Reduced anxiety and depression scores on General Health Questionnaire-28 and Depression Anxiety Stress Scales with probiotic yoghurt or capsule	

Table 1. Probiotic interventional studies in healthy human volunteers (continued)					
Nishihira et al (2014)	238; healthy adults	Randomized, placebo-controlled, double-blind trial; 12 weeks	*L. gasseri* SBT2055, *B. longum* SBT2928 (1.5×10^9 colony-forming units/g)	No change in overall General Health Questionnaire-28 scores	Significant improvement in anxiety/insomnia subscale of the General Health Questionnaire. Elevated natural killer cell activity and reduced serum adrenocorticotrophic hormone in probiotic group
Chung et al (2014)	36; healthy older adults over 65 years	Randomized, placebo-controlled, double-blind trial; 12 weeks	*L. helveticus* IDCC3801	No effect on mood as measured by Geriatric Depression Scale Short Form	Improvements in cognitive function in probiotic group. No effect on Perceived Stress Scale or brain-derived growth factor
Shinkai et al (2013)	300; healthy older adults over 65 years	Randomized, placebo-controlled, double-blind trial; 20 weeks	Tablet containing 2×10^9 or 2×10^{10} *L. pentosus* strain b240	No effect on Profile of Mood States Scale	Probiotic reduced the incidence of the common cold in elderly adults, and improved their perception of their general health
Östlund-Lagerström et al (2016)	290; healthy older adults over 65 years	Randomized, placebo-controlled, double-blind trial; 20 weeks	*L. reuteri* DSM17938 (1×10^8 colony-forming units/g)	No significant effect on Hospital Anxiety and Depression Scale, Perceived Stress Scale	No effect on Perceived Stress Scale