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Gut microbiota, the pharmabiotics they produce and host health

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A healthy gut microbiota plays many crucial functions in the host, being involved in the correct development and functioning of the immune system, assisting in the digestion of certain foods and in the production of health-beneficial bioactive metabolites or 'pharmabiotics'. These include bioactive lipids (including SCFA and conjugated linoleic acid) antimicrobials and exopolysaccharides in addition to nutrients, including vitamins B and K. Alterations in the composition of the gut microbiota and reductions in microbial diversity are highlighted in many disease states, possibly rendering the host susceptible to infection and consequently negatively affecting innate immune function. Evidence is also emerging of microbially produced molecules with neuroactive functions that can have influences across the brain–gut axis. For example, γ-aminobutyric acid, serotonin, catecholamines and acetylcholine may modulate neural signalling within the enteric nervous system, when released in the intestinal lumen and consequently signal brain function and behaviour. Dietary supplementation with probiotics and prebiotics are the most widely used dietary adjuncts to modulate the gut microbiota. Furthermore, evidence is emerging of the interactions between administered microbes and dietary substrates, leading to the production of pharmabiotics, which may directly or indirectly positively influence human health.

Gut mircobiota: Pharmabiotics: Gut–brain axis

The microbial ecosystem residing in the human gut consists of over 100-fold more genes than the human genome[1–3] and is tantamount to a virtual organ. To a large extent, intestinal ecological conditions are set by the host and resident commensals must adapt to this environment. Host–microbe, environment–microbe and microbe–microbe interactions may also dictate the composition of this microbial community. A symbiotic relationship exists between the gut microbiota and host such that both partners benefit; the host provides protection and nutrients for the micro-organisms to flourish within[4], whereas the microbiota contribute to food digestion, inhibit the growth of potential invading pathogens, convert harmful compounds into less toxic substances and produce bioactive molecules, which play a role in host physiology[5]. Disruptions to this symbiotic relationship can occur, for example, during certain disease states and during adoptive pathogenesis by certain commensal gut microbes causing small intestinal bacterial overgrowth[6,7] and/or translocation to other tissues and organs[8,9]. However, it remains unclear whether disease development is causal or consequential of an altered intestinal microbiota, with an increasing body of evidence describing a link between the two.

Microbial colonisation of the infant intestine begins at birth[10]. Extrinsic factors contribute to the initial colonisation of the infant gut[11,12], including mode of delivery[13], feeding regime[14,15], gestational age at birth[16]

Abbreviations: CLA, conjugated linoleic acid; CNS, central nervous system; EPS, exopolysaccharides; GABA, γ-aminobutyric acid; IBD, inflammatory bowel disease; LPS, lipopolysaccharide; T1D, type-1-diabetes; T2D, type-2-diabetes.

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and antibiotic therapy[17,18]. Intestinal establishment of a healthy microbiota is believed to have a profound impact on the development and maturation of the immune system[19]. Vaginally born infants are initially colonised by faecal and vaginal bacteria from the mother, whereas infants delivered by Caesarean-section render the gut susceptible to colonisation by maternal skin microbiota and bacteria from the hospital environment[13,20,21]. It has been shown that vaginally born babies have higher numbers of *Lactobacillus* and *Bifidobacterium*, compared with infants delivered by Caesarean-section[13]. The weaning process determines the transition of an unstable infant microbiota to a more complex adult-like microbial ecosystem[10,22]. The development and diversification of the gut microbiota continues into adulthood and is further influenced by several factors, including diet and environment[23]. To a large extent, the gut microbiota remains relatively stable throughout adulthood, unless perturbed by extrinsic or host factors, including antibiotic treatment and inflammation, respectively.

The tools used for studying the link between gut microbial diversity and health status have improved our knowledge of the host–microbe relationship significantly. Culture-independent analysis of the composition and functional capacity of the gut microbiome targets the 16S rRNA gene, due to its presence in all prokaryotes with the existence of variable domains that allow different taxa to be identified. Although compositional studies generate a large volume of data, they fail to provide direct information regarding the microbial viability or the functional potential of the populations present and so the knowledge generated is somewhat limited in these aspects. Metagenomic studies go beyond the 16S rRNA gene to sequence small fragments of metagenomic DNA at random to characterise the full genetic content and functional potential of the microbial community[2,24,25]. The development of methods used to analyse gene expression (metatranscriptomics), protein products (metaproteomics) and metabolic profiles (metabolomics) of the gut microbiota has further enabled such studies to identify the microbial activity and to link this with compositional analysis, to determine host–microbe interactions[26].

Dietary interventions with probiotics and prebiotics, due to the dynamic nature of the gut microbial ecosystem, have become an attractive means of self-manipulating the microbiota to improve health status, and have been extensively reviewed[27,28]. Probiotics are defined as 'live micro-organisms which when administered in adequate amounts confer a health benefit on the host'[29] and have been shown to improve intestinal barrier function, modulate the immune system and enhance the host defence system by competing against pathogens for nutrients and binding sites. In addition, numerous probiotic intervention studies have revealed their functional capacity to improve certain gastrointestinal disorders, for example, irritable bowel syndrome, inflammatory bowel disease (IBD)[30,31] and antibiotic-associated diarrhoea[32,33]. *Bifidobacterium* and *Lactobacillus* are the main genera of micro-organisms used as probiotics as many of them can survive gastrointestinal transit, have the capability to adhere to intestinal epithelial cells and are regarded as safe[34]. Prebiotics are non-digestible food ingredients that selectively stimulate the growth of beneficial indigenous microbes already established within the gut such as bifidobacteria and lactobacilli[35]. Typically, prebiotics must reach the large intestine unaltered, resisting host digestion, absorption and adsorption to be fermented by the gut microbiota. Commonly used prebiotics include inulin, fructo-oligosaccharides and galacto-oligosaccharides[36]. The fermentation of prebiotics by the gut microbiota generates SCFA, such as butyric and acetic acids, which are linked with numerous health benefits *in vivo*[35,36]. This review will focus on the importance of the microbiota to host health and in establishing a healthy immune system and describes some of the known beneficial bioactive metabolites produced by the microbiota that impact on health.

Role of the microbiota in establishing a healthy immune system

During the first year of life, the immature developing gut microbial ecosystem rapidly shapes the maturation of the infant immune system, while the immune system also influences the gut microbiota[37]. From birth, breast milk provides passive transfer of maternal antibodies to the infant which shapes both the immature immune system and gut microbiota[4]. Much of the information regarding the influence of gut microbes on the host immune system is generated from studies using germ-free animals, i.e. those born and reared without exposure to micro-organisms such that the immune responses have not been influenced by interactions with molecules of commensal and pathogenic micro-organisms. Consequently, germ-free animals show defects in both the development of the immune system and in immune responses. One of the first immunological defects observed in these animals was a marked reduction in antibodies produced within the intestine[38]. Furthermore, germ-free animals show extensive defects in the development of gut-associated lymphoid tissue and have fewer and smaller Peyer's patches and mesenteric lymph nodes, compared with animals housed under specific pathogen-free conditions[39–42]. Intestinal epithelial cells have many immunological functions; they secrete and respond to various cytokines and express molecules that directly interact with lymphocytes and line the gut to form a physical barrier between the luminal contents (including the microbiota) and the underlying cells of the immune system[43]. Germ-free mice demonstrate a reduced number of these cells, whereby their function is compromised[44,45] and demonstrate decreased cell turnover rates of these cells[46]. Furthermore, animals lacking a gut microbiota are more susceptible to infection due to a poorly developed immune system; e.g. germ-free guinea-pigs challenged with the enteric pathogen *Shigella flexneri* demonstrated a decrease in the immune resistance to infection coupled with an increase in mortality[47], while infection with

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the intracellular pathogen *Listeria monocytogenes* in germ-free mice resulted in decreased pathogen clearance, compared with conventionalised animals[48]. Deliberate colonisation of the sterile gut of these animals either with a single microbial species or a defined species mixture, termed 'gnotobiotics' is a powerful technological tool for determining which host immune functions are genetically encoded and which require interactions with microbes[49]. For example, colonisation of germ-free animals with a single bacterium, *Bacteroides fragilis*, has been shown to protect against inflammation in an animal model of experimental colitis[50,51]. Collectively, these observations suggest that developmental defects through the absence of a gut microbial ecosystem compromise immune function of the host at the tissue, cellular and molecular levels and highlight a role of the microbiota in the establishment of a functional immune system.

Implications of a perturbed gut microbiota on immune function and health

Antibiotic therapy soon after birth has been shown to impact gut microbiota composition up to 8 weeks after treatment[17,18]. This could have a negative impact on the development of the immune system, predisposing the infant to development of asthma, obesity and allergies[17,18]. Thus, disruptions in the host–microbe relationship can undoubtedly predispose to disease, from infancy to adulthood. There has been a rapid increase in the development of disorders such as IBD, asthma, rheumatoid arthritis, diabetes and obesity, particularly within developed, Western populations. Indeed, the role of a perturbed gut microbial ecosystem in such diseases is becoming more evident, although future work is needed to decipher the mechanisms involved.

Inflammatory bowel disease

IBD comprises a group of disorders characterised by severe intestinal inflammation and is characterised as either Crohn's disease or ulcerative colitis, based on the location of the gastrointestinal tract affected. Although the exact causes of IBD remain unclear, the onset of both conditions is generally thought to be due to an overall disruption in the host–microbe relationship, and not by a single causal organism[52,53]. Recently reviewed, numerous studies which indicate a role of the gut microbiota in the manifestation of IBD generally conclude that the gut microbiota are involved in the development of mucosal lesions causing intestinal inflammation[54]. Inflammatory damage in IBD has been linked to alterations in the relative abundances of *Enterobacteriaceae, Ruminococcaceae* and *Leuconostocaceae*[55] and an overall decrease in bacterial diversity[56,57]. The incidence of *Clostridium difficile* carriage, an opportunistic pathogen frequently linked with antibiotic-associated diarrhoea, has been reported to be over 8-fold higher in patients suffering from IBD, compared with healthy controls[58].

Type-1 and type-2 diabetes

The incidence of both type-1- (T1D) and type-2- (T2D) diabetes have increased dramatically in recent decades. Although genetic factors play a role in disease onset, particularly in predisposing individuals to T1D, T2D is principally linked to obesity associated insulin resistance. Recent studies demonstrate disruptions to the host–microbe relationship and gut microbial composition and diversity associated with both T1D and T2D. Compositional sequencing studies have revealed a reduction in the relative proportions of Firmicutes, while Bacteroidetes were enriched in T2D subjects, compared with healthy controls[59]. Identification of gut microbial markers associated with the moderate degree of microbial disruption in patients with T2D could be useful in the future management of this disease[60]. Furthermore, increases in opportunistic pathogens such as *Clostridium* were identified as contributing to disruption of host–microbe interactions associated with T2D[60]. Creating a link between the gut microbiota and T1D is more difficult, since genetic factors play a more significant role in this disease. However, evidence indicates that alterations in the intestinal microbiota are associated with T1D and subsequent insulin dependence in various models of the disease. While one study demonstrated that the stool of bio-breeding diabetes-resistant rats contained higher relative abundances of *Lactobacillus* and *Bifidobacterium*, compared with bio-breeding diabetes-prone rats[61], others have reported that lactate-producing species such as *Lactobacillus, Lactococcus* and *Bifidobacterium* were increased in the stool of children who tested positive for T1D-associated autoimmunity[62,63]. Furthermore, low relative abundances of two of the most common *Bifidobacterium* species, *B. adolescentis* and *B. pseudocatenulatum* have been associated with autoimmunity in children who tested positive for at least two T1D-associated autoantibodies[64].

Obesity

Excessive energy intake over expenditure is the main cause of obesity, since host-negative feedback signals are insufficient to maintain normal weight in circumstances of plentiful food/energy supply. Although lifestyle, genetic factors, diet and exercise undoubtedly contribute largely to this modern epidemic, an increasing body of evidence suggests that disruptions to the host–microbe relationship also contribute[65–68]. Identifying specific populations which may be associated with weight gain has been the subject of much debate, often differing among various models of obesity in both rodent and human subjects. Genetically (ob/ob) and diet-induced obese mice have been shown to harbour an increased Firmicutes:Bacteroidetes ratio, compared with their lean counterparts[66]. Furthermore, weight loss in human subjects has been linked with decreased Firmicutes:Bacteroidetes ratio[1], but yet the relevance of the Firmicutes:Bacteroidetes ratio in obesity remains

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unclear[69]. The gut microbiota also increase the dietary energy-harvesting capacity of the host[70] and conventionally raised mice have been shown to contain 40 % more body fat than their germ-free counterparts, while colonisation with a conventional gut microbiota induced hepatic lipogenesis and increased lipid storage in adipocytes[3].

Obesity is also associated with low-grade inflammation, which may be linked to host–microbe interactions. Data from several studies have revealed that the lipopolysaccharide (LPS) endotoxin derived from certain components of the gut microbiota contributes towards obesity-associated inflammation. Endogenous LPS is continuously produced in the gut as a consequence of inactivation of Gram-negative bacteria, since LPS is a component of the Gram-negative bacterial cell wall and acts through the Toll-like receptor 4/MyD88/NF-κB-signalling pathway. LPS-induced inflammation could also be an early factor which triggers high-fat diet-induced metabolic diseases, otherwise known as metabolic endotoxemia[71]. It has been shown that high-fat feeding increased plasma LPS levels throughout the day, compared with controls, resulting in significant increases in fasting blood glucose, insulin, liver TAG content, body weight and proinflammatory cytokine mRNA expression, similar to mice that were infused with LPS[71]. Further studies examined the effect of changes in the gut microbiota leading to LPS-induced metabolic endotoxemia[72]. It was revealed that while plasma LPS levels were increased following high-fat feeding relative to controls, this result was overturned in high-fat diet-fed mice following antibiotic treatment[72]. Such studies reveal that disruptions to host–microbe interactions within the gut following obesity and high-fat diet may generate increased gastrointestinal levels of microbial-derived LPS endotoxin, associated with metabolic endotoxemia.

Microbial metabolism of choline and CVD

Choline is a water-soluble essential nutrient, an important component of cell membranes and mediates lipid metabolism and VLDL synthesis in the liver[73]. In addition, choline is a precursor to the neurotransmitter acetylcholine, which has important functions in cognition, as discussed later. While small quantities of choline are continuously synthesised by the host, it is mostly obtained from foods such as red meats and eggs. From infancy, breast milk is an important source of choline and the US Food and Drug Administration requires that infant formula not made from cow's milk be supplemented with choline. Much like a choline-deficient diet, microbial metabolism of choline decreases the bioavailable levels of this essential nutrient and triggers non-alcoholic fatty liver disease[74]. The gut microbiota play a role in the transformation of dietary choline to trimethylamine with subsequent metabolism in the liver to the toxic methylamine, trimethylamine-N-oxide[74,75]. Excess plasma levels of the pro-atherosclerotic metabolite trimethylamine-N-oxide and its metabolites are associated with CVD[76]. One recent study highlighted a direct link between increased plasma trimethylamine-N-oxide levels, increased risk of major adverse cardiovascular events and the gut microbiota[77]. Plasma trimethylamine-N-oxide levels were suppressed following antibiotic treatment, but reappeared following antibiotic withdrawal[77].

Host–microbe interactions, generation of long-chain PUFA and microbial metabolite production with health effects

The products of human enteric microbial metabolism often act as signalling molecules, developing 'intelligent communication systems' in the body. These pharmabiotics can exert beneficial health effects, which directly impact host intestinal function but may also affect the liver and brain[78]. Host–microbe interactions can together co-metabolise dietary components to produce a large array of molecules with beneficial impacts on health. Commensal bacteria have been shown to synthesise essential vitamins such as vitamin K_2 and B vitamins[79], can alter *n*-3 PUFA metabolism to generate increased levels of long-chain PUFA metabolites such as EPA and DHA[80,81], can produce conjugated fatty acid derivatives of PUFA such as conjugated linoleic acid (CLA) and conjugated α-linolenic acid[82,83] and can increase production of SCFA[81]. The beneficial impacts of some of these bioactive compounds on host health are reviewed later and summarised in Table 1.

Vitamin synthesis

Some commensals of the human gut microbiota possess the ability to synthesise menaquinone (vitamin K_2), as well as many of the water-soluble B vitamins such as biotin, cobalamin, folate, nicotinic acid, panthotenic acid, pyridoxine, riboflavin and thiamine[92]. In particular, many *Bifidobacterium* strains have been shown to exhibit vitamin production capabilities[93–95]. Vitamin K is a lipophilic vitamin which acts as a co-factor for the enzyme γ-carboxylase, which converts specific glutamyl residues in a limited number of proteins to γ-carboxyglutamyl (Gla) residues, responsible for high-affinity binding of calcium ions[92]. The daily requirement for vitamin K is fulfilled by dietary phylloquinone, present in plants and to an undetermined extent, by bacterially produced vitamin K_2[92]. Vitamin K is important for blood clotting, bone and vascular health and deficiencies have been associated with low bone mineral density[96,97], increased risk of fracture[98,99] and CVD[100].

Vitamin B_{12} is a type of cobalt corrinoid, particularly of the cobalamin group and is solely synthesised by some bacteria and archea. Vitamin B_{12} biosynthesis was first identified in *Propionibacteria freudenreichii*, now used in the commercial production of the vitamin[101]. Furthermore, *Lactobacillus reuteri* CRL1098 was shown to be the first lactic acid producing bacterial strain

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Table 1. Effects of probiotic metabolite production on host metabolic and psychiatric health

Metabolite	Probiotic strain	Model	Trial duration	Health effects	Reference
Metabolic health					
CLA	*Lactobacillus rhamnosus* PL60	C57Bl/6J mice	8 weeks	Reduced body weight, reduced white adipose tissue and no presence of liver steatosis. Anti-obesity effect	(84)
	Lactobacillus plantarum PL62	C57Bl/6J mice	8 weeks	Reduced body weight, reduced serum and leptin levels. Anti-obesity effect	(85)
	Bifidobacterium breve NCIMB 702258	BALB/c mice, SCID mice, swine	8–10 weeks	Reduced inflammatory profile and higher concentrations of *n*-3 fatty acids in the adipose tissue	(80)
n-3 long-chain PUFA	*B. breve* NCIMB 702258	BALB/c mice	8 weeks	Increased liver EPA and brain DHA concentrations	(86)
		C57BL/6J mice	8 weeks	Increased arachidonic acid and DHA concentrations in the brain	(81)
SCFA	*B. breve* DPC 6330	C57BL/6J mice	8 weeks	Both strains increased propionate concentrations in the caecum	(81)
	B. breve NCIMB 70228				
EPS	*Lactococcus lactis subsp cremoris* SBT 0495	F-344 rats	7 d	Serum cholesterol level of rats fed the ropy fermented milk were the lowest among the three treatments	(87)
	Lactobacillus kefiranofaciens	BALB/c mice	2, 5 and 7 d	Positive influence of EPS on systemic immunity and maintenance of intestinal homoeostasis	(88)
Psychiatric health					
GABA	*Lactobacillus brevis* FPA3709	Sprague Dawley rats	28 d	Similar antidepressant effects to a commonly used antidepressant drug in the forced swin test	(89)
	Lactobacillus rhamnosus JB-1	BALB/c mice	28 d	Reduced stress and corticosterone and reduced anxiety and depression-related behaviour	(90)
Serotonin	*Bifidobacterium infantis* 35624	Sprague-Dawley rats	14 d	Elevated the plasma levels of the serotonergic precursor, tryptophan	(91)

CLA, conjugated linoleic acid; EPS, exopolysaccharides; GABA, γ-aminobutyric acid.

capable of producing a cobalamin-like compound with an absorption spectrum resembling that of standard cobalamin[102]. The genetic pathway responsible for the *de novo* synthesis of vitamin B_{12} by *L. reuteri* has previously been described for two *L. reuteri* strains[103]. More recently, the presence of bifidobacterial genes, predicted to be involved in the biosynthesis of several B vitamins has been identified in bifidobacteria residing in faecal samples of adult subjects[104–106].

PUFA and conjugated fatty acid synthesis

PUFA contain two or more double bonds and are classified as either *n*-3, *n*-6 or *n*-9, based on the location of the last double bond relative to the terminal methyl end of the molecule. Linoleic acid (18:2*n*-6; precursor to the *n*-6 series of fatty acids) and α-linolenic acid (18:3*n*-3; precursor to the *n*-3 series of fatty acids) are the simplest members of each family of PUFA and are essential fatty acids. PUFA regulate a wide variety of biological functions, ranging from blood pressure and blood clotting, to the development and functioning of the brain and nervous system. It has been shown that the gut microbiota not only affects fat quantity[3], but also affects fat quality[80,86] in various animal models. CLA refers to a family of positional and geometric isomers of linoleic acid, which have been associated with several health benefits. The CLA isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12 are most often studied for their beneficial *in vitro* and in some cases *in vivo* health effects associated with various types of cancer, atherosclerosis, obesity, diabetes, as well as an ability to improve immune function, body composition and bone formation[107–119]. It has recently been shown that plasma CLA metabolite concentrations in human subjects following dietary CLA supplementation were comparable with those previously observed in experimental animal models and sufficient enough to exert health benefits[120]. Considerable species variations among bifidobacteria have been observed for PUFA and CLA productions. Although *Bifidobacterium breve* has been reported as one of the most efficient CLA producers among various strains tested[121,122], *Bifidobacterium bifidum*[122] and *Bifidobacterium dentium*[121] have also demonstrated good conversion rates *in vitro*. It has been shown that administered CLA-producing strains of bifidobacteria are metabolically active in the gastrointestinal tract of mice and pigs[80]. Furthermore, administration of *B. breve* NCIMB 702258 in combination with linoleic acid resulted in modulation of tissue fatty acid composition, significantly increasing levels of *cis*-9, *trans*-11 CLA in the

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liver of both mice and pigs[80]. Increased tissue concentrations of *n*-3 long-chain PUFA, EPA and DHA were also found in the adipose tissue of both mice and pigs[80]. Furthermore, the ratio of arachidonic acid to EPA in the liver and adipose tissue was reduced following *B. breve* supplementation, coupled with an anti-inflammatory cytokine profile in the host[80]. Both EPA and DHA have previously been shown to exert anti-inflammatory properties[123]. In a related study, it was found that administration of *B. breve* NCIMB 702258 in combination with α-linolenic acid was associated with alterations in the fatty acid composition of the brain, with elevated levels of EPA and DHA[81]. Such studies have demonstrated that manipulation of the gut microbiota with metabolically active strains may represent a therapeutic strategy for various disorders related to inflammation in the host, through the production of long-chain PUFA and PUFA-derived conjugated fatty acids.

Production of SCFA

SCFA are the end products of anaerobic gut microbial fermentation of undigested dietary fibres and have important functions in host energy metabolism. Indeed, SCFA play a key role in the prevention and treatment of metabolic and bowel disorders and certain types of cancer[124–128]. The positive influence of SCFA treatment on ulcerative colitis and Crohn's disease have been demonstrated in various clinical studies[129–133]. Butyrate is the primary energy source for cellular metabolism in the colonic epithelium[134]. The colonic epithelial cells of germ-free mice are severely energy-deprived and are characterised by increased activation of AMP-activated protein kinase, which senses cellular energy status[135]. SCFA also regulate gene expression in the host by binding to the G-protein-coupled receptors, GPR41 and GPR43 to impact on several different cellular functions in the host, depending on the cell type[136]. For example, SCFA suppress inflammation through GPR43 signalling in immune cells[137,138] and modulate secretion of the insulin secreting and antidiabetic hormone glucagon-like peptide-1 in the distal small intestine and colon[139].

Microbial production of exopolysaccharides

Many organisms including some resident microbes of the gut microbial ecosystem have the ability to synthesise exopolysaccharides (EPS) with a large variation in composition, charge and molecular structure[140]. EPS-producing strains are responsible for a 'ropy' phenotype and are beneficial in the food and health industries. Health benefits associated with EPS include immunostimulatory effects[88,141], blood cholesterol-lowering effects[87,142] and prebiotic effects[143,144]. β-Glucan is a water-soluble fibre found in cereals, as well as in yeast, bacteria, algae and mushrooms[145]. The EPS β-glucan has been reported to have many health promoting properties, including immunomodulatory effects[146–149], lowering serum cholesterol levels[145,150], antiosteoporotic[151], antitumourigenic, anticytotoxic and antimutagenic effects[152,153]. Furthermore, oat β-glucan has also been associated with the ability to modulate satiety, thus controlling appetite[154,155]. Heterologous expression of the pediococcal glycotransferase (*gtf*) gene responsible for the synthesis and secretion of the two substituted (1,3) β-D-glucan in *Lactobacillus paracasei* NFBC 338 increased the stress tolerance of the probiotic, due to EPS production[140]. Furthermore, *B. breve* UCC2003 has been shown to produce two EPS which have been associated with an increased resilience of this strain to tolerate acid and bile while reducing the intestinal colonisation levels of pathogenic *Citrobacter rodentium*[156]. Thus, EPS production is thought to be important not only in host interactions, but also for protection against pathogenic infection.

The gut–brain axis: microbial metabolite production with implications on host psychiatric health

The gut–brain axis is a bidirectional communication system between the brain and the gut, including the metabolically complex gut microbiota which integrates neural, hormonal and immunological signalling between the gut and the brain[157]. The gut microbiota and the metabolites they produce may also modulate the peripheral nervous system and central nervous system (CNS) to influence brain development and function[158]. To date, numerous studies have demonstrated the importance of the gut microbiota in the stress response[159,160] and neurodevelopmental disorders[161–163]. Commensal microbiota have demonstrated the ability to interact with the serotonergic system in the host by regulating the development of the hypothalamus–pituitary–adrenal axis, a neuroendocrine system that controls reactions to stress[160]. Recent studies have demonstrated that germ-free mice display a reduction in anxiety-like behaviour[164,165], compared with conventionally colonised mice, possibly through an enhanced hypothalamus–pituitary–adrenal response. Another study using germ-free mice described how in the absence of gut microbiota, mice exhibited deficits in social motivation and preference for social novelty, behavioural characteristics indicative of disruptions in distinct normal social behaviours[162]. Probiotic intervention has proven successful for the treatment of psychiatric disorders such as anxiety[90,166], depression[91] and autism[167]. *Bifidobacterium infantis* 35 624, when administered to a maternal separation animal model of depression, exhibited antidepressant properties[91], *Lactobacillus rhamnosus* JB-1 has also demonstrated antianxiety and antidepressant properties through activation of the vagus nerve in mice, compared with broth-fed controls[90] and *B. fragilis* administration alleviated autistic-like behavioural impairments in communication, social behaviour, social abnormalities and restricted/repetitive behaviour in mice symptomatic of this disorder, compared with autistic, untreated controls[167]. Furthermore, administration of *B. breve* NCIMB 702258 to mice had a significant impact on

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the fatty acid composition of the brain[81]. Mice that received the bacteria for 8 weeks exhibited higher concentrations of bioactive fatty acids, arachidonic acid and DHA, compared with unsupplemented controls[81], whereby these bioactive fatty acids have a role in neurotransmission and protection against oxidative stress[168,169].

A broad range of microbes, either probiotics or commensals can manufacture and secrete neurochemicals which can positively impact on mental health and thus, could be used for the treatment of CNS disorders, such as anxiety and depression. Recently defined, a psychobiotic is 'a live micro-organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness'[170]. Moreover, disruptions in the composition of the gut microbiota may lead to a deterioration of gastrointestinal, neuroendocrine and immune pathways, which could in turn lead to alterations in gut–brain interactions and consequently result in disease[171]. The gut microbiota produce a range of neurotransmitters and neuromodulators, bioactive metabolites which impact on host psychiatric health, only some of which have been demonstrated *in vivo* (Table 1).

Gamma-aminobutyric acid

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter of the vertebrate CNS and is the main inhibitory neurotransmitter in the brain. Dysfunctions of GABA have been linked with anxiety and depression[172,173]. Certain strains of *Lactobacillus* and *Bifidobacterium* secrete GABA via the same biosynthetic pathway as in neuronal tissue involving conversion of glutamate by the action of the enzyme glutamate decarboxylase and vitamin co-factor pyridoxal phosphate[174]. Furthermore, the GABA producing capability of some bacterial strains is thought to protect the organism from the acidic environment of the stomach[175]. Several human-derived lactobacilli and bifidobacteria were screened for their ability to produce GABA from monosodium glutamate, and it was found that five strains had this ability[176]. Of these strains, *Lactobacillus brevis* and *B. dentium* were the most efficient GABA producers[176]. Ko *et al.*[89], recently demonstrated GABA production in black soyabean milk by *L. brevis* FPA3709 and its 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[89]. At the level of gene expression, ingestion of *L. rhamnosus* JB-1 altered the mRNA expression of both $GABA_A$ and $GABA_B$, two GABA receptors which have been implicated in anxiety and depression[90].

Serotonin

Serotonin is a metabolite of the amino acid tryptophan and plays an important role in the regulation of a number of brain functions, including mood[170]. The vast majority of antidepressant drugs work to increase serotonin levels in the brain and some studies have shown that bacteria can synthesise serotonin *in vivo*. For example, plasma serotonin levels were shown to be nearly 3-fold higher in conventional mice than in their germ-free counterparts[177]. Oral ingestion of *B. infantis* 35 624 increased the plasma levels of tryptophan, precursor to serotonin, suggesting that commensal bacteria have the ability to influence tryptophan metabolism and could potentially act as antidepressants[91]. This effect on tryptophan metabolism may be mediated by the impact of the microbiota on the expression of indoleamine-2,3-dioxygenase, a key enzyme in the physiologically dominant kynurenine pathway of tryptophan metabolism[158]. Early life stress induces changes in the gut microbiota and is a known risk factor for depression in adulthood[178]. This phenomenon has been shown in rhesus monkeys, whereby prenatal stressors have been shown to alter the microbiome by reducing the overall numbers of bifidobacteria and lactobacilli[179].

Catecholamines and acetylcholine

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. Tsavkelova *et al.*[180], identified a wide range of bacteria, which produce mmol quantities of dopamine[180] and which could be used for the treatment of Parkinson's disease, Alzheimer's disease and other major depressive disorders whereby dysfunctions in catecholamine neurotransmission are implicated. In addition, bacteria which constitute the normal gut microbiome in mice have been shown to be capable of the production of norepinephrine *in vivo*[181]. Acetylcholine is a neurotransmitter found in the CNS and peripheral nervous system 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*[182–184] and a microbial metabolite.

Conclusion

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[185]. Consequently it is not surprising that alternations in the microbial consortium are being found to be associated with a number of disease states such as IBD, diabetes, obesity, anxiety and depression. Disturbances to the delicate host–microbe relationship may disrupt development of the immune system, which may in turn result in disease. The gut microbiota have the ability to produce a variety of

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metabolites that exert beneficial effects on biological and neurological functions. Probiotics, prebiotics and dietary PUFA offer the potential to modulate the gut microbiota with knock-on health effects. Microbe manipulation to strengthen the host–microbe symbiotic relationship may be crucial for the future prevention of immune and psychiatric-related disorders.

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Conflicts of Interest

None.

Authorship

E. P. and C. S. wrote the manuscript; J. F. C., G. F. F., R. P. R. and T. G. D. made substantial contributions to the overall content of the manuscript and all authors had responsibility for the final content.

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