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Psychobiotics; A Promise for Neurodevelopmental Therapy

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Abstract

The inseparable association between man and microbes has long been known and some of their benefits are well documented. However, the use of bacteria as therapeutics has attracted much interest. Much is known about the ability of gut microbes to enhance immunity, lower cholesterol levels, improve gut barrier functions and many more benefits. Although it is evident that the gut and brain communicate through biochemical signaling which often involves the gut microbiota, it is still not clear whether or not gut manipulations through probiotic administration can correct or treat neurological problems. Many studies have shown that patients with neurodegenerative diseases also have gut dysbiosis and other studies have also shown the ability of certain gut bacteria to synthesize neurotransmitters. Yet, do these probiotics produce enough neuroactive chemicals to influence physiology? Are there precautions to be taken, since such probiotics may cause undesirable effects? This review discusses our current knowledge on the ability of probiotics to affect the central nervous system (CNS) and their potential use in neurodegenerative therapy. Some knowledge gaps left in this area of research have also been highlighted.

Keywords: Probiotics; Gut dysbiosis; Psychobiotics; Neurodegenerative therapy

Introduction

Microbes are ubiquitous as they are found everywhere on the planet. It is therefore not surprising that the human body is made of 90% bacteria cells and only 10% human cells making us a cocktail of human and bacteria cells tightly associated in a mutualistic relationship [1]. These microbes may play very essential roles in the development of the fetus and that may be why they are present in amniotic fluids, amniotic cord blood, and even in healthy neonatal meconium [2,3]. The human gut microbiome (the collection of all the microorganisms living in association with the human gut) consists of three enterotypes namely, Bacteroides (most abundant and most variable genus), Prevotella and Ruminococcus [4]. Over 1500 gut bacteria species are present in the human body [5] with more than 95% of them residing in the gut [6]. These bacteria contain over 8 million distinct genes encoding for several enzymes and proteins which influence host metabolism. A balance in the richness of the gut bacteria is therefore required for homeostasis. In various pathological conditions such as chronic anxiety, depression, autism and celiac disease [7,8], disease-associated dysbiosis are characterized by an imbalance in the levels, the reciprocal abundance, the presence and/or localization of normal gut bacteria species, rather than an overgrowth of well-defined pathogenic bacteria (as observed in *C. difficile* infection). Such disease-associated alterations in the microbiome are usually caused by genetic and environmental factors such as drugs, diet, toxins and pathogens [9]. Over the years, studies with germ free (GF) mice have shown that majority of the biochemical compounds and metabolites circulating in the blood are as a result of the gut microbial activities and these compounds affect mammalian behavior and endocrine response [10]. Heijts et al. [11] observed that early microbial colonization process was required to initiate brain signaling mechanisms that affect neuronal circuits involved in motor control and anxiety in mice. Their study shows the role of gut microbes in the normal mammalian brain development. Strong associations between microbial pathogenic infections and neurodevelopmental disorders such as autism and schizophrenia during perinatal periods have also been reported [12]. Many other studies have shown interesting correlations between emotional state and gut functionality [13,14] and the gut bacteria have been implicated in this process. Therefore, the strong association between gut dysbiosis, intestinal permeability and neurological dysfunction suggests that modifying the gut microbiota may provide a promising therapeutic option in emotional and neurodegenerative ailments. The search for probiotics that can affect cognitive functions has therefore increased recently. Such probiotics are called psychobiotics. Dinan et al. [15] defined psychobiotics as live organisms that when ingested in adequate amounts produce beneficial health effects to patients suffering from psychiatric illness. This work highlights how the gut microbiota and the brain interact and how this knowledge may be helpful in the treatment and management of diseases associated with the central nervous system (CNS).

The gut–brain communication pathway

The gut is embedded with neurons of the enteric nervous system, a subdivision of the autonomic nervous system that directly controls the gastrointestinal tract. During early embryogenesis, a neural crest is formed which latter differentiates into the enteric nervous system and the CNS [16]. These two systems are later connected by the vagus nerve (the tenth cranial nerve that runs from the brain stem down to the abdomen) during development [17]. To control gut activity patterns, the brain sends signals through command neurons to the vagus nerve which regulates the volume of signals and relays the signal to the gut interneurons in a bidirectional fashion. This is made possible by the abundance of command neurons and interneurons in the myenteric plexus and submucosal plexus of the gut. The plexus contain

glial cells, mast cells and sensory nerve endings for monitoring digestion and sending feed signals back to the spinal cord and the brain [18]. The enterochromaffin (EC) cells in the intestinal mucosa produce about 90% of the body's serotonin [19] and this brain neurotransmitter is thought to affect behaviors such as appetite, emotional, motor, cognitive and autonomic behaviors [20]. However, germ-free mice produce low levels of serotonin (about 60% lower than conventional mice) but increased levels of serotonin are observed when the mice are recolonized with normal gut microbes implying the impact of gut microbes on the production of the neurotransmitter [21]. This indicates how gut microbes communicate with the brain to affect physiology and shows the interesting prospects of using probiotics to restore brain function associated disorders in patients.

Gut dysbiosis and neuropathology

Many CNS disorders are associated with dysbiosis in the gut resulting in gastrointestinal disturbance [22]. Autism spectrum disorder (ASD) is a developmental disability that can cause significant social, communication and behavioral challenges caused by a combination of genetic and environmental risk factors. Gastrointestinal disturbances are common among children with ASD. The genera Prevotella, Coprococcus, and unclassified Veillonellaceae were significantly reduced in autistic children with high levels of Enterobacteriaceae [23]. They also have increased levels of *Ruminococcus torques* [24] which degrade mucus in the gut to induce gut barrier dysfunction. Children with autism also have higher levels of Clostridium species relative to healthy subjects [25]. Ileal and caecal mucosa biopsy specimens of children diagnosed with autism and gastrointestinal dysfunction contain *Sutterella* species but the bacteria were absent in children with only gastrointestinal dysfunction [2,26]. This finding reveals a potential role of Sutterella species in autism disorder but their actual role in the pathogenesis of the disease is still unknown. Parkinson's disease (PD) is another disease that has been linked with gut dysbiosis. Neurodegeneration in PD results from both genetic and environmental factors. These factors result in the progressive impairment or deterioration of neurons in the substantia nigra of the brain that produce dopamine. The decrease in dopamine levels results in abnormal brain activity. Meanwhile, as most PD patients usually experience constipation several years before the onset of the motor symptoms, that could show a possible link between gut dysbiosis and the brain in the pathogenesis of the disease [27]. Patients with PD have shown to have significantly higher levels of putrefaction bacteria such as *Proteus* and *Klebsiella* species in their small intestine and this contributes to the high urinary indoxyl sulfate concentrations in PD patients relative to healthy controls [28,29]. Furthermore, they have reduced Prevotellaceae and significantly increased Enterobacteriaceae which is positively associated with the severity of postural instability and gait difficulty [28,30]. In fact, the presence of Prevotella in the human gastrointestinal tract is inversely correlated with PD. Members of the genus Prevotella synthesize mucin, thiamine and folate as well as neuroactive short chain fatty acids [28]. A reduction in these bacteria therefore indicates a compromise in gut epithelial integrity due to low mucin synthesis, thereby enhancing gut permeability and systemic exposure to bacteria endotoxins [31]. Another disease thought to be linked with gut dysbiosis is celiac disease. The disease is an autoimmune disorder triggered by dietary gluten in genetically susceptible individuals. However, environmental factors such as infections may trigger changes in the gut of people who have such genes that make them more prone to abnormal immune response when they consume gluten. Many celiacs (up to 50%) develop peripheral neuropathy, ataxia, epilepsy (in some children) and even dementia in adults [32]. Celiac disease in children is usually preceded by gastroenteritis that suggests the involvement of gut microbes in the genesis of the disease [8]. Analyses of the duodenal microbiota of children with celiac disease revealed significant abundance of Bacteroides and *E. coli* and a significant reduction in Lactobacillus and *Bifidobacterium* compared to healthy controls [33]. It is thought that the presence of high levels of Gram-negative and potentially pro-inflammatory bacteria in the duodenum of celiac children might contribute to the symptoms of the disease that could favor the pathological process [34,35]. Meanwhile, other studies have proposed that alterations in the gut mucosal glycosylation in some celiacs can cause changes in the bacterial flora which might lead to a functional change in the gut [8]. It is therefore not clear whether celiac disease is caused by the abnormal glycosylation of the gut mucosa or it is the changed microbiota that triggers the abnormal glycosylation of the gut. However, since most patients with neuropathological cases including cerebral palsy, chronic depression and Rett syndrome experience gastrointestinal disorders, that may be linked with the gut bacteria [36-38].

Psycobiotics and neurodegenerative therapy

Many gut bacteria have been found to synthesize and respond to neuroactive compounds similar to those produced in the host and this gives a clue to the point where the two kingdoms (prokaryotae and animalia) communicate [39]. One such biologically active compound is γ-aminobutyric acid (GABA), a brain neurotransmitter that suppresses anxiety and depression [40]. Human intestinally derived strains of *Lactobacillus brevis* DPC6108 and *Bifidobacterium dentium* have been shown to produce large amounts of GABA when cultured in media containing monosodium glutamate [41]. The ability of these intestine bacteria to synthesize neurochemicals shows that the balanced gut microbes in healthy people regulate GABA levels to ensure homeostasis while dysbiosis can be traced to a deficiency in this neurotransmitter causing depression. Indeed, depressed patients have been found to have significantly low levels of GABA in their cerebrospinal fluid [42] as well as in their prefrontal, dorsolateral, and occipital cortex [43]. To study the ability of probiotics in preventing depression in humans, Steenbergen et al. [44] administered a multispecies probiotic containing *Lactobacillus brevis* W, *Bifidobacterium lactis* W, *Lactobacillus acidophilus* W37, *Bifidobacterium bifidum* W2, *Lactobacillus salivarius* W2, *Lactobacillus casei* W5, and *Lactococcus lactis* (W19 and W58) to 20 healthy individuals while the control group received a placebo for 4 weeks. After 4 weeks, the consumers of the probiotics showed a significant overall reduction in the cognitive reactivity to sad mood. Studies on the ability of probiotics to treat patients with depression and anxiety are however needed to validate this claim. Another symptom associated with depression is the alteration in the expression of central GABA receptor mRNA [45]. Interestingly, probiotic *Lb. rhamnosus* (JB-1) appeared to reduce the levels of stress-induced corticosterone and increase GABA Aα2 receptor mRNA expression in the hypothalamus while reducing the expression of GABA Aα2 receptor mRNA in the amygdala and prefrontal cortex in animal models [46] causing a reduction in stress. These researchers assumed that *Lb. rhamnosus* (JB-1) may influence brain and behavior by facilitating vagal firing [47]. Though the study showed how probiotics may affect mood and behavior, it is possible that other neurotransmitter systems and/neuropeptides may also be involved in the reduction of anxiety-like behavior and not just the regulation of the GABA receptors.

Other probiotics are known to modulate the production of serotonin in the brain. Serotonin synthesis in the brain depends on the levels of tryptophan and some probiotics have been found to elevate the levels of serum tryptophan [48]. Low serotonin levels have been linked with depression and hence probiotics that elevates tryptophan synthesis may potentially enhance serotonin synthesis. Chronic administration of *Bifidobacteria infantis* to rats has shown to increase plasma tryptophan levels relative to control groups and the increased tryptophan levels affect central neurochemical functions [49]. This phenomenon may account for why Rao et al. [48] observed a significant reduction in anxiety scores compared to controls after orally administering *Lactobacillus casei* strain Shirota to 39 patients with chronic fatigue syndrome for 2 months. However, since these researchers did not examine the bowel functions during the study despite an increase in fecal Lactobacillus and Bifidobacteria levels, it is possible that the reduced anxiety was due to an improvement in bowel functions.

Another means by which probiotics affect mood is their ability to modulate pain in the gut. Oral administration of *Lactobacillus acidophilus* has shown to reduce pain by inducing opioid and cannabinoid receptors in the intestinal epithelial cells [50]. The opium and cannabinoid systems are involved in mood regulation, pain, reward and addictive behavior. Mice lacking mu-opioid receptors do not exhibit morphine analgesic and addictive behaviors as compared with the wild type mice [51]. The ability of *Lactobacillus acidophilus* to induce opioid and cannabinoid receptors therefore opens new doors to enhancing pain relief in diseases associated with compromised gut epithelial barrier functions such as IBD through probiotic supplementation.

Conclusion

Although there is growing evidence to show the potential of probiotics to treat depression, anxiety and even improve symptoms associated with autism in animal models, their mechanisms of action and duration of the health effects are still not clearly understood. This information is important because many of the ingested probiotics pass out in feces and it is unclear whether they would leave lasting effects or the therapy (probiotic consumption) would have to be continued forever. In fact, the ability of probiotics to synthesize neuroactive compounds make them potential delivery vehicles for neurodevelopmental therapy as Lyte [52] proposed. However, do these consumed probiotics produce significant amounts of neurochemicals capable of causing any physiological effect in humans? The consumption of fermented foods date back to centuries and many fermented foods contain neuroactive compounds from some of these psycobiotics, however very little is known about the ability of these foods to treat neurodegenerative diseases or have any impact on mood. Much more human studies are therefore needed to substantiate the psychoactive ability of probiotics. It will also be helpful to know whether the tryptophan produced by probiotics is really related to the synthesis of serotonin. Further studies are also needed to better understand how gut bacteria induce EC cells to produce serotonin as observed by Yano et al. [21].

References

1. Montiel-Castro AJ, González-Cervantes RM, Bravo-Ruiseco G, Pacheco-López G (2013) The microbiota-gut-brain axis: neurobehavioral correlates, health and sociality. Front Integr Neurosci 7: 70.
2. DiGiulio DB, Romero R, Amogan HP, Kusanovic JP, Bik EM, et al. (2008) Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. PLoS One 3: e3056.
3. Ardissone AN, de la Cruz DM, Davis-Richardson AG, Rechcigl KT, Li N, et al. (2014) Meconium microbiome analysis identifies bacteria correlated with premature birth. PLoS One 9: e90784.
4. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, et al. (2011) Enterotypes of the human gut microbiome. Nature 473: 174-180.
5. Li J, Jia H, Cai X, Zhong H, Feng Q, et al. (2014) An integrated catalog of reference genes in the human gut microbiome. Nat Biotechnol 32: 834-841.
6. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, et al. (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464: 59-65.
7. Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, et al. (2013) Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 155: 1451-1463.
8. Tjellström B, Stenhammar L, Högberg L, Fälth-Magnusson K, Magnusson KE, et al. (2005) Gut microflora associated characteristics in children with celiac disease. Am J Gastroenterol 100: 2784-2788.
9. Carding S, Verbeke K, Vipond DT, et al. (2015) Dysbiosis of the gut microbiota in disease. Microb Ecol Health Dis 26: 26191.
10. Galland L (2014) The gut microbiome and the brain. J Med Food 17: 1261-1272.
11. Diaz Heijtz R, Wang S, Anuar F, Qian Y, Björkholm B, et al. (2011) Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci USA 108: 3047-3052.
12. Finegold SM, Molitoris D, Song Y, Liu C, Vaisanen ML, et al. (2002) Gastrointestinal microflora studies in late-onset autism. Clin Infect Dis 35: S6-6S16.
13. Beaumont W (1977) Nutrition Classics. Experiments and observations on the gastric juice and the physiology of digestion. By William Beaumont. Plattsburgh. Printed by F. P. Allen. 1833. Nutr Rev 35: 144-145.
14. Cannon WB (1909) The influence of emotional states on the functions of the alimentary canal. Am J Med Sci 137: 480–487.
15. Dinan TG, Stanton C, Cryan JF (2013) Psychobiotics: a novel class of psychotropic. Biol Psychiatry 74: 720-726.
16. Sasselli V, Pachnis V, Burns AJ (2012) The enteric nervous system. Dev Biol 366: 64-73.
17. Anderson RB, Newgreen DF, Young HM (2000) Neural crest and the development of the enteric nervous system: Madame Curie Bioscience Database. Austin (TX): Landes Bioscience.
18. Grundy D (2004) What activates visceral afferents? Gut 53 Suppl 2: 5-8.
19. Reigstad CS, Salmonson CE, Rainey JF, Szurszewski JH, Linden DR, et al. (20115) Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. FASEB J 29: 1395-403.
20. Frazer A, Hensler JG (1999) Serotonin involvement in physiological function and behavior. In: Siegel GJ, Agranoff BW, Albers RW (eds). Basic Neurochemistry: Molecular, Cellular and Medical Aspects. (6th edn). Philadelphia: Lippincott-Raven.
21. Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, et al. (2015) Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 161: 264-276.
22. Rhee SH, Pothoulakis C, Mayer EA (2009) Principles and clinical implications of the brain-gut-enteric microbiota axis. Nat Rev Gastroenterol Hepatol 6: 306-314.
23. Kang DW, Park JG, Ilhan ZE, Wallstrom G, Labaer J, et al. (2013) Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. PLoS One 8: e68322.
24. Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, et al. (2013) Increased abundance of *Sutterella* spp. and *Ruminococcus torques* in feces of children with autism spectrum disorder. Mol Autism 4: 42.

25. Adams JB, Johansen LJ, Powell LD, Quig D, Rubin RA (2011) Gastrointestinal flora and gastrointestinal status in children with autism-comparisons to typical children and correlation with autism severity. BMC Gastroenterol 11: 22.
26. Williams BL, Hornig M, Parekh T, Lipkin WI (2012) Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of Sutterella species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. MBio 3.
27. Felice VD, Quigley EM, Sullivan AM, O'Keeffe GW, O'Mahony SM, et al. (2016) Microbiota-gut-brain signaling in Parkinson's disease: Implications for non-motor symptoms. Parkinsonism Relat Disord pii: S1353-8020: 30066-9.
28. Scheperjans F, Aho V, Pereira PAB, Koskinen K, Paulin L, et al. (2014) Gut microbiota are associated with Parkinson's disease and clinical phenotype: a case-control study. Mov Disord 29: 1548.
29. Cassani E, Barichella M, Cancello R, Cavanna F, Iorio L, et al. (2015) Increased urinary indoxyl sulfate (indican): new insights into gut dysbiosis in Parkinson's disease. Parkinsonism Relat Disord 21: 389-393.
30. Forsyth CB, Shannon KM, Kordower JH, Voigt RM, Shaikh M, et al. (2011) Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. PLoS One 6: e28032.
31. Freeman HJ (2008) Neurological disorders in adult celiac disease. Can J Gastroenterol 22: 909-911.
32. Nadal I, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y (2007) Imbalance in the composition of the duodenal microbiota of children with coeliac disease. J Med Microbiol 56: 1669-1674.
33. Forsberg G, Fahlgren A, Hörstedt P, Hammarström S, Hernell O, et al. (2004) Presence of bacteria and innate immunity of intestinal epithelium in childhood celiac disease. Am J Gastroenterol 99: 894-904.
34. Cinova J, De Palma G, Stepankova R, Kofronova O, Kverka M, et al. (2011) Role of intestinal bacteria in gliadin-induced changes in intestinal mucosa: study in germ-free rats. PLoS One 6: e16169.
35. Erkin G, Culha C, Ozel S, Kirbiyik EG (2010) Feeding and gastrointestinal problems in children with cerebral palsy. Int J Rehabil Res 33: 218-224.
36. Trivedi MH (2004) The link between depression and physical symptoms. Prim Care Companion J Clin Psychiatry 6: 12-16.
37. Motil KJ, Caeg E, Barrish JO, Geerts S, Lane JB, et al. (2012) Gastrointestinal and nutritional problems occur frequently throughout life in girls and women with Rett syndrome. J Pediatr Gastroenterol Nutr 55: 292-298.
38. Wall R, Cryan JF, Ross RP, Fitzgerald GF, Dinan TG, et al. (2014) Bacterial neuroactive compounds produced by psychobiotics. Adv Exp Med Biol 817: 221-239.
39. Gabbay V, Mao X, Klein RG, Ely BA, Babb JS, et al. (2012) Anterior cingulate cortex Î³-aminobutyric acid in depressed adolescents: relationship to anhedonia. Arch Gen Psychiatry 69: 139-149.
40. Barrett E, Ross RP, O'Toole PW, Fitzgerald GF, Stanton C (2012) Î³-Aminobutyric acid production by culturable bacteria from the human intestine. J Appl Microbiol 113: 411-417.
41. Sanacora G, Saricicek A (2007) GABA ergic contributions to the pathophysiology of depression and the mechanism of antidepressant action. CNS Neurol Disord Drug Targets 6: 127-140.
42. Price RB, Shungu DC, Mao X, Nestadt P, Kelly C, et al. (2009) Amino acid neurotransmitters assessed by proton magnetic resonance spectroscopy: relationship to treatment resistance in major depressive disorder. Biol Psychiatry 65: 792-800.
43. Steenbergen L, Sellaro R, van Hemert S, Bosch JA, Colzato LS (2015) A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. Brain Behav Immun 48: 258-264.
44. Fatemi SH, Folsom TD, Rooney RJ, Thuras PD (2013) mRNA and protein expression for novel GABAA receptors Î¸ and Ï2 are altered in schizophrenia and mood disorders; relevance to FMRP-mGluR5 signaling pathway. Transl Psychiatry 3: e271.
45. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, et al. (2011) Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci USA 38: 16050-16055.
46. Perez-Burgos A, Wang B, Mao YK, Mistry MK, Neufeld KAM, et al. (2013) Psychoactive bacteria *Lactobacillus rhamnosus* (JB-1) elicits rapid frequency facilitation in vagal afferents. Am J Physiol Gastrointest Liver Physiol 304: G211-G220.
47. Rao AV, Bested AC, Beaulne TM, Katzman MA, Iorio C, et al. (2009) A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. Gut Pathog 1: 6.
48. Desbonnet L, Garrett L, Clarke G, Bienenstock J, Dinan T (2008) The probiotic *Bifidobacterium infantis*: an assessment of potential antidepressant properties in the rat. J Psychiatr Res 43: 164-74.
49. Rousseaux C, Thuru X, Gelot A, Barnich N, Neut C, et al. (2007) *Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors. Nat Med 13: 35-37.
50. Nogueiras R, Romero-Picó A, Vazquez MJ, Novelle MG, López M, et al. (2012) The opioid system and food intake: homeostatic and hedonic mechanisms. Obes Facts 5: 196-207.
51. Moles A, Kieffer BL, D'Amato FR (2004) Deficit in attachment behavior in mice lacking the mu-opioid receptor gene. Science 304: 1983-1986.
52. Lyte M (2011) Probiotics function mechanistically as delivery vehicles for neuroactive compounds: Microbial endocrinology in the design and use of probiotics. Bioessays 33: 574-581.