Probiotics function mechanistically as delivery vehicles for neuroactive compounds: Microbial endocrinology in the design and use of probiotics

Mark Lyte

I hypothesize here that the ability of probiotics to synthesize neuroactive compounds provides a unifying microbial endocrinology-based mechanism to explain the hitherto incompletely understood action of commensal microbiota that affect the host's gastrointestinal and psychological health. Once ingested, probiotics enter an interactive environment encompassing microbiological, immunological, and neurophysiological components. By utilizing a trans-disciplinary framework known as microbial endocrinology, mechanisms that would otherwise not be considered become apparent since any candidate would need to be shared among all three components. The range of neurochemicals produced by probiotics includes neurochemicals for which receptor-based targets on immune and neuronal elements (intestinal and extra-intestinal) have been well characterized. Production of neurochemicals by probiotics therefore allows for their consideration as delivery vehicles for neuroactive compounds. This unifying microbial endocrinology-based hypothesis, which may facilitate the selection and design of probiotics for clinical use, also highlights the largely unrecognized role of neuroscience in understanding how microbes may influence health.

Keywords:
hormones; microbial endocrinology; neurochemicals; probiotics

Introduction

Probiotic bacteria are increasingly being employed in an ever-widening spectrum of diseases as well as in general health [1, 2]. The putative health benefits gained from the ingestion of probiotics have been widely reported in the scientific literature as well as lay press. However, definitive mechanism(s) have yet to be identified for the ability of orally administered bacteria to modulate a number of biological processes ranging from the production of inflammatory cytokines by immune cells within the gastrointestinal tract to the adhesion of pathogenic bacteria to the mucosal gut wall. Whereas multiple mechanisms may be operative in each of these situations, an alternative hypothesis as described herein is that there may be a shared mechanism that essentially links the neural and immune responses to probiotic administration that leads to the claimed prophylactic effects.

Critically, recent studies that have demonstrated the ability of probiotics to influence psychological states imply that the mechanism(s) by which probiotics influence the host may extend beyond those which address their well-recognized ability to influence immune-related pathways. For example, administration of the probiotic *Bifidobacterium infantis* to rats subjected to a forced swim test resulted in neurochemical alterations in addition to attenuation of pro-inflammatory responses that suggested a potential antidepressant capability for the administered probiotic [3]. In human volunteers, as well as in a rat model, administration of a probiotic formulation consisting of *Lactobacillus helveticus* R0052 and *B. longum* R0175A significantly attenuated psychological distress and reduced anxiety-like behavior, respectively [4].

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Department of Pharmacy Practice, Texas Tech University Health Sciences Center, Lubbock, TX, USA

Corresponding author:
Mark Lyte
E-mail: mark.lyte@ttuhsc.edu

This ability of a probiotic to function as an anxiolytic may have profound clinical applications given the well-documented occurrence of psychosocial abnormalities that accompany a number of gastrointestinal disorders such as those associated with chronic intestinal inflammation [5]. As such, the examination of hitherto unsuspected novel mechanism(s) by which probiotics directly influence central nervous system function is warranted.

Spectrum of probiotics: Mechanistic issues

The diversity of probiotic organisms that have been employed in both animal and clinical studies is very large [6]. Increasingly, the selection of probiotics seems to be as much dictated by the need for use of a proprietary probiotic to establish market share as by any defined mechanism of action. Further complicating the identification of specific mechanisms has been the diversity of measures utilized to assess effectiveness that have spanned the gamut from measurement of inflammatory cytokines in patients diagnosed with inflammatory bowel disease to an improvement in the psychological profile of healthy volunteers. The lack of commonly shared, readily identifiable, mechanisms of action by which to evaluate probiotics will continue to prove a hindrance to their acceptance and use within the medical community. Defining potential mechanisms of action for probiotic bacteria will enable decisions regarding which patient populations are likely to benefit from probiotic treatment and encourage increased utilization of these treatments among health care professionals. It is therefore crucial to identify the mechanisms of action by which probiotics may prove beneficial for individuals. By employing a completely new, trans-disciplinary approach combining microbiology and neurophysiology to the issue of defining mechanism(s) of action, the hypothesis introduced in this paper will, if supported by subsequent in vitro and in vivo experimental work outlined below, identify a new, previously hitherto, unknown and unsuspected mechanism of action.

Microbial endocrinology: Where microbiology meets neuroscience

The first report that a bacterium can respond to a mammalian neuroendocrine hormone occurred in 1929 prompted by the observation that a high percentage of otherwise healthy patients administered epinephrine to relieve simple urticaria (itching) unexpectedly died of fulminating gas gangrene [7]. Over the ensuing three decades, increasing numbers of reports noted that the temporal presence of neurohormones, especially those belonging to the stress-related catecholamine family, at the time of infection had an influence on subsequent growth and pathogenesis of a number of bacterial strains, including that of *Clostridium perfringens* (reviewed in ref. [8]). However, the mechanism that these early reports ascribed to such phenomena was one in which the neurochemicals suppressed local immunity thereby allowing the infectious agent to rapidly multiply unimpeded [9]. That a bacterium could *directly* respond to a neurochemical and alter its growth and pathogenic capabilities was not proposed and demonstrated until the early 1990s [8, 10, 11]. This intersection of microbiology with neuroscience has been termed microbial endocrinology [8, 10]. Evidence of its growing recognition as an interdisciplinary field addressing inter-kingdom signaling in health and disease is shown by the recent publication of the first book solely dedicated to it as a sub-discipline within microbiology [12] as well as the recognition within the medical community of its relevance to the pathogenesis and treatment of infectious disease [13–15]. As regards its application to the field of probiotics, microbial endocrinology addresses the ability of probiotics to both synthesize and respond to neuroactive compounds as a mechanism by which host biological processes, both physiological and neurological, may be influenced. Inherent in an understanding by which a microbial endocrinology-based approach may be relevant to the understanding of the ability of probiotics to influence host health is that production of neuroactive components by a probiotic suggests the presence of possible receptors on the probiotic. If so, then production of the same neuroactive compounds by the host may be expected to influence the probiotic as well. This neurochemical-mediated "two-way street" is one of the principles that undergirds the microbial endocrinology construct [16, 17].

That a micro-organism, such as a probiotic bacterium, should be able to produce a neurochemical that is exactly the same as one found in mammalian systems may seem surprising (Table 1). However, what is commonly considered to be exclusive to vertebrate neurochemicals and related receptors are in fact widely dispersed throughout nature. For example, the stress-related neuroendocrine hormone family of catecholamines has also been demonstrated in bacteria [18], plants [19], insects [20], and fish [21]. Indeed, the presence of the complete biosynthetic pathway for catecholamines in bacteria has led to the theory that cell-to-cell signaling in vertebrates may be due to late horizontal gene transfer from bacteria [18]. The ability of bacteria to not only produce, but also respond to host neurochemicals forms the basis of the emerging field of microbial endocrinology (for review see [8]).

Bacteria are well recognized within the community of scientists engaged in microbiological safety analysis of food products to be prodigious but unwanted producers of neurochemicals. For example, histamine which is more widely known for its role in allergy and anaphylaxis than as a neurotransmitter [22], is produced in large quantities by certain bacteria which may contaminate fish or shellfish products [23] requiring testing to insure that histamine levels do not exceed government guidelines for food poisoning.

Hypothesis: Neurochemical-producing probiotics act as delivery vehicles for neuroactive compounds

Given the ability of probiotic micro-organisms to produce neuroactive substances which have exactly the same structure as their host counter parts I therefore hypothesize that they act

Table 1. Diversity of neurochemicals isolated from various microbial species (as from review [25])

Genus	Neurochemical
Lactobacillus, *Bifidobacterium*	GABA
Escherichia, *Bacillus*, *Saccharomyces*	Norepinephrine
Candida, *Streptococcus*, *Escherichia*, *Enterococcus*	Serotonin
Bacillus, *Serratia*	Dopamine
Lactobacillus	Acetylcholine

essentially as neuroactive compound delivery vehicles affecting host physiology through the provision of neurochemicals. This hypothesis (Table 1) provides a central mechanism for neurochemicals recognized to exist in all three systems involved in health and disease, those being immunological, neurophysiological, and microbiological (Fig. 1).

Importantly, it is not the intent of this hypothesis to obviate a role for direct modulation of immune responsiveness by probiotics [24]. Indeed, this microbial endocrinology-based hypothesis proposes a mechanism by which probiotic bacteria modulate immune responsiveness. The demonstration that probiotic production of neurochemicals and possibly receptor-based recognition of prokaryotic as well as eukaryotic-produced neurochemicals [17], provide a shared mechanism affecting the neural and immune compartments both intestinally and extra-intestinally may therefore provide new mechanistic information that will improve scientific knowledge of how probiotics may function. Furthermore, by proposing that the central mechanism linking receptor-based mechanisms in immunological, neurophysiological, and microbiological components is that of probiotic production of neuroactive substances, this hypothesis provides not only a mechanistic basis to understand the action of probiotics but also a rationale for their selection and design for the treatment of various clinical conditions.

Testing the hypothesis

Bacteria have been well documented to produce an extensive range of neurochemicals (Table 1 and refs. [8, 25]) for which receptor-based mechanisms of action have been well studied in intestinal and extra-intestinal host physiology for decades. For example, the production of GABA by probiotic bacteria occurs via the same biosynthetic pathway as in neuronal tissue involving conversion of glutamate by the action of the enzyme L-glutamic acid decarboxylase and vitamin co-factor pyridoxal phosphate [26]. Thus, neurochemicals such as GABA may be viewed as a common shared language enabling inter-kingdom signaling between prokaryotes (e.g. probiotic bacteria) and eukaryotes (e.g. vertebrates).

This recognition of inter-kingdom signaling provides a step-by-step mechanistic approach to test the validity of the hypothesis that production of neurochemicals by probiotics represents a heretofore unrecognized mechanism by which probiotics influence intestinal and extra-intestinal host physiology (Table 2). That a large and robust array of different molecular techniques and reagents exist and have been validated for use in demonstrating the role of neurochemicals produced by eukaryotic cells on both neuronal and immune physiology, allows for a methodological approach that easily transitions to the study of bacterial-produced neurochemicals and their effect on eukaryotic systems (Table 2). The emergence of metabolomics-based measurement of neurochemicals associated with the gut microbiome [27] is but one example of such an approach (Table 3).

A concern of many probiotic studies has been that while the administration of a given probiotic may be associated with a change in a specific physiological measure such as reduction in a specific immune cytokine, the precise mechanism demonstrating a cause and effect relationship is often lacking. At best, such studies are correlational and the lack of a specific demonstrable mechanistic pathway detracts from its applicability to conditions that differ from that of the initial study. In an effort to address this aspect of experimental design, I have proposed a step-by-step experimental system to test the hypothesis that administration of neurochemical producing probiotics can effect changes in host physiology and as such may provide a mechanistic platform for the rational use and design of probiotics (Table 2). Central to the experimental

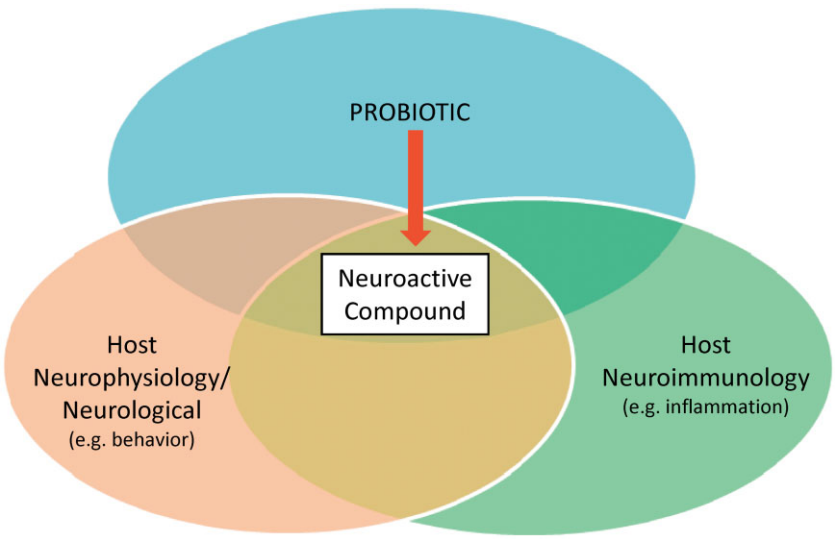

Figure 1. Venn diagram illustrating probiotic-produced neuroactive compounds as common elements influencing both neurophysiological/neurological and neuroimmunological host aspects.

Table 2. Sequential design to evaluate ability of neurochemical-producing probiotics to influence disease pathobiology

Step	Comments
Identify neurochemical of interest to be produced by probiotic based on desired physiological and/or behavioral effect in host.	Physiological and/or behavioral measures should be readily quantifiable. Measures that are receptor-based with known antagonists readily available are preferred as can subsequently be employed at in vivo steps involving animal models.
Screen candidate probiotic in vitro for neurochemical production using robust assay to determine if neurochemical of interest as well as other neurochemicals are produced.	An example of a metabolomics-based screen is given in Table 3. More than one microbiological growth medium should be used. Preferably a medium that reflects the gut environment should also be employed.
Define kinetics (i.e. time dependent achievable intra- and extra-cellular concentrations) of neurochemical production.	Identify in vitro growth conditions which result in sustained levels of neurochemical production throughout growth period.
Obtain non-producer mutant (either through in vitro screening or site-directed mutagenesis procedure).	A mutant that does not produce the neurochemical will provide critical control for in vivo experiments.
Conduct time and dose-dependent per oral administration of neurochemical-producing probiotic in normal animals to determine ability of probiotic to produce neurochemical in vivo. Employ vehicle -only animals as control.	Measure levels of neurochemical of interest in intestinal luminal fluid and plasma. Determine time-dependent colonization of gut tissue using quantitative PCR. Perform gross pathology and immunohistopathology of relevant tissue and compare to control (vehicle only) animals.
Perform per oral administration of probiotic in an animal model which involves a neurochemical-responsive element.	Animal models of specific disease pathology or behavior are suitable candidates. Select dosage of neurochemical-secreting probiotic from prior step that is found to result in high and sustainable levels of neurochemical within the gut. If known receptor antagonists are available, give antagonist to block neurochemical-responsive element of disease or behavioral process.
Perform control experiments utilizing per oral administration of mutant (non-neurochemical-secreting) probiotic.	Quantifiable changes in animal model that are obtained by administration of neurochemical-secreting probiotic in above step should not be present (or at lower levels) with mutant strain.

Table 3. Example of a metabolomics-designed screen to evaluate candidate probiotics for production of neurochemicals that can influence host physiology and/or behavior

Acetylcholine
Dihydroxyphenylacetic acid
L-DOPA
Dopamine
Dopamine-4-O-sulfate
Epinephrine
GABA
Histamine
Norepinephrine
Norepinephrine-3-O-sulfate
Serotonin
Tyramine

approach shown in Table 2 is a sequential research plan that first seeks to elucidate the in vitro parameters of neurochemical production by the specific probiotic. Critically, it should not be assumed that the probiotic only produces one type of neurochemical. For example, probiotics belonging to *Lactobacilli* and *Bifidobacterium* have been shown to produce more than one neurochemical (Table 1). A number of factors may influence the in vitro production by a probiotic of a given neurochemical with the medium composition being of particular importance. For any given probiotic, a range of different media are typically available to support in vitro growth. More than one medium should be examined as the variability in material composition of each medium may have a determining effect on the production of a particular neurochemical especially if the substrate for that neurochemical is not present in the medium formulation.

GABA serves as a prototypical example of a neurochemical that may be selected for testing in the system approach outlined in Table 2 as it satisfies a number of the fundamental requirements. First, it is a neurochemical which is produced in vitro in large (micro- to millimolar) quantities by a range of probiotic micro-organisms notably *Lactobacillus* and *Bifidobacterium* (Table 1). The levels of GABA that can be achieved in vitro by probiotic organisms are quite large. For example, in the production of fermented foodstuffs, such as Japanese funa-sushi and Chinese traditional *paocai*, which employ lactobacilli as part of the manufacturing processing, GABA levels in the millimolar range have been demonstrated in the final products [28–30]. Secondly, as the predominant inhibitory neurotransmitter in the nervous system, GABA also serves a receptor-mediated role in a number of immunological (i.e. down-regulation of cytokine release by proinflammatory cells release [31]) as well as intestinal neurophysiological (i.e. secretion of neuropeptides by intrinsic and extrinsic intestinal nerve fibers [32, 33]) processes. As such GABA-sensitive elements in the gut have often been implicated in the pathophysiology of intestinal diseases such as implammatory bowel disease (IBD), the potential use of a GABA-secreting probiotic to ameliorate a specific pathophysiology condition such as IBD

makes for an ideal system with which to test the validity of the hypothesis. Thus, production of GABA by probiotic bacteria could reduce inflammation in colitis via both neuroimmune and neurophysiological mechanisms.

As indicated in Table 2, a mutant probiotic that does not secrete the neurochemical of interest provides for a critical test of the hypothesis. The in vitro identification of sets of high and non-GABA producing probiotics could be performed using standard growth methodology combined with analysis of GABA production in place of the more involved generation of mutants by site-directed mutagenesis. The feasibility of finding high and non-GABA producing isolates has already been reported for *L. brevis* strains from Chinese traditional *paocai* [30]. Once identified, candidate probiotic strains would then be administered to both normal and inflammatory bowel disease-model animals (i.e. dextran sulfate-induced colitis [34]). Changes in gut inflammation could be followed by a combined approach assessing markers of inflammation such as tissue histology and production of pro-inflammatory mediators and coupling such measurements with assessment of GABA levels in the intestinal tract [27] as well as enumeration of numbers of probiotic bacteria in the lumen and mucosal surface in order to establish a direct cause and effect relationship. Thus, following the step-by-step methodology outlined in Table 2 as applied to probiotic-produced GABA and its potential amelioration of gut inflammation would provide initial demonstration of the viability of the proposed hypothesis and would forge the way forward for human clinical trials.

Indeed, the ability of various probiotic strains to produce GABA in high concentrations has led to the introduction of GABA-related functional foods such as a GABA-enriched bread [35], although no specific use for such a product was identified or suggested. The clinical use of GABA produced by a probiotic has been proposed as regards dermatological applications. The production of GABA in grape must beverages by *L. plantarum* has recently been shown to be effective in regulating immune gene transcription in an in vitro human epithelial skin model [36]. Interestingly, analysis of yogurts which may be fortified with up to five different probiotic strains has shown GABA levels in the low millimolar range and similarly probiotic-containing capsules contain appreciable levels of GABA in the micromolar range (Lyte, M.; unpublished results).

The amount of a neurochemical, such as GABA for example, found within, or produced by, probiotics is most likely sufficient to influence localized immune and neurophysiological process in the gut as both immune and neuronal cells have been well documented to respond to nanomolar concentrations of GABA [31, 32, 37–39]. The delivery of neurochemicals by probiotics may therefore either be in the amount already contained in the bacterium at time of ingestion or that which is actively produced once inside the gastrointestinal tract. Thus, in delivering a neuroactive chemical to a specific anatomical site in which various cellular processes are influenced, the presence of neurochemical containing/producing probiotic bacteria can be viewed essentially as delivery vehicles for neuroactive compounds.

Considerations in hypothesis testing strategy

In addition to Table 2, microbial endocrinology-based experimental design raises a number of potential issues, both in vitro and in vivo, that should be addressed prior to the testing of the hypothesis. These considerations mainly address factors which may govern the production and delivery of a neurochemical by a particular probiotic micro-organism.

The question of the degree of intestinal colonization following ingestion of the probiotic carries important physiological consequences for the microbial endocrinology-based theory of probiotics acting in the capacity of delivery vehicles for neuroactive compounds. Given the relatively poor ability of probiotic bacteria to successfully colonize large segments of the intestine it is not surprising that increasing the daily frequency of probiotic ingestion increases the purported beneficial effects on physiological measures such as inflammation as well as for psychological measures such as anxiety-like behavior. This is wholly consistent with the theory of probiotics acting as delivery vehicles for neuroactive compounds since continued administration of a neurochemical that suppresses cytokine production (i.e. GABA) would be expected to be more efficacious if administered multiple times over a defined time period than at a single time point. As such, the selection of probiotic strains that combine successful colonization with robust production of the desired neurochemical should be viewed as dual essential criteria in the design of any in vivo application. The possibility of establishing a neurochemical-secreting probiotic within the intestinal environment may offer substantial therapeutic benefit over more conventional drug dosing regimens since actively growing probiotic organisms within the gut would provide a continual supply of the neuroactive compound that would presumably avoid the spikes and valleys associated with per oral administered pharmacotherapy.

Secondly, one of the aspects of probiotic selection that is often overlooked is the composition of foods that either may be present at the time of probiotic administration or are already present within the gut. For example, given that the production of GABA by probiotic bacteria is dependent on the presence of glutamate and pyridoxal phosphate as substrate and enzyme co-factor, respectively [26], any protocol that tests the ability of a specific GABA-secreting probiotic to influence a host physiological or disease process should consider the use of foods either containing or fortified with sufficient levels of glutamate and pyridoxal phosphate instead of solely relying on basal (and unknown) levels of these two compounds required for GABA synthesis. Thus, specific consideration should be given to the composition of either the food carrier that the probiotic is administered in, or the meal with which the probiotic is consumed, that specific substrates and co-factors necessary for the probiotics to synthesize the neurochemical of interest are provided along with the probiotic itself.

Clinical implications

Testing of a microbial endocrinology-based hypothesis of probiotic action will allow not only the identification of a

novel mechanism by which probiotics function in the host, but also the molecular and cellular targets for probiotic-produced neurochemical action. The use of a trans-disciplinary framework such as microbial endocrinology in which a common neurochemical-based mechanism involving microbiological, immunological, and neurophysiological components is being proposed implies that candidate neurochemicals should satisfy the dual criteria that bacterial production of the candidate neurochemical in question has been previously demonstrated in either culture or a food product and that a eukaryotic receptor for the neurochemical has also been shown within the host.

Of consequence, this highly innovative approach of selecting probiotics based on their neurochemical production profile will lead to the identification of new probiotic strains that may prove more efficacious since the mechanism of action can now be identified and provide the rationale for the selection of new probiotic strains that are clinically targeted in their use and application. Indeed, validation of this hypothesis in pre-clinical models will enable targeted selection of potentially highly effective probiotics for clinical trials for patients with inflammatory bowel diseases such as colitis.

Although the principal focus of this hypothesis may understandingly be directed toward its application within the gastrointestinal tract, it should be appreciated that extra-intestinal effects of neurochemical-secreting probiotics are also possible. The transport of neurochemicals produced within the gut by commensal as well as pathogenic bacteria to extra-intestinal sites such as the liver and brain has been shown in both human and animal studies [27, 40, 41]. Active uptake of a large variety of substances including neurochemicals from the gut lumen into the portal circulation represents a pathway by which neurochemicals produced within the gut may exert extra-intestinal effects such as on behavioral changes. Evidence that this is indeed a viable pathway can be seen in the recent metabolomics-based report that in mice circulating plasma levels of neurochemicals such as serotonin was due to direct uptake from the gut lumen [27]. Thus, consistent with the hypothesis that probiotics acting as neuroactive compound delivery vehicles may effect changes in the local gut environment, it may be further proposed that such neurochemical-secreting probiotics may have extra-intestinal effects at sites such as the brain that may ultimately influence overt physiological states such as behavior. In this context, the recent report [4] that administration of a probiotic mixture containing probiotic strains that belong to genera that typically contain producers of neurochemicals such as GABA (Table 1) resulted in a reduction of anxiety-like behavior in both human and animal subjects suggests that one possible mechanism may have been through the provision of a probiotic-derived neurochemical that exerted its effects within the central nervous system. While the authors of this study did not examine nor propose a microbial endocrinology-based mechanism (i.e. probiotic production of a neurochemical), it is nonetheless intriguing to speculate that such a mechanism may have played such a role.

This ability of probiotics to affect central nervous system processes is perhaps one of the most exciting recent developments in probiotic research as evidenced, for example, by the findings of anxiolytic activity of a *Bifidobacterium-Lactobacillus* probiotic formulation in human volunteers and in rats [4]. While the pathways and mechanisms, both neural and immune, which are involved in the gut-brain axis, have been the subject of intensive study for decades, it is only more recently that the role of the intestinal microbiota in the gut-brain axis has begun to be elucidated (for review see [42]).

Emerging research has shown that variations in the composition of the intestinal microbiota itself may in fact play a determining role in psychological states through alterations in nervous system processes (for review see [43]). Consistent with the sequential design proposed in Table 2 for the evaluation of neurochemical-producing probiotics to influence disease pathobiology, this model can also be modified for use in evaluating the ability, and elucidating the mechanisms, by which neurochemical-secreting probiotics may influence nervous system function and thereby behavior. For example, a human-based trial of a neurochemical-producing probiotic that is being evaluated for the ability to influence negative affective states in IBD should include collection of plasma, fecal, and urine components to enable metabolomic screening prior to, during, and following cessation of probiotic administration. Levels of a specific neurochemical, along with markers of neuronal function, could then be quantitatively measured (for example utilizing a metabolomics-based approach) and their relationship to the performance of the subjects in appropriate psychological testing for the specific behavior could then be assessed thereby enabling direct cause and effect relationships to be rigorously evaluated.

As with the use of any drug-based system, there may be unforeseen and hence potentially undesired consequences. This truism in conventional drug-based therapy equally applies to the proposed use of probiotics as neurochemical delivery vehicles as clinical-based knowledge concerning the administration of neurochemicals is incomplete at best. Further, a robust screening approach, such as the metabolomics screen shown in Table 3, should be employed to understand, as completely as possible the spectrum of neurochemicals that may be produced by any one probiotic under a variety of growth conditions. The finding of potentially harmful neurochemicals, such as histamine, would be sufficient to exclude that probiotic from consideration. Although histamine has not yet been reported for any probiotic micro-organism, it reinforces the need for neurochemical screening to avoid potential clinical issues.

Concluding thoughts

This unifying microbial endocrinology-based hypothesis, which may facilitate the selection and design of probiotics for clinical use based on the production and delivery of neuroactive chemicals by bacteria, also highlights the largely unrecognized role of neuroscience in understanding how microbes may influence health. The realization that microbes produce a wide spectrum of neuroactive compounds extending from GABA to somatostatin [8, 25] suggests that the consequences of such neuroactive compound production, as well as the mechanisms governing such interactions, are yet to be discovered.

The recent report describing the ability of probiotics to alleviate psychological distress in human volunteers and anxiolytic-like activity in rats [4] lends further support to the increasing evidence that the gut microbiota can influence nervous system function (for reviews see [42, 43]). As such, the elucidation of the mechanism(s) by which probiotics can influence nervous system function will be critical in determining the utility and appropriateness of probiotics in the clinical arena. In proposing that probiotics act as neuroactive compound delivery vehicles due to their production of neurochemicals, this paper proposes a novel, innovative interdisciplinary microbial endocrinology-based hypothesis that may further understand the mechanism(s) by which probiotics may function in the treatment of host pathobiology as well as affect behavior.

While GABA has been employed as an example to demonstrate the utility of the microbial endocrinology-based hypothesis, it is by no means limiting the application only to micro-organisms that are capable of producing GABA. As shown in Table 1, the spectrum of neurochemicals produced by micro-organisms is quite large and varied. Thus, one can envision the ''mining'' of micro-organisms, especially those deemed safe for human or animal consumption, for neurochemical secreting capacity. Micro-organisms identified through screening processes, such as metabolomics (Table 3), as capable of secreting neurochemicals could then be employed according to the hypothesis-testing methodology shown in Table 2 and discussed above.

Critically, this microbial endocrinology-based hypothesis thus provides for a unifying model that can guide the selection of probiotics based on a matching of the specific probiotic organism's capacity to produce a particular neurochemical and the physiological or behavioral condition that is responsive to that neurochemical. In this way, probiotic treatment could be tailored to treat the pathology and/or symptomology associated with specific disease or psychological states.

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References

1. **Gareau MG**, **Sherman PM**, **Walker WA.** 2010. Probiotics and the gut microbiota in intestinal health and disease. *Nat Rev Gastroenterol Hepatol* **7**: 503–14.
2. **Pagnini C**, **Saeed R**, **Bamias G**, **Arseneau KO**, et al. 2010. Probiotics promote gut health through stimulation of epithelial innate immunity. *Proc Natl Acad Sci USA* **107**: 454–9.
3. **Desbonnet L**, **Garrett L**, **Clarke G**, **Bienenstock J**, et al. 2008. The probiotic *Bifidobacteria infantis*: an assessment of potential antidepressant properties in the rat. *J Psychiatr Res* **43**: 164–74.
4. **Messaoudi M**, **Lalonde R**, **Violle N**, **Javelot H**, et al. 2011. Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. *Br J Nutr* **105**: 755–64.
5. **Irvine EJ.** 2004. Review article: patients' fears and unmet needs in inflammatory bowel disease. *Aliment Pharmacol Ther* **20**: 54–9.
6. **Iannitti T**, **Palmieri B.** 2010. Therapeutical use of probiotic formulations in clinical practice. *Clin Nutr* **29**: 701–25.
7. **Renaud M.** 1930. Role favorisant des perturbations locales causees par l'adrenaline. *C R Seances Soc Biol Fil* **103**: 1052–4.
8. **Lyte M.** 2004. Microbial endocrinology and infectious disease in the 21st century. *Trends Microbiol* **12**: 14–20.
9. **Evans DG**, **Miles AA**, **Niven JSF.** 1948. The enhancement of bacterial infections by adrenaline. *Br J Exp Pathol* **29**: 20–39.
10. **Lyte M.** 1993. The role of microbial endocrinology in infectious disease. *J Endocrinol* **137**: 343–5.
11. **Lyte M.** 1992. The role of catecholamines in gram-negative sepsis. *Med Hypotheses* **37**: 255–8.
12. **Lyte M, Freestone PPE**, eds. 2010. *Microbial Endocrinology: Interkingdom Signaling in Infectious Disease and Health*. New York: Springer.
13. **Everest P.** 2007. Stress and bacteria: microbial endocrinology. *Gut* **56**: 1037–8.
14. **Stewart PS.** 2003. New ways to stop biofilm infections. *Lancet* **361**: 97.
15. **Singer M.** 2007. Catecholamine treatment for shock–equally good or bad? *Lancet* **370**: 636–7.
16. **Lyte M.** 2009. Reciprocal gut-brain evolutionary symbiosis provokes and amplifies the postinjury systemic inflammatory response syndrome. *Surgery* **146**: 950–4.
17. **Lyte M.** 2010. The microbial organ in the gut as a driver of homeostasis and disease. *Med Hypotheses* **74**: 634–8.
18. **Iyer LM**, **Aravind L**, **Coon SL**, **Klein DC**, et al. 2004. Evolution of cell-cell signaling in animals: did late horizontal gene transfer from bacteria have a role? *Trends Genet* **20**: 292–9.
19. **Kulma A**, **Szopa J.** 2007. Catecholamines are active compounds in plants. *Plant Sci* **172**: 433–40.
20. **Pitman RM.** 1971. Transmitter substances in insects: a review. *Comp Gen Pharmacol* **2**: 347–71.
21. **Guerrero HY**, **Caceres G**, **Paiva CL**, **Marcano D.** 1990. Hypothalamic and telencephalic catecholamine content in the brain of the teleost fish, *Pygocentrus notatus*, during the annual reproductive cycle. *Gen Comp Endocrinol* **80**: 257–63.
22. **Benarroch EE.** 2010. Histamine in the CNS: multiple functions and potential neurologic implications. *Neurology* **75**: 1472–9.
23. **Ienistea C.** 1971. Bacterial production and destruction of histamine in foods, and food poisoning caused by histamine. *Nahrung* **15**: 109–13.
24. **Reiff C**, **Kelly D.** 2010. Inflammatory bowel disease, gut bacteria and probiotic therapy. *Int J Med Microbiol* **300**: 25–33.
25. **Roshchina VV.** 2010. Evolutionary considerations of neurotransmitters in microbial, plant, and animal cells. In Lyte M, Freestone PPE, eds; *Microbial Endocrinology: Interkingdom Signaling in Infectious Disease and Health*. New York: Springer. pp. 17–52.
26. **Komatsuzaki N**, **Nakamura T**, **Kimura T**, **Shima J.** 2008. Characterization of glutamate decarboxylase from a high gamma-aminobutyric acid (GABA)-producer, *Lactobacillus paracasei*. *Biosci Biotechnol Biochem* **72**: 278–85.
27. **Wikoff W**, **Anfora A**, **Liu J**, **Schultz P**, et al. 2009. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci USA* **106**: 3698–703.
28. **Higuchi T**, **Hayashi H**, **Abe K.** 1997. Exchange of glutamate and gamma aminobutyrate in a *Lactobacillus* strain. *J Bacteriol* **179**: 3362–4.
29. **Komatsuzaki N**, **Shima J**, **Kawamoto S**, **Momose H**, et al. 2005. Production of gamma-aminobutyric acid (GABA) by *Lactobacillus paracasei* isolated from traditional fermented foods. *Food Microbiol* **22**: 497–504.
30. **Li HX**, **Gao DD**, **Cao YS**, **Xu HY.** 2008. A high gamma-aminobutyric acid-producing *Lactobacillus brevis* isolated from Chinese traditional paocai. *Ann Microbiol* **58**: 649–53.
31. **Bjurstom H**, **Wang J**, **Ericsson I**, **Bengtsson M**, et al. 2008. GABA, a natural immunomodulator of T lymphocytes. *J Neuroimmunol* **205**: 44–50.
32. **Krantis A.** 2000. GABA in the mammalian enteric nervous system. *News Physiol Sci* **15**: 284–90.
33. **Page AJ**, **O'Donnell TA**, **Blackshaw LA.** 2006. Inhibition of mechanosensitivity in visceral primary afferents by GABAB receptors involves calcium and potassium channels. *Neuroscience* **137**: 627–36.
34. **Johansson ME**, **Gustafsson JK**, **Sjoberg KE**, **Petersson J**, et al. 2010. Bacteria penetrate the inner mucus layer before inflammation in the dextran sulfate colitis model. *PLoS One* **5**: e12238.
35. **Coda R**, **Rizzello CG**, **Gobbetti M.** 2010. Use of sourdough fermentation and pseudo-cereals and leguminous flours for the making of a

functional bread enriched of gamma-aminobutyric acid (GABA). *Int J Food Microbiol* **137**: 236–45.
36. **Di Cagno R**, **Mazzacane F**, **Rizzello CG**, **De Angelis M**, et al. 2010. Synthesis of gamma-aminobutyric acid (GABA) by *Lactobacillus plantarum* DSM 19463: functional grape must beverage and dermatological applications. *Appl Microbiol Biotechnol* **86**: 731–41.
37. **Bhat R**, **Axtell R**, **Mitra A**, **Miranda M**, et al. 2010. Inhibitory role for GABA in autoimmune inflammation. *Proc Natl Acad Sci USA* **107**: 2580–5.
38. **Nakajima K**, **Tooyama I**, **Kuriyama K**, **Kimura H.** 1996. Immunohistochemical demonstration of GABAB receptors in the rat gastrointestinal tract. *Neurochem Res* **21**: 211–5.
39. **Poulter MO**, **Singhal R**, **Brown LA**, **Krantis A.** 1999. GABA(A) receptor subunit messenger RNA expression in the enteric nervous system of the rat: implications for functional diversity of enteric GABA(A) receptors. *Neuroscience* **93**: 1159–65.
40. **Yurdaydin C**, **Walsh TJ**, **Engler HD**, **Ha JH**, et al. 1995. Gut bacteria provide precursors of benzodiazepine receptor ligands in a rat model of hepatic encephalopathy. *Brain Res* **679**: 42–8.
41. **Minuk GY.** 1986. Gamma-aminobutyric acid (GABA) production by eight common bacterial pathogens. *Scand J Infect Dis* **18**: 465–7.
42. **Collins SM**, **Bercik P.** 2009. The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. *Gastroenterology* **136**: 2003–14.
43. **Forsythe P**, **Sudo N**, **Dinan T**, **Taylor VH**, et al. 2010. Mood and gut feelings. *Brain Behav Immun* **24**: 9–16.