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Probiotics to prevent mental disorders

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Abstract Increased intestinal permeability has been associated with a diversity of gastro-intestinal diseases. Moreover, the intestinal barrier is involved in the bidirectional communication between the GI-tract and the central nervous system. Research has shown that probiotics can positively influence the gut microbiota and intestinal barrier function. The aim of this article is to summarize the research performed with the probiotic formulation (Ecologic® Barrier) that has been specifically developed to strengthen the intestinal barrier function and by this alleviate or prevent mental disorders. In rodent studies this formulation has demonstrated to reduce depressive-like behavior, and in human studies to decrease sensitivity to depression and migraine frequency. This and other research will further unravel the working mechanisms of probiotics on the gut-brain axis and could reveal a potential for a broader range of gut-brain related disorders.

INTRODUCTION

Increased intestinal permeability, also called a "leaky gut", is associated with various gastrointestinal disorders, including Inflammatory Bowel Disease (IBD), coeliac disease and Irritable Bowel Syndrome (IBS) (1). Moreover, a connection between a "leaky gut" and conditions such as metabolic syndrome, depression and autism is also becoming increasingly more pronounced (2-4).
Preclinical research has shown that certain probiotics can strengthen the intestinal barrier and reduce intestinal permeability (5). Probiotics have also shown to affect the activity of the gut microbiome and even influence communication between the gut and the brain, which is known as the gut-brain axis (6). Initial evidence from clinical research supports the potential of probiotics as a supplementary treatment strategy for brain-related problems such as; autism, ADHD, depression, Alzheimer's disease and Parkinson's disease (7).
The probiotic formulation Ecologic® Barrier has been specifically developed to support the intestinal barrier functioning and has shown *in vitro* to strengthen the epithelial barrier and inhibit pro-inflammatory activation of the immune system. This paper will summarize the results of *in vivo* trials performed with this probiotic on brain functioning.

INTESTINAL BARRIER: COMPOSITION AND FUNCTION

An ideal intestinal barrier consists of closely packed epithelial cells. The luminal side of the barrier is populated by 10^{13}-10^{14} micro-organisms, known as the microbiome. The microbiome is essential for digestion of food, vitamin production (B and K) and protection against pathogens, with a high microbiome diversity in the gut being associated with good health (8).
The intestinal barrier fulfils an essential role in maintaining homeostasis and protecting against the harmful effects of antigens and toxins. The intestinal barrier function is influenced by various factors, such as genetic predisposition, age, infections, diet, stress, and certain medication. Negative effects can lead to increased permeability caused by disturbance of barrier integrity. This results in bacterial components, such as microbiome metabolites, insufficiently digested food particles and other immunomodulatory components, entering more easily into the internal environment, where they do not belong.

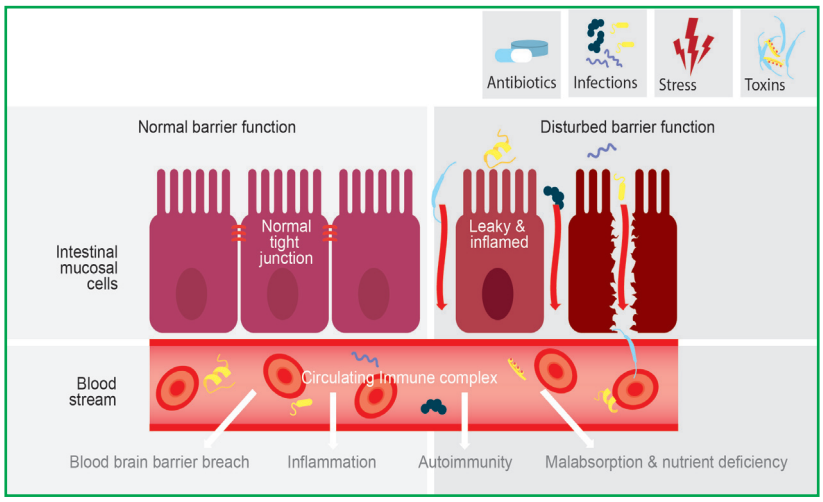
GUT-BRAIN CONNECTION

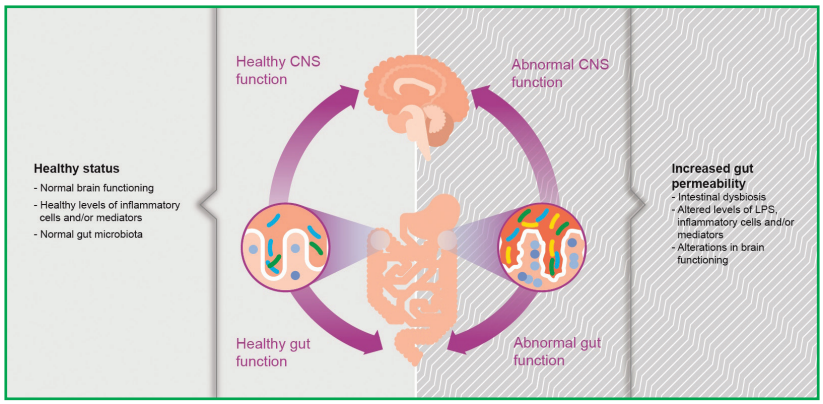
Recent research has started to unravel the connections between the gut and the central nervous system. Communications have been found to move in two directions; not only are messages send from the brain to the gut, but also from the gut to the brain (9). The exact mechanisms of gut-brain communications have not yet been elucidated, but it is thought to involve neural, hormonal, metabolic and immunological routes (5). Evidence on these mechanisms primarily come from animal studies. Braniste and colleagues showed that the blood-brain barrier permeability was increased in germ free mice compared to their colonized counterparts (10). Ingestion of a butyrate producing bacteria normalized the barrier function, even only offering butyrate had this effect. Also in rodents they found that ingestion of a *Lactobacillus* species normalized emotional behaviour and expression of GABAergic receptors in the brain.
This effect was not seen when the vagus nerve was cut, which suggests that the effect of the probiotic was through the neural route (11). The immunological pathway was investigated using

mice who underwent bile duct ligation, this inflammatory state induces sickness behaviour. This effect was less apparent when the mice used a probiotic mixture. They also looked at the microglia cells in the brain, as expected, they were less activated in the probiotic group (12). This research suggests that neuro-inflammation might be an important factor in gut-brain axis related diseases.

PRECLINICAL EVIDENCE SUPPORTING THE EFFECT OF THE MICROBIOME ON THE CENTRAL NERVOUS SYSTEM

Preclinical research and early clinical evidence support the hypothesis that the intestinal microbiome can affect brain functioning. Preclinical research has shown that the microbiome can stimulate the regulatory effects of microglia (immune cells in the brain) and therefore can control neuro-inflammatory processes (7). Apart from the fact that the intestinal barrier and the blood brain barrier (BBB) have similar structures, various studies have shown changes in BBB permeability after introducing microbiomes or metabolites of microbes to germ-free animals (2). The effect of the microbiome on an organism is generally investigated in germ-free animals because this allows the causal relationship between microbiome and health to be properly researched.





Some mice species are known to differ in behaviour and physiology as well as in composition of the microbiome. Using these differences the effect of the microbiome on animal behaviour was investigated. From both mice species they used germ-free individuals and transplanted the microbiome of the other strain. What they found is that this fecal transplantation lead to transfer of the behavioural characteristics. This is initial proof for a causal relationship between the microbiome and behaviour independent of the autonomous nervous system or other genetic aspects (13). Metabolites produced by micro-organisms in the gut have neuro-active properties (14). One of the most important examples is certain strains of *Lactobacillus* and *Bifidobacterium* can produce and secrete gamma-aminobutyric acid (GABA); a very important inhibitory neurotransmitter associated with anxiety and depression (15, 16). Furthermore, the serotonin precursor tryptophan was increased in the plasma of rats after consumption of *Bifidobacterium infantis* (17). Also, some specific species are known to produce norepinephrine, serotonin and dopamine (18). Moreover, polyunsaturated fatty acids (PUFA) like EPA and DHA, and short chain fatty acids (SCFA) like propionate and butyrate are produced by the microbiota and seem to affect brain functioning (19).
In summary, preclinical research has provided evidence for the importance of the microbiome for the gut-brain connection.

PROBIOTICS

Probiotics are defined by the World Health Organization as "live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host" (20, 21).
Factors that contribute to good probiotic functionality are; survival though the gastro-intestinal tract , metabolic activity, adherence to the intestinal mucosa and/or production of antimicrobial substances (22). A daily intake of minimum 10^9 to 10^{10} cfu/ day seems to be required to show an effect. Research has shown that multispecies probiotics are more effective compared to single-strain probiotics.

ECOLOGIC® BARRIER

Properties of bacteria can differ significantly between strains and species. For example, a specific strain can have good qualities to inhibit pathogens, but can have no influence on the gut barrier function. For each disorders different working mechanisms of probiotics are most relevant. Therefore, the composition of targeted indication specific multispecies probiotic requires a specific composition per indication. Ecologic® Barrier is a multispecies probiotic that has been developed to optimise barrier function and to reduce low-grade inflammation. The product contains the following bacterial strains: B. *bifidum* W23, B. *lactis* W52, L. *acidophilus* W37, L. *brevis* W63, L. *casei* W56, L. *salivarius* W24, Lc *lactis* W19, Lc. *Lactis* W58, in a carrier matrix of maize starch and maltodextrins with a viable cell count of 2.5 *109 cfu/gram. The advised dosage is 2 grams, twice daily. The product has been developed by Winclove Probiotics, Amsterdam the Netherlands. The probiotic strains were selected based on the following criteria: in vitro strengthening of the epithelial barrier, inhibition of mast-cell activation, inhibition of pro-inflammatory cytokines and decreasing lipopolysaccharide load.
Preclinical research has shown that this multispecies probiotic can strengthen the epithelial barrier and can inhibit pro-inflammatory activation of the immune system (23).

		Pre-intervention	Post-intervention
LEIDS-r			
Aggression	Placebo	8.80 (0.94)	8.45 (0.98)
	Probiotics**	8.68 (0.94)	6.25 (0.98)
Control	Placebo	7.65 (0.80)	6.70 (0.82)
	Probiotics	7.25 (0.83)	5.80 (0.82)
Hopelessness	Placebo	5.60 (0.85)	4.70 (0.74)
	Probiotics	4.75 (0.85)	4.0 (0.74)
Risk aversion	Placebo	9.50 (0.93)	9.25 (0.87)
	Probiotics	10.00 (0.93)	7.95 (0.87)
Rumination	Placebo	11.75 (0.90)	11.85 (0.93)
	Probiotics***	11.20 (0.90)	8.25 (0.93)
Acceptance	Placebo	1.40 (0.34)	1.35 (0.37)
	Probiotics	0.90 (0.34)	1.10 (0.37)
Total	Placebo	44.70 (3.24)	42.30 (3.51)
	Probiotics***	42.75 (3.24)	33.35 (3.51)

* p<.05
** p<.01
***p<.001

Table 1. Mean pre- and post-intervention scores and standard error of the means (shown in parentheses) on the LEIDS-r in the Placebo and Probiotics groups. Asterisks indicate significant treatment effect of differences between pre- and post-intervention assessments.

Since this probiotic has shown in vitro positive effects on intestinal barrier function the product has been tested further in various in vivo models to investigate effects on brain functioning.

(CLINICAL) EVIDENCE WITH ECOLOGIC® BARRIER

A first study with Ecologic® Barrier was performed in collaboration with Aarhus University, Denmark. Healthy rats were given Ecologic® Barrier or placebo for 8 weeks. After this period the animals performed a forced swim test, a typical screening for depressive-like behaviour in rodents. The more depressed rats are, the less they move. The group of animals that received probiotics moved significantly more compared to the group of animals that received a placebo (24).

The effects of Ecologic® Barrier on vulnerability to depression has also been investigated in a human trial. In a randomized placebo-controlled trial performed at Leiden University the Netherland, 40 healthy students were given Ecologic® Barrier or placebo for 4 weeks. Before and after the intervention a validated questionnaire (the Leiden Index of Depression Sensitivity) was filled out. This questionnaire measured cognitive reactivity response to sad mood, which is known to give a good estimation of likelihood to become depressed.

The LEIDS-r scale consists of six subscales that measure vulnerability with respect to:

- Aggression (e.g., when I feel down, I lose my temper more easily);
- Hopelessness/suicidality (e.g., when I feel down, I more often feel hopeless about everything; when I feel sad, I feel more that people would be better off if I were dead);
- Acceptance/coping (e.g., when I am sad, I feel more like myself);
- Control/perfectionism (e.g., I work harder when I feel down);
- Risk aversion (e.g., when I feel down, I take fewer risks);
- Rumination (e.g., when I feel sad, I more often think about how my life could have been different).
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At baseline there were no differences between the two groups, but after 4 weeks the sensitivity to depression score decreased significantly in the probiotic group compared to the placebo group, see Table 1. The most pronounced decreases were found in the categories aggression and rumination (25).

In another study, Ecologic® Barrier has been tested in an open-label pilot in collaboration with Wageningen University and Hospital Gelderse Vallei in Ede, the Netherlands, in patients that suffer from migraine. Twenty-seven patients received a daily dose (5*109 cfu/day) of Ecologic® Barrier for 12 weeks. Patients kept a headache diary and filled out two validated headache questionnaires at t=0 and t=12 weeks. The trial showed that supplementation with Ecologic® Barrier decreased migraine frequency with 23% and severity with 13% (26). A follow-up of this pilot study is currently being analysed in collaboration with the research groups.

FUTURE RESEARCH

These first results are promising and will lead to further research. The University of Leiden has received a European Joint Translational Grant to continue this research in collaboration with Spanish and German research groups. They will perform a randomised, placebo-controlled trial to investigate the effect of multiple probiotic formulations, including Ecologic® Barrier, on depressed mood and cognitive function in a large group of elderly people. In addition, a study is being performed in collaboration with the University of Technology Sydney in Australia in a depressed patient population. They also plan to perform a follow-up study in a population using antidepressants. Moreover, the Radboud UMC in Nijmegen are undertaking another randomized control trail using imaging techniques (fMRI) to investigate the effect of Ecologic® Barrier on brain activity in relation to cognitive functioning and performing activities among a group of healthy women.

CONCLUSION

(Clinical) evidence supports a role of gut microbiota and probiotics in brain related disorders. A wide range of gut-brain related disorders are thought to be linked to a disturbed intestinal barrier function. The outcome of studies investigating probiotics such as Ecologic Barrier® are very positive and has prompted further research into the exact mechanisms and effects in patient populations. New insights into the mechanisms involved