

## PROBIOTICS AND MANAGEMENT OF AUTISM SPECTRUM DISORDERS

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Review

### ABSTRACT

Studies on gut microbiota have revealed surprisingly diverse effects on human immune system and the disturbances in composition of the commensal microorganisms have shown to increase the incidence of diseases. The microorganisms from these metabolites are helpful in the regulation in the host system. This present review focuses on the intricate mechanisms of immune modulation by probiotic microorganisms and the pathways. Gut microbiome is an important modulator of brain functions as well as behaviour. This review specifically deals with the analysis of microbiota of gut in autism spectrum disorders. They are a group of neurological illness which causes difficulty in social behaviour and communication.



**Keywords:** Autism Spectrum Disorders, Probiotics, Microbiome, Gut Brain Axis

### INTRODUCTION

Probiotic is axiom of the modern period, which means “for life”. This term is used for the bacteria which is not harmful but helpful for the host system in various ways (Bagchi, 2014). Human gut is a reservoir of over 100–1000 different microbial species, which constitutes ‘the human-microbiome super-organism’. In particular, they are vital in the eupepsia, defence function, and the brain-gut responses (George Kerry et al., 2018). Probiotics are able to modulate, stimulate and control the immune response of host by activating particular genes in host cells. They also regulate the release of gastrointestinal hormone and modulate the response of brain via bidirectional signalling of neurons (Kristensen et al., 2016). According to recent studies the type of microflora changes with aging. Human gut microbiota majorly consists of organisms belonging to five different phyla *Verrucomicrobia*, *Actinobacteria*, *Firmicutes*, *Proteobacteria* and *Bacteroidetes*. Among these *Firmicutes* and *Bacteroidetes* constitute about 90% of the total bacterial species. The ratio of *Bacteroidetes* to *Firmicutes* decreases with aging and a noticeable decline of *Bifidobacteria* concentration has been reported (Lloyd-Price et al., 2016). The concentration of commensal organisms is minimal in stomach due to inhibitory acidic conditions; however, it gradually increases to very high concentrations in the colon. Here the microbiota is dominated by gram-negative anaerobic bacteria (Giorgetti et al., 2015). Health benefits of probiotics are obtained mostly through transient colonization of probiotic bacteria and restoration of normal healthy microbiota of the gut. *Lactobacillus*, *Bifidobacterium* and *Saccharomyces* are three extensively studied and commonly used probiotic strains. Investigators are trying to discover novel species of probiotic bacteria (Leuschner et al., 2010).

### BENEFICIAL EFFECTS OF PROBIOTICS

Mechanisms by which probiotics affect gut microflora are (1) competition with pathogenic microorganisms for nutrients (2) bioconversion (sugars into fermentation products) (3) production of growth substrates (extracellular polymeric substances or vitamins) (4) production of antimicrobial substances (5) competition for binding receptors (6) improvement of epithelial barrier (7) reduction of inflammation (8) stimulation of innate and adaptive immune response. Adhesion of probiotics initiates the repair of the barrier function by secreting antimicrobial substances or proteins, promote mucous secretion through the exclusion of pathogens and vital for the immune system functioning (Hirano et al., 2003; Perdigón et al., 2002; Schiffri et al., 1997). All these mechanisms are inter-related and help in elimination of pathogens.

Probiotic organisms affect composition of microbiota by competing for substrate availability. In oral cavity, *Bifidobacterium* strains (*B. adolescentis*) compete with *Porphyromonas gingivalis* and reduce vitamin K (growth substrate)

concentration (Hojoet et al., 2007). Repair of intestinal tissues is facilitated by several mechanisms (1) secretion of organic acids like lactic, formic and phenylacetic acid which take down the pH and induces growth inhibition of harmful microorganisms (2) building short chain of fatty acids that show benefits like reduction of obesity in mice (3) making of antimicrobial compounds such as hydrogen peroxide and bacteriocins (Ammoret et al., 2006; Choi and Chang, 2015; Hassan et al., 2012; Macouzet et al., 2009; Ouwehand and Vesterlund, 2004; Tharmaraj and Shah, 2009) (4) Inhibition of proinflammatory cytokines by probiotics result in suppression of inflammation and stimulate immune response (Gill et al., 2001, 2000; O’Hara et al., 2006; Sheilet et al., 2004; So et al., 2008).

### GUT-BRAIN AXIS

Gut-brain axis is the two way communication between gastrointestinal tract (GIT) and brain. The scaffolding of it consists of GIT, enteric nervous system (ENS), central nervous system (CNS), neuroimmune systems, parasympathetic and sympathetic arms of the autonomic nervous system and neuroendocrine. Signals from brain affect the sensory, motor and secretory function (Grenham et al., 2011). It is synchronized at the immune hormonal, immunological levels and neural for homeostasis sustainment (Cryan and Dinan, 2012; Matsumoto et al., 2013). Various evidences have revealed that the modification in the microbiota of gut affects brain function as well as modulates behaviour. Cholecystokinin (CCK) is out of the various rich neuropeptides and a ‘brain – gut peptides’ member (Liddle, 1994). The postprandial release of CCK plays an important role in intestinal feedback control of gastrointestinal function. Therefore, CCK plays a vital role in nutrients entry into the small intestine and its digestion (Dockray, 1976; Dockray et al., 1978; Muller et al., 1977; Rehfeld and Kruse-Larsen, 1978; Robberecht et al., 1978; Straus et al., 1977).

Probiotics modulate and improve stress response, anxiety and mood signs in chronic fatigue patients and irritable bowel syndrome (IBS) (Lakhan and Kirchgessner, 2010). Variation in the composition of microbiota of gut may be linked with pathological process of neurological disorders including autism, stress, Parkinson’s disease, Alzheimer’s disease and depression (Dash et al., 2015; Dinan and Cryan, 2017; Inoue et al., 2016; Kelly et al., 2016; Mahony et al., 2015; Pistollato et al., 2016; Scheperjans, 2016). Fermented milk consumption which contains the probiotics mixture has been reported to play a role in sensation in healthy women and emotion balance (Tillisch et al., 2013). In a study, the response of conservatively reared specific pathogen-free (SPF) mice and female germ-free (GF) mice were compared. Female germ-free mice showed an elevated response of stress in comparison to the SPF mice. Distorted level of gene expression of BDNF (brain-derived neurotrophic factor), serotonin and glutamate receptors which have been implicated in anxiety were also seen in the

GF mice (Neufeld et al., 2011). Blood brain barrier (BBB) permeability was lower in SPF mice as compared to GF mice. Several cases have shown that gut microbiome of ASD patients have modifications in the configuration of the

metabolic products and faecal flora. However, the fundamental mechanisms are still remained unknown. [Table 1].

**Table 1** List of probiotic microorganisms and their observed effects on nervous system

MICRO-ORGANISMS	RESPONSE	ORGANISM	REFERENCE
<i>Lactobacillus helveticus</i> and <i>B. longum</i>	Decrease the level of anxiety and serum cortisol	Human and rats	(Messaoudiet al., 2011)
<i>Lactobacillus reuteri</i>	Alter immune system Decreases anxiety Stimulates corticosterone rise	Mice	(Bravo et al., 2011; Ma et al., 2004)
<i>Lactobacillus rhamnosus</i>	Decline in stress-induced corticosterone which lowered depression-related behaviours and anxiety.	Mice	(Bravo et al., 2011)
<i>Lactobacillus reuteri</i> and <i>L. rhamnosus</i>	Alterations in GABA <sub>A</sub> and GABA <sub>B</sub> which changes mRNA expressions Alter depression-related behaviours and anxiety	Mice	(Bravo et al., 2011)
<i>Bacillus infantis</i>	Antidepressant properties	Sprague-Dawley rats	(Cryan et al., 2005; Desbonnet et al., 2008)
	Relieve stress induced changes	Preclinical model of IBS	(Desbonnet et al., 2010)
<i>Lactobacillus casei</i> (Shirota)	Decline of anxiety but more improvement in mood	Chronic fatigue syndrome patients	(Benton et al., 2007; Rao et al., 2009)
<i>Lactobacillus acidophilus</i> , <i>Bacillus lactis</i> and <i>Lactobacillus fermentum</i>	Recovery of weak synaptic transmission Repair the hippocampus for its LTP Reduction in the level of glucose in blood as well as 8-OHdG factor by increasing the insulin level Activation of superoxide dismutase.	Diabetic rats	(Davari et al., 2013)

Probiotics lower the concentration of inflammatory cytokines and reduce the oxidative stress. Probiotic consumption has been shown to increase BDNF: low BDNF levels have been correlated with anxiety and depression (Bergamiet al., 2008; Brenner et al., 2009; Logan and Katzman, 2005; O’Leary et al., 2009; O’Mahony et al., 2005). Probiotics can control various gut-brain axis features and simultaneously provide potential benefits in the management of depressive behaviours, stress and anxiety. Moreover, disorders, like ASD, mood disorders and inflammatory bowel disease, and others are linked to abnormal gut microbiota (Li et al., 2017).

**POTENTIAL RELATIONSHIPS BETWEEN THE MICROBIOTA AND ASD**

The GIT mucosa has millions of neurons which consist of ENS and regulate GI function. The gut microbiota effects mammalian brain differentiation and successive adult behaviour. GI barrier had been shown to be faulty in case of ASD in mice model. The faulty GI barrier allows the bacterial products and toxins entry into the bloodstream and affects the behaviour of the brain (Hsiao et al., 2013; Onoreet al., 2012). Disturbances in the gut microbiota composition and their metabolic products were seen in ASD patients and in ASD animal models (Borre et al., 2014; de Magistris et al., 2010; Kushaket al., 2016). Behavioural manifestations of ASD such as nervousness, self-harm and anger have been proved to be linked to GI micro-organisms (Angelis et al., 2015; Buie et al., 2010; Mead and Ashwood, 2015). White blood cells and cytokines like IL-6, IL-1β, TNF-α and IFN-γ are there in the cross BBB and circulation. On brain endothelial cells, TNF-α and IL-1β attach and stimulate immune responses (de Theijeet al., 2011; Li et al., 2009). The concentration of these cytokines is also modulated by probiotics. ASD affected individuals have higher serum concentrations of lipopolysaccharides as compared to the healthy individuals. Lipopolysaccharides are the cell wall components of Gram-negative bacteria (Emanuele et al., 2010). Microbial products like acetate and propionate can change the BBB function (Branisteet al., 2014). Studies found that intestinal components of tight junction (TRIC, CLDN-1 and OCLN), the BBB and gut barrier were compromised and levels of claudin (MMP-9, CLDN-12, CLDN-5 and CLDN-3) were higher in the patients from ASD as compared to the control

mice models (Fiorentino et al., 2016). Studies showed that microbiota modification and GI barrier defects in a mouse resulted in the ASD characteristics development in the model. Concentration of bacteria belonging to *Porphyromonada*, *Prevotella*, *Lachnospira* and unclassified *Bacteroidales* in progeny of maternal immune activation mothers were found to be higher than the control progeny; whereas, the concentration of *Ruminococca*, *Erysipelotricha*, and *Alcaligena* were richer in the control individuals (Hsiao et al., 2013). The valproic acid induced ASD mice models also demonstrate variations in Firmicutes and Bacteroidetes ratio (Theijeet al., 2014a). Table 2 summarizes the various researches that compare the gut microbiota in ASD and control subjects. Maternal immune activation (MIA) models are produced by triggering the maternal immune system by infectious bacteria or their products. This results in alterations in cytokines and immunological effectors that are delivered to the foetus, producing in abnormal CNS phenotypes.

The gut microflora secretes neuroactive substances such as dopamine (DA), histamine and GABA that either activate or inactivate the CNS with the help of cranial nerve X (Eisenstein, 2016; Spiller and Major, 2016). Mice researches have confirmed the gut microbiota’s role on modulation of concentrations of different neuroactive substances (Clarke et al., 2013; Diamond et al., 2011; Diaz Heijtz et al., 2011; Neufeld et al., 2011). Antibiotics have been shown to decrease gut microbiota which increases depression-like behaviours and damages learning. Further, glucocorticoid receptor intensity and corticotrophin-releasing hormone receptor 1 levels were seen altered in previous studies (Hoban et al., 2017).

5-HT or Serotonin transporter is synthesized in intestines as well as in brain, which regulates mood and cognition (Cryan et al., 2000). It was found that there is a connection between the GI symptoms and whole-blood serotonin levels in ASD patients (Marler et al., 2016). Theijeet al. (2014b) studied the changes in murine model of ASD induced by prenatal exposure to valproic acid (VPA). Valproic acid exposed male offspring showed reduced social behaviour, lower concentration levels of serotonin in prefrontal cortex and amygdala and other changes like epithelial cell loss as well as increased expression of neuroinflammatory markers.

**Table2** Comparison of gut microbiota of ASD and normalsubjects

ASD compared to Normal	Proposed mechanism	Samples	Reference
Lesser quantity of the genera <i>Prevotella</i> , <i>Coprococcus</i> , and <i>unclassified Veillonellaceae</i>	None	Gut	<a href="#">(Kang et al., 2013)</a>
Higher levels of <i>Firmicutes</i> and <i>Bifidobacterium</i> plus lower levels of <i>Clostridium</i> , <i>Lactobacillus</i> , <i>Bacteroidetes</i> , <i>Sarcina</i> , <i>Desulfovibrio</i> and <i>Caloramator</i>	None	Gut	<a href="#">(Adams et al., 2011; De Angelis et al., 2013; Finegold, 2011; Finegold et al., 2010, 2002)</a>
Increased levels of the group <i>Clostridium histolyticum</i> ( <i>Clostridium</i> clusters II and I)	Neurotoxins might exert systemic effects Decrease in <i>Clostridium</i> shows improvement in ASD children	Fecal	<a href="#">(Parracho, 2005; Sandler et al., 2000)</a>
<i>Candida</i> was two times more abundant in autistic patients	<i>Candida</i> discharges toxins and ammonia that can induce autistic like behaviours Deteriorate dysbiosis	Gut	<a href="#">(Burrus, 2012; Iovene et al., 2017; Strati et al., 2017)</a>

**CONCLUSION**

The latest scientific papers prove the relation between human health and gut microbiota. Disturbances in composition of gut microbiota are known in many diseased related conditions including chronic inflammation, autoimmune and neurological disorders. In autistic children a study of the fecal samples showed to have abnormally higher concentrations of *Clostridium histolyticum* group (*Clostridium* clusters II and I) in contrast to healthy children and in which neurotoxins released by clostridium being ultimately responsible for the disturbances in the gut brain axis that might cause autistic like behaviour. The current usage of antibiotics and other antibacterial agents on the intestinal microbiota has showed to have some harmful effects while on the other hand, probiotics with beneficial effects of useful bacteria has microorganisms stimulating the growth of other microorganism which creates a appreciative environment for the viability of microorganisms that consequently have a beneficial effect on the host. Reduced concentration of *Clostridium* improved the autistic like behaviour in children which proves that probiotics can be a new medication therapy for autistic individuals. Probiotics have been shown to restore the natural healthy flora of the gut providing benefits to the host. Strong scientific evidence has been accumulated that illustrates the two way communication path between gut microbiota and the nervous system and its role in maintaining the neurological health. These researches are not only a way forward in unravelling several baffling patho-physiologies in complex disorders but raise a hope for possible therapeutic interventions. Well-designed studies in model organisms or other disease models would yield a greater insight into these problems and would yield novel therapeutic targets and drugs.

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## ABBREVIATIONS

- 8-OHdG – 8-hydroxydeoxyguanosine
- ASD – Autism spectrum disorder
- BBB – Blood brain barrier
- BDNF – Brain-derived neurotrophic factor
- CCK – Cholecystokinin
- CNS – Central nervous system
- DA – Dopamine
- DCs – Dendritic cells
- ENS – Enteric nervous system
- GF – Germ-free
- GI – Gastrointestinal
- GIT – Gastrointestinal tract
- IBD – Inflammatory bowel disease
- IBS – Irritable bowel syndrome
- IECs – Intestinal epithelial cells
- IFN – Interferon
- Ig – Immunoglobulin
- IL – Interleukin
- LPS – Lipopolysaccharide
- MAPK – Mitogen-activated protein kinase
- NF-κB – Nuclear Factor- κB
- NK – Natural killer
- SPF – Specific pathogen-free
- TNF-α – Tumor necrosis factor alpha