Multispecies probiotics; an overlooked piece of the puzzle in fighting depression?

Elsbeth Pekelharing, MSc
Science Liaison
Winclove’s Company DNA

60+ customers
30+ countries

70+ formulations

25 years
dedicated to PROBIOTIC
DEVELOPMENT, research
and manufacturing

Family owned
100% SELF
FINANCED

100+ strains
10 prebiotics

FOOD
supplements, FSMP & MD

100+ strains
10 prebiotics

100 million
daily dosages sold in 2015

PhDs +10%
Winclave probiotics

Goal: indication specific probiotics that improve quality of life

• Evidence based; development in collaboration with universities, academic hospitals and research institutes

• High quality technical aspects; optimal survival through, and activity in GI-tract

• User friendly; Safe to use, no side effects.

Shelf life 2 years @ room temperature (25°C)
Winclave Research

Our Focus Areas

- **38** RESEARCH PROJECTS started, ongoing, or finished in 2015
- **82%** of research in 2015 were in HUMANS
- **>40** External research partners worldwide
- **>50** peer-reviewed PUBLICATIONS over the last 10 years
- **5** MANUSCRIPTS IN THE RESEARCH PIPELINE for publication in the 2016
Our position within Probiotic Landscape

- Human Use
- MD, FSMP, FS
- Probiotics
- Evidence-based
- Multi-species
- Medical endorsed
Evidence based Ecologic formulations
Our Microbes

The gut contains between 500 - 2000 different individual species of MICROORGANISMS.

Our bodies are MORE MICROBE THAN HUMAN.

Genetically 150X more microbe than HUMAN.

100,000,000,000,000 (1x10^{14}) bacteria

UNIQUE Personal microbiota
Gut-Brain: Bidirectional communication

Mayer et al. J Neurosci 2014
Publications on: Microbiota & Brain

Source: Pubmed (March, 2016)
Germ-free mice: higher stress response

Sudo et al were the first to demonstrate that germfree mice have an exaggerated stress response than mice with a (pathogen free) microbiota.

This enhanced responsivity can be reversed by probiotics.
Mechanisms in gut-brain axis

**Neurological pathway**
- Vagus nerve
- Enteric nervous system
- Neurotransmitters (serotonin, GABA)

**Humoral pathway**
- Gut and endocrine hormones

**Metabolic pathway**
- Bacterial metabolites (eg SCFA)

**Immune pathway**
- Cytokines

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*Cryan & Dinan, Nat Rev Neurosci. 2012 Oct;13(10):701-12*
Metabolic pathway

The gut microbiota influences blood-brain barrier permeability in mice


Evans Blue:

SCFAs or metabolites produced by bacteria affect BBB permeability
Neurological pathway – vagus nerve

Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve

Javier A. Bravo\textsuperscript{a,1}, Paul Forsythe\textsuperscript{b,c,1}, Marianne V. Chew\textsuperscript{b}, Emily Escaravage\textsuperscript{b}, Hélène M. Savignac\textsuperscript{a,d}, Timothy G. Dinan\textsuperscript{a,e}, John Bienenstock\textsuperscript{b,f,2}, and John F. Cryan\textsuperscript{a,d,g,2}
Immune pathway


Probiotics Improve Inflammation-Associated Sickness Behavior by Altering Communication between the Peripheral Immune System and the Brain

**A**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sham</th>
<th>BDL</th>
<th>Sham</th>
<th>BDL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time spent in social investigation (seconds/10 minute observation period)</strong></td>
<td>[Bar chart showing data]</td>
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</tbody>
</table>

**B**

<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>Time spent being immobile (seconds/10 minute observation period)</strong></td>
<td>[Bar chart showing data]</td>
<td></td>
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</tbody>
</table>
Immune modulation to the brain by probiotics

Debilitating sickness behaviour
Changes in brain function
Microglial activation
Cerebral monocyte infiltration
Higher systemic TNF-α

Probiotics

Immune modulation to the brain by probiotics

D'Mello et al (2015)
Neurobiology of Disease

Probiotics Improve Inflammation-Associated Sickness Behavior by Altering Communication between the Peripheral Immune System and the Brain
Barrier function in gut-brain-axis


**FIGURE 2 | Potential neuropsychiatric consequences of a dysregulated intestinal barrier.** Activation of brain-gut-microbiota Axis signaling pathways via a compromised intestinal barrier with potential effects on mood, anxiety, cognition and social interaction.
Barrier function in gut-brain-axis

- Proinflammatory cytokines
- Low grade inflammation
- Neuroinflammation
- Cognitive dysfunction & altered behaviour

LPS in circulation → Proinflammatory cytokines → Low grade inflammation

Intestinal Mucosal Cells
- Normal tight junction
- Leaky and Inflamed

Blood Stream
- Blood Brain Barrier Breach
- Inflammation
- Autoimmunity
- Malabsorption & nutrient deficiency

Circulating immune complex
Microbiota management

Definition
Live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host.

(WHO)

Selection criteria

- **Stimulate regulatory response**
  - IL-10 production

- **Inhibit pro-inflammatory response**
  - Inhibition mast cell activation
  - LPS breakdown

- **Barrier function**
  - Trans epithelial resistance
Effect of strains on barrier function

Stressor (TNF-α + strain)
Formulation

• Eight bacterial strains
  - *Bifidobacterium bifidum* W23
  - *Bifidobacterium lactis* W52
  - *Lactobacillus acidophilus* W37
  - *Lactobacillus brevis* W63
  - *Lactobacillus casei* W56
  - *Lactobacillus salivarius* W24
  - *Lactococcus lactis* W19
  - *Lactococcus lactis* W58

• Concentration: 2,5 billion cfu/gram

• Corn starch, maltodextrine, vegetable protein, mineral mix (potassium magnesium manganese sulfate)
Depression – rodent study

- Aarhus University, Denmark
- Placebo controlled study with 40 healthy rats
- Multispecies probiotic 8 weeks
  Ecologic®Barrier (5x10⁹ cfu/day)
- Forced swim test (7 min)
Depression – human study

- Leiden University, the Netherlands, 2013-2014
- Randomized, placebo-controlled study
- 40 healthy students
- Multispecies probiotic (Ecologic® Barrier) or placebo
- 4 weeks, 2 gram daily
- Questionnaire: Leiden Index of Depression Sensitivity, Revised
Depression – human study

A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood

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Attention for Steenbergen publication
Eating your way out to longevity and well-being

Posted on November 17, 2015 in Human Potential, Pharma, Social

Within twenty years Europe will face a situation where the largest population cohort will be those over the age of 65. Can food
Influence of the Multispece Ecologic® BARRIER on Pa Intestinal Barrier Function

Saskia Van Hermer, Geline Ornel
Research and Development Wincove Probiotics, Amsterdam, Email: GOrnell@wincove.com
Received 24 June 2014; revised 26 August 2014; accepted 25 October 2014. Copyright © 2014 by authors and Scientific Research Publisher. This work is licensed under the Creative Commons Attribution http://creativecommons.org/licenses/by/4.0/.

Abstract
The intestinal epithelium is a single cell layer that faces against the external environment. A proper functioning maintains optimal health. A disrupted intestinal barrier development of parasitic and inflammatory disease confer a health benefit to the host and have been associated with associated diseases. The ability to control the amounts of bacterial in Ecologic® BARRIER barrier in various ways.

Keywords: Probiotics, Ecologic® BARRIER, Intestinal Barrier Func.

1. Introduction
The intestinal barrier is the largest interface between the mucosal surfaces that is subject to the effects of gut bacteria and other bacteria in the gastrointestinal tract. The ability to control the amounts of bacterial in Ecologic® BARRIER barrier in various ways.


The effects of the multispecies probiotic mixture Ecologic® BARRIER on migraine: results of an open-label pilot study


Abstract
Migraine prevalence is associated with gastrointestinal dysorxia and inflammation. Probiotics may therefore reduce the frequency and intensity of symptoms, and reduce the number of clinical effects and adverse reactions of probiotic therapy. (Ecologic® Barrier, 2.5 × 109 CFU/50 g and 10 billion CFU/mg) and intensity of migraine in a diary and complete.

Henry Ford Hospital Headache Disability Inventory (HDI) was measured every 4 weeks by comparing the remission to 90% patients who took 95% of the supplements. Obstructive treatment only.


A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood

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Abstract
Background: Recent developments in the role of the human microbiota in cognitive and affective functions have led to the hypothesis that probiotic supplementation may act as an adjuvant strategy to reduce negative thoughts and mood disorders.

Purpose: To test the effects of multispecies probiotics on cognitive reactivity to sad mood.

Methods: A randomized controlled trial was conducted in healthy volunteers. Participants were randomly assigned to receive 4 weeks of probiotic supplementation or a placebo intervention. The primary outcome was measured using the Leiden Index of Depression and Anxiety Scale.

Results: Participants who received the probiotic intervention showed a significant reduction in cognitive reactivity to sad mood, which was largely accounted for by reduced rumination and negative thoughts. These results provide the first evidence that the intake of probiotics may reduce negative thoughts associated with sad mood.

Keywords: Probiotics, Depression, Cognitive reactivity

1. Introduction
Migraine in a disorder with a lifetime prevalence of 15-33% (Lauer et al., 1999). Several studies suggest that migration can be considered as a complex multifocal inflammatory disorder (Monteith and Goodbey, 2011). The pathophysiology of migraine is not fully understood (Sasson et al., 2010). Associations were found between migraine and gastrointestinal disorders including irritable bowel syndrome, inflammatory bowel disease and Crohn's disease (Van Hermer et al., 2015). These associations have been found in two directions: patients who have more often gastrointestinal disorders compared with healthy controls.

Keywords: Gastrointestinal disorders, Probiotics, Migraine

1. Introduction
The intestine and the brain are intimately connected via the gut-brain axis, which involves bidirectional communication via neuro-endocrine and immune pathways (Grossman, 1998; Graham et al., 2011; Mayer, 2011; Mayer et al., 2016). In recent years, it has become increasingly evident that the communication also involves interactions with the intestinal microbiota, release immune activating and other signaling molecules that may play an important role in regulating the brain and behavior (Mayer, 2011; Ogino and Jänne, 2013). For example, the microbiota produce neurotransmitter substances and their precursors (e.g., tryptophan) which also affect the brain's endogenous systems.
In vivo studies

- Gut brain general
  - RCT with fMRI (ongoing)
  - Proof of principle (ongoing)

- Depression
  - Animal model
  - RCT pilot (Steenbergen et al, 2015)
  - RCT in elderly (Leiden University, ongoing)
  - RCT in depressive patient (ongoing) follow up planned with antidepressant

- Migraine
  - Open label pilot (Roos et al, 2015)
  - RCT (analyzing)
Thank you for your attention

Any questions?