

PROBIOTICS FOR MENTAL HEALTH AND WELLBEING

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Introduction

A psychobiotic was recently defined as a live organism that, when ingested in adequate amounts, produces a mental health benefit (Dinan, 2013). These psychoactive probiotic bacteria can produce neuroactive compounds which act via the brain-gut axis, through the vagus nerve, spinal cord or neuroendocrine systems. Evidence is starting to accumulate supporting the efficacy of probiotics in the area of mental health, such as IBS, and depression, as researchers identify which strains of bacteria can affect the nervous system, and start to map pathways by which psychobiotics can exert their effects.

Bacterial colonisation of the gut plays a major role in postnatal development and the maturation of key systems that influence central nervous system (CNS) programming and signaling, including the immune and endocrine systems. Individually, these systems have been implicated in many CNS disorders and collectively they form an important tri-directional pathway of communication between the microbiota, the gut and the brain in health and disease. There is increasing evidence that the commensal microbiota plays a role in early programming of the stress system, and is responsiveness to triggers. The routes of communication between the microbiota and brain are not fully elucidated but include neural, humoral, immune and metabolic pathways. The concept of a microbiome-brain-gut axis is emerging which suggests that modulation of the gut microflora may be a tractable strategy for developing novel therapeutics for complex mental health disorders, where there is a huge unmet medical need.

Probiotics

"Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO 2001, as updated by Hill et al, 2014)

The first known reference to the health promoting effects of probiotics can be found in the Persian bible which states that Abraham owed his longevity to fermented milk products which he consumed daily (Genesis 18:8). The concept of using live microbes to colonise the gut is attributed to Nobel Prize

recipient, Eli Metchnikoff, who hypothesised in 1907 that "the dependence of the intestinal microbes on food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes" (Mechnikoff, 1907). Fermented foods were common in parts of Europe and it was known that lactic-acid bacteria fermented milk inhibited the growth of proteolytic bacteria, because of the low pH produced by the fermentation of lactose.

Based on observations of longevity in certain rural populations in Europe, e.g. Bulgaria and the Russian steppes, whose diets consisted largely of LAB fermented milk, Metchnikoff proposed that consumption of fermented milk would “seed” the intestine with harmless lactic-acid bacteria, decrease the intestinal pH and that this would suppress the growth of proteolytic bacteria. He introduced milk fermented with “Bulgarian Bacillus” into his own diet and found his health benefited. Friends in Paris soon followed his example and doctors began prescribing a sour milk diet for their patients.



The probiotic concept is not new, but is undergoing transition as knowledge of the gut microbiota in health and disease becomes translated to the clinic. Operationally, a probiotic represents a mimic of and/or supplement to the normal gut microbiota. The reality is that many bacteria are claimed to be probiotic but very few have been subjected to rigorous investigation. Much consumer skepticism has arisen because of media portrayal of probiotics as essentially all being the same. But as the molecular basis of host-microbe interactions are being elucidated, the selection criteria for probiotics and their distinct mechanisms of action are improving. Most probiotics are from the genus *Lactobacillus* or *Bifidobacterium*, which lack the external pro-inflammatory lipopolysaccharide chains found on pathogenic bacteria, such as *E. coli* and *Salmonella*. The host gut, therefore, is tolerant to extensive colonization by beneficial bacteria, which lower the risk of infection by competing with pathogens. These innocuous species are detected and monitored by the intestinal immune system from early-life, but do not illicit an immune response because they lack the necessary inflammatory elements, whilst supporting the development of a robust anti-inflammatory immune response that minimises damage to the gut (Sansonetti, 2009). Thus, probiotics can help maintain a healthy balance between the anti- and pro-inflammatory responses that, via the circulation,

impart physiological advantages to all organs, including the brain.

The idea that *Lactobacillus* strains may improve quality of life and mental health is not a new one. Dr. George Porter Phillips first reported in 1910 that a gelatin-whey formula with live lactic acid bacteria improved depressive symptoms in adults with melancholia (Phillips, 1910). In more recent times, following a review of increasing clinical evidence from other conditions with depressive symptoms, such as IBS, the use of probiotics as an adjunct therapy in the treatment of depression was first proposed in 2005, based on mounting evidence (Logan, 2005). Since then, there has been considerable activity in the field, indicating that the resident gut microbiota, and certain probiotic strains, can influence mental health.

Microbiota And Host Mental Health

The microbiome-brain-gut axis, the communication system between the brain, gut and GI microbiota, integrates neural, hormonal and immunological inputs from these three systems (Collins, 2012; Moloney 2014). The gut microbiota, and the metabolites they produce, may also modulate the peripheral and central nervous systems to influence brain development and function (Forsythe, 2010). In recent years, an increasing number of studies have demonstrated the importance of the gastrointestinal microbiota in the stress response (Neufeld, 2011; Sudo, 2004) and in neurodevelopmental disorders (de Theije 2011; Desbonnet, 2013; Finegold, 2010). Commensal microbiota have demonstrated the ability to interact with the serotonergic system, which plays an important role in mood, by regulating the development of the hypothalamus-pituitary-adrenal axis (HPA), the core neuroendocrine system that controls reactions to stress (Sudo, 2004). Some recent germ free (GF) studies, in which germ free mice lack any microbiota, have shown that the non-colonised mice show a reduction in anxiety like behaviour, compared to conventional mice, possibly through an enhanced HPA response (Heijtza, 2011; Clarke 2013), and how GF mice had behavioural characteristics which indicate deficits in normal social behavior, such as social motivation and preference for social novelty (Desbonnet, 2013).

In animal models, a number of intervention studies with probiotic strains have proven successful in the treatment of mental health conditions. In a maternal separation model of depression in mice, *Bifidobacterium infantis* 35624 demonstrated antidepressant properties (Desbonnet, 2008), while *Lactobacillus rhamnosus* JB-1 exhibited anti-anxiety and anti-depressant properties, compared to brotched control mice, through activation of the vagus nerve (Bravo, 2011). *B. fragilis* reduced autism-like behavioral deficits in communication, social behavior, and repetitive behaviour, in mice symptomatic of autism, compared to autistic, placebo treated controls (Hsiao, 2013).

Probiotic strains that produce neurotransmitters

It is clear from a variety of in vitro and preclinical models that a broad range of bacteria can manufacture and secrete essential neurochemicals. Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain, and regulates many physiological and psychological processes. Dysfunction in the GABA system is implicated in anxiety and depression (Cryan, 2005; Schousboe, 2007). Certain strains of *Lactobacillus* and *Bifidobacterium* can produce GABA from monosodium glutamate and it has been suggested that the microbially produced GABA in the gut may have an effect on the brain-gut axis.

Of 5 strains identified in a screen to identify GABA producers, *Lactobacillus brevis* and *Bifidobacteria dentium* were the most efficient (Barrett, 2012).

Lactobacillus brevis FPA3709, another GABA producer, administration to rats resulted in an antidepressant effect similar to that of fluoxetine, a common antidepressant drug, but without the side-effects such as appetite and weight loss (Ko, 2013). At the level of gene expression, ingestion of *L. rhamnosus* JB-1 altered the mRNA expression of both the GABA A and B receptors, which have been implicated in anxiety and depression (Bravo, 2011).

Serotonin (5-HT), a metabolite of the amino acid tryptophan, plays an important role in the regulation of a number of brain functions, including mood. Most antidepressant drugs act through increasing serotonin levels, and some studies have shown that

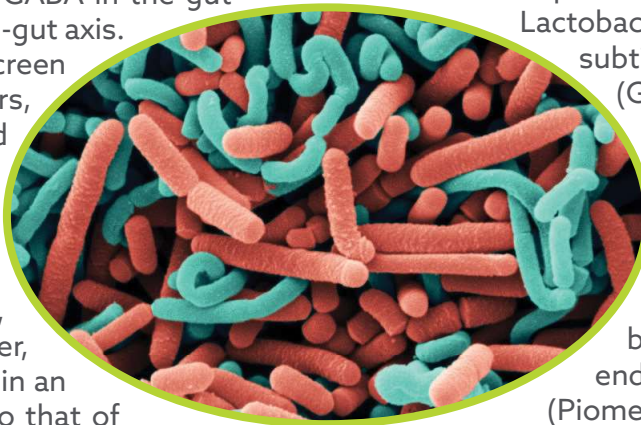
the microbiota can synthesise serotonin.

The serotonin levels in the plasma of conventional mice are significantly higher than germ free mice, which have no GI microbiota (Wickoff, 2009), indicating that the microbiota can influence serotonin levels. In addition, oral ingestion of the probiotic *Bifidobacterium infantis* 35624 increased levels of the serotonin precursor, tryptophan, in the plasma of rats, suggesting that the strain may have potential as an antidepressant (Desbonnet, 2008). *Candida*, *Streptococcus*, *Escherichia*, and *Enterococcus* strains produce serotonin (Lyte, 2011).

Catecholamines, such as dopamine and norepinephrine, are the major neurotransmitters that mediate a variety of CNS functions such as motor control, cognition, memory processing, emotion and endocrine regulation. *Escherichia*, *Bacillus*, and *Saccharomyces* produce norepinephrine (also called noradrenaline) (Asano, 2012), the neurotransmitter most responsible for vigilant concentration, while *Bacillus* and *Serratia* have the potential to produce dopamine, which plays a role in reward-motivated behaviour (Lyte, 2011). Acetylcholine is a neurotransmitter found in the central and peripheral nervous systems which plays a critical role in cognitive function, particularly in memory and learning. Previous studies have shown that acetylcholine is

both a component of bacterial strains, including *Lactobacillus plantarum* and *Bacillus subtilis* and a microbial metabolite (Girvin, 1954; Rowatt, 1948; Horiuchi, 2003).

Endocannabinoids are lipid molecules that act as neurotransmitters/neuromodulators in the brain, which contains specific endocannabinoid receptors (Piomelli, 2003), that also engage with Δ 9-tetrahydrocannabinol, the active constituent of cannabis, a plant long known for its psychotropic properties. The endocannabinoid system and the gut microbiota can impact on the development of obesity and related disorders (Muccioli, 2010). In addition, a *Lactobacillus acidophilus* strain modulates expression of cannabinoid receptors in the spinal cord (Rousseaux, 2007).



Clinical Evidence

Since first suggested as an adjunct therapy in the treatment of depression in by Logan and Katzmann in 2005, a number of placebo-controlled clinical trials have been conducted with probiotics to explore potential benefits in the context of mental health.

IBS: Irritable bowel syndrome is a disorder of the brain-gut axis and is associated with a high degree of comorbid depression and anxiety (Clarke, 2009). Our group and others have conducted several well-designed studies of probiotics in IBS (O'Mahony, 2005; Veiga, 2014; Dapoigny, 2012). O'Mahony et al. carried out a double blind, parallel, placebo-controlled study comparing two different probiotic strains, *B. infantis* and *L. salivarius*. The *B. infantis* 35624 strain resulted in significant improvement in the IBS patients, though a reduction in proinflammatory cytokines. That the *L. salivarius* strain did not demonstrate a therapeutic benefit indicated that the effect was strain specific. *Lactobacillus casei rhamnosus* LCR35 was shown to have some efficacy, according to the hospital anxiety and depression (HAD) scale, in a subgroup of IBS patients, those suffering from diarrhoea (Dapoigny, 2012).

Anxiety: In a 2011 double-blind, placebo-controlled, randomized parallel group study, volunteers received either a probiotic combination (*L. helveticus* R0052 and *B. longum*) or placebo for 30 days and were assessed using a number of validated depression and coping checklists (Messaoudi, 2011). Daily administration of the probiotic combination significantly reduced psychological distress in volunteers, as measured by the Hopkins Symptom Checklist scale, the Hospital Anxiety and Depression Scale, and by the Coping Checklist. Furthermore, urinary free cortisol levels were significantly reduced by the probiotics, indicating a potential mechanism for the improvement in psychological symptoms observed.

Mood and memory: A double blind, placebo controlled trial (Benton, 2007) found that the consumption of a probiotic-containing milk drink improved mood. 132 physically healthy subjects, with a mean age 61.8 years, participated in a 3-week intervention study, with 124 completing the study. Mood and cognition were measured at baseline and after 10 and 20 days of consumption. Overall there was no statistical effect, but when the third of the participants with the lowest baseline mood were considered, they selectively responded by reporting themselves as happy rather than depressed after taking the probiotic. Post hoc analysis indicated a trend for those with failing memory to benefit from the probiotic, although the effect failed to reach significance.

Chronic fatigue syndrome: In a study of patients with chronic fatigue syndroms, a complex illness in

which patients demonstrate increased anxiety, 39 subjects were treated daily with 2.4×10^{10} CFU of *Lactobacillus casei* strain Shirota or a placebo (Rao, 2009) for 2 months. Overall, there was a significant improvement in anxiety among those taking the active *Lactobacillus casei* Shirota compared with the placebo, providing further support for the view that a probiotic may have psychotropic effects.

Conclusions

The director of the National Institute of Mental Health, Thomas Insel, in a 2012 blog on the major advances of that year, discussed studies on the GI microbiota as among the most important published, concluding that "our bodies are a complex ecosystem in which human cells represent a paltry 10% of the population. But beyond the sheer numbers, we now know little about the profound diversity of this ecosystem and striking individual differences. How these differences in our microbial world influence the development of brain and behavior will be one of the great frontiers of clinical neuroscience in the next decade". Pharmabiotics produced by the gut microbiota can undoubtedly influence a variety of physiological and metabolic systems/processes in the human body. At a local level they can induce changes in the gut epithelium and the enteric nervous system, while at a more systemic level processes as wide-ranging as immune function and CNS signalling may be affected. In addition to the direct action of probiotics, the likelihood that diet itself can catalyze bacterial communication with the brain should also be considered, but is outside the scope of this paper.



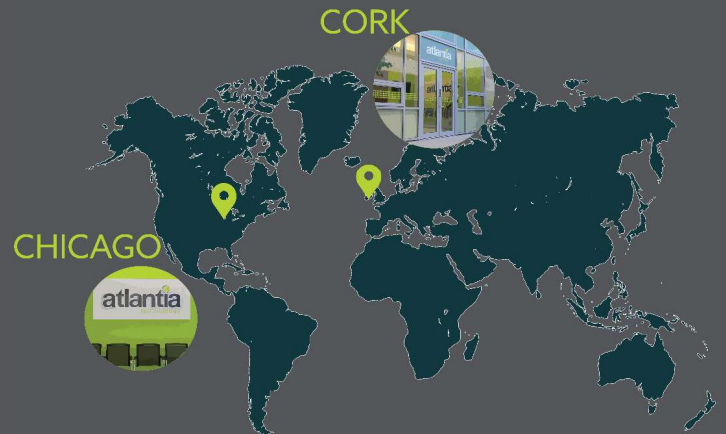
OUR CLINICAL EXPERTISE

PROBIOTICS FOR MENTAL HEALTH AND WELLBEING

Atlantia Food Clinical Trials Ltd has considerable experience in conducting clinical studies exploring the potential health benefits of probiotics, in a variety of health areas including digestive health, cardiovascular health and mental and cognitive health. In this whitepaper we explore a relatively new area in the field of probiotics, namely the use of probiotics to promote mental health and wellbeing.

In light of EFSA rejections of all probiotics health claims to date, based largely on lack of strain characterisation and poorly designed human intervention studies, Atlantia has developed a "whole solution" for client companies to assist in getting a positive EFSA response for a probiotic.

- ✓ We provide end to end solutions for the entire clinical study process
- ✓ ICH GCP Standard trials
- ✓ Regulatory environment expertise, including EFSA and FDA
- ✓ One company, with multi-centre capability



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