Stable *Bifidobacterium* colonization and personalized therapy

**Jens Walter**

Therapeutic modulation of the gut microbiota

Therapeutic disruption of dysbiosis
- Antibiotics
- Probiotics
- Dietary intervention/Prebiotics
- Fecal transplantation

Dysbiosis-related diseases
- Chronic gastrointestinal infections
- Antibiotic-associated diarrhoea
- Pseudomembranous colitis
- Inflammatory bowel disease
- Necrotizing enterocolitis

Health

Microbiota diversity reduced
- Elevated Enterobacteriaceae/opportunistic pathogens
- Skewed SCFA profile
- Disruption of mucosal barrier
- Host inflammatory response initiated

Invited review

Therapeutic modulation of intestinal dysbiosis

Alan W. Walker\textsuperscript{a,*}, Trevor D. Lawley\textsuperscript{b}

\textsuperscript{a} Pathogen Genomics Group, Wellcome Trust Sanger Institute, Hinxton, UK
\textsuperscript{b} Bacterial Pathogenesis Laboratory, Wellcome Trust Sanger Institute, Hinxton, UK

Pharmacological Research 69 (2013) 75–86
How to modulate the gut microbiota?

Antibiotics
Bacteriophages
Fecal transplantation
Diet
  Probiotics
  Prebiotics
  Fiber, Resistant starches
  Whole grains
  Other food components
Rare
Strain-specific effects
- Neurological effects
- Immunological effects
- Endocrinological effects
- Production of specific bioactives

Frequent
Species-level effects
- Vitamin synthesis
- Direct antagonism
- Gut barrier reinforcement
- Bile salt metabolism
- Enzymatic activity
- Neutralization of carcinogens

Widespread
Among studied probiotics
- Colonization resistance
- Acid and SCFA production
- Regulation of intestinal transit
- Increased turnover of enterocytes
- Competitive exclusion of pathogens
- Normalization of perturbed microbiota
Probiotics have limited effect on the resident microbiota

454 Pyrosequencing Analysis on Faecal Samples from a Randomized DBPC Trial of Colicky Infants Treated with *Lactobacillus reuteri* DSM 17938

Stefan Roos¹, Johan Dicksved⁴, Valentina Tarasco², Emanuela Locatelli², Fulvio Ricceri³, Ulf Grandin⁵, Francesco Savino²*

Associations between the human intestinal microbiota, *Lactobacillus rhamnosus* GG and serum lipids indicated by integrated analysis of high-throughput profiling data

Leo Lahti¹,²,⁶, Anne Salonen¹,²,⁶, Riina A. Kekkonen⁴, Jarkko Salojärvi⁴, Jonna Jalanka-Tuovinen¹, Airi Palva¹, Matej Orešič⁵ and Willem M. de Vos¹,²,³

Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials

Nadja B. Kristensen¹, Thomas Bryrup, Kristine H. Allin, Trine Nielsen, Tue H. Hansen and Oluf Pedersen
<table>
<thead>
<tr>
<th>Day and Lactobacillus strain</th>
<th>Rate of reisolation (log no. of bacteria/g of feces) from volunteer no.:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSM 12246</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19070-2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHCC 2329</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHCC 3137</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGG</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSM 12246</td>
<td>—</td>
<td>6.7</td>
<td>7.5</td>
<td>—</td>
<td>5.9</td>
<td>—</td>
<td>7.7</td>
<td>6.4</td>
<td>—</td>
<td>5.7</td>
<td>6.7</td>
<td>6.7</td>
<td>6.7</td>
</tr>
<tr>
<td>19070-2</td>
<td>8.2</td>
<td>7.7</td>
<td>7.4</td>
<td>5.0</td>
<td>5.8</td>
<td>—</td>
<td>4.7</td>
<td>6.7</td>
<td>—</td>
<td>7.7</td>
<td>7.5</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>CHCC 2329</td>
<td>7.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>—</td>
<td>6.7</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHCC 3137</td>
<td>—</td>
<td>—</td>
<td>7.9</td>
<td>6.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.7</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGG</td>
<td>—</td>
<td>5.0</td>
<td>—</td>
<td>7.6</td>
<td>7.7</td>
<td>6.0</td>
<td>—</td>
<td>6.0</td>
<td>5.0</td>
<td>—</td>
<td>7.7</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSM 12246</td>
<td>6.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19070-2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHCC 2329</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHCC 3137</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGG</td>
<td>5.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td>5.0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSM 12246</td>
<td>ND</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19070-2</td>
<td>ND</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHCC 2329</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHCC 3137</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGG</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* – no reisolations.

*b* ND, not determined.
Persistence of *Lactobacillus reuteri* in the human gastrointestinal tract

Quantitative evaluation of synbiotic strategies to improve persistence and metabolic activity of *Lactobacillus reuteri* DSM 17938 in the human gastrointestinal tract

Monchaya Rattanaprasert, Stefan Roos, Robert W. Hutkins, Jens Walter
Why such limited impact? An ecological perspective

Characteristics of microbe(s) that influence engraftment
• Dose, frequency, and activity
• Traits to overcome habitat filters and facilitate competition
• Genetic and species diversity
• Phenotypic plasticity
• Adaptability

(A) Introduction into the gastrointestinal tract
(B) Establishment of a viable population
(C) Growth and persistence upon the acquisition of resources
(D) Impact

Host related mechanisms
• Habitat filtering (stomach pH, bile acids and defensins)
• Epithelial selection

Microbiome related mechanisms
• Competition
• Antagonism (inhibition) or facilitation
• Predation

Effects on the microbiome
• Shift and displacement of resident taxa
• Alteration of community function

Walter et al, in preparation, based on Mallon et al., Trends in Microbiology, 2015
Will the real gut microbiota please stand up!

‘autochthonous’ (found where they were formed)

Bacteria that have occupied an ecological niche in the GIT.

They have a long-term association with a particular host and form stable populations of characteristic size in a particular region of the gut (Savage, 1977).

‘allochthonous’ (formed in another place)

Bacteria that are just passing through.

They may represent autochthonous members of a more proximal area (oral cavity) that have been dislodged (shed), or derived from ingested food and water.
What happens when a core member is introduced into the human gut?

Bifidobacterium longum

Genomic variation landscape of the human gut microbiome

Siegfried Schlössner1, Manimezhian Arumugam1, Shinichi Sunagawa1, Makedonka Mitrova2, Julien Tap1, Ana Zhu1, Alison Waller1, Daniel R. Mende1, Jens Roat Kultima1, John Martin2, Karthik Koru1, Shamil R. Sunyaev3, George M. Weinstock2 & Peer Bork1,4

3 January 2013 | Vol 493 | Nature
How does a core member of the human microbiome behave after being introduced into the human gut as a probiotic?

Stable Engraftment of *Bifidobacterium longum AH1206* in the Human Gut Depends on Individualized Features of the Resident Microbiome

Maria X. Maldonado-Gómez, Inés Martínez, Francesca Bottacini, Amy O’Callaghan, Marco Ventura, Douwe van Sinderen, Benjamin Hillmann, Pajau Vangay, Dan Knights, Robert W. Hutkins, and Jens Walter

Maldonado-Gómez et al., 2016, Cell Host & Microbe 20, 515–526

October 12, 2016 © 2016 Elsevier Inc.
**Experimental Design**

**Group 1 : Probiotic**

**Group 2 : Placebo**

**Period 1**

- Fecal samples collection
- GSRS
- Sequencing and typing

**Week**

1. Baseline Treatment
2. Test of Persistence (TOP)

**Period 2**

1. Baseline Treatment
2. Test of Persistence (TOP)

**Follow up**

**Group 1**

**Group 2**

**n=22**

- **Probiotic**
  - *B. longum subsp. longum AH1206*
  - (10^{10} cells/day)

- **Placebo**
  - *Maltodextrin*

Maria X. Maldonado-Gómez
Ph.D. Candidate
Survival through the gastrointestinal tract and persistence

Proportion of strain AH1206 among total cultured bifidobacteria

Log$_{10}$ B. longum spp. longum AH1206 bacterial cells per gram of feces
Persistence of AH1206 in the human gut

Cell numbers of AH1206 in fecal samples (Log_{10} cells per gram)

-qRT-PCR

Detection limit

Persisters

Non-persisters

Days of consumption

Days of Washout

Detection limit

qRT-PCR

Persisters

Non-persisters
Confirmation of prolonged persistence

AH1206-unique genes in the metagenomic data set

<table>
<thead>
<tr>
<th>Baseline</th>
<th>14 days probiotic consumption</th>
<th>~200 days TOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persisters</td>
<td>A, B, D, G, H, I, J</td>
<td>*</td>
</tr>
</tbody>
</table>

$ p = < 0.0001 $  
$ * p = 0.0313 $  

Isolates from 3 subjects displayed the same sequence type as AH1206
Are ecological characteristics of the resident community associated with colonization?

Inés Martínez
Maria X. Maldonado-Gómez
Does community membership determine persistence?

**B. longum**

- $p = 0.0022$
- m.i. 6.12

16S rRNA Oligotyping:
- Relative Abundance (%)

Metagenomics:
- Abundance (mapped reads/total reads)

qRT PCR:
- Log10 (cells/gram of feces)

Dan Knights
University of Minnesota
Charles Darwin’s naturalization hypothesis proposes that colonization is less likely when the colonizing species is related to members of the invaded community, because evolutionary closeness intensifies competition among species that share similar resources.

“As the species of the same genus usually have, though by no means invariably, much similarity in habits and constitution, and always in structure, the struggle will generally be more severe between them” (The origin of Species, 1859, p. 60).
Is the functional composition different in communities of persisters and non-persisters?

70 genes with differential abundance between P and NP
- 93% enriched in NP

13 highly discriminative genes identified by Random Forest analysis

<table>
<thead>
<tr>
<th>Gene Description</th>
<th>P:NP</th>
<th>NP:P</th>
<th>Mean Imp.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permease facilitator - g 1830</td>
<td>2.27</td>
<td>0.0274</td>
<td>2.27</td>
<td>0.0274</td>
</tr>
<tr>
<td>RNA methyltrans - g 0009</td>
<td>2.18</td>
<td>0.0184</td>
<td>2.18</td>
<td>0.0184</td>
</tr>
<tr>
<td>MSF transporter – g 0323</td>
<td>2.03</td>
<td>0.0016</td>
<td>2.03</td>
<td>0.0016</td>
</tr>
<tr>
<td>β - galactosidase - g 0877</td>
<td>1.72</td>
<td>0.0035</td>
<td>1.72</td>
<td>0.0035</td>
</tr>
<tr>
<td>3-oxoacyl-ACP reductase - g 1048</td>
<td>1.68</td>
<td>0.0166</td>
<td>1.68</td>
<td>0.0166</td>
</tr>
<tr>
<td>Amino acid transporter - g 1568</td>
<td>1.30</td>
<td>0.0219</td>
<td>1.30</td>
<td>0.0219</td>
</tr>
<tr>
<td>Dipeptide-binding ABC transp. - g 0143</td>
<td>1.26</td>
<td>0.0166</td>
<td>1.26</td>
<td>0.0166</td>
</tr>
<tr>
<td>LacI family transcrip regul - g 1077</td>
<td>1.16</td>
<td>0.0179</td>
<td>1.16</td>
<td>0.0179</td>
</tr>
<tr>
<td>dTDP4-dehydrohamnose reductase - g 0411</td>
<td>1.14</td>
<td>0.0040</td>
<td>1.14</td>
<td>0.0040</td>
</tr>
<tr>
<td>Membrane protein - g 0791</td>
<td>1.06</td>
<td>0.0047</td>
<td>1.06</td>
<td>0.0047</td>
</tr>
<tr>
<td>LacI family transcrip regul - g 1833</td>
<td>1.04</td>
<td>0.0013</td>
<td>1.04</td>
<td>0.0013</td>
</tr>
<tr>
<td>Tetracycline resist related - g 0186</td>
<td>1.03</td>
<td>0.0028</td>
<td>1.03</td>
<td>0.0028</td>
</tr>
<tr>
<td>Hypothetical protein - g 1574</td>
<td>1.01</td>
<td>0.0080</td>
<td>1.01</td>
<td>0.0080</td>
</tr>
</tbody>
</table>
Genes that predict colonization show inter-individual variability in the resident *B. longum* populations (WMS)

- **LacI-type response repressor** – g 2015
- **Permeases of the major facilitator superfamily** – g 1830

**Functional genes that predict persistence are not phylogenetically conserved!**
Is the functional composition different in communities of persisters and non-persisters?

70 genes with differential abundance between P and NP
- 93% enriched in NP
- 38% CHOs metabolism

13 highly discriminative genes identified by Random Forest analysis
**Hypothesis:** Predictive genes encoding for specific functional traits determine resource opportunities

1. Strain AH1206 would possess the functional traits to successfully occupy the niche.
2. Differences in the relative abundance of these genes should disappear after AH1206 colonization.
91% of the discriminative genes had homologues

amino-acid similarity >98%, 100% coverage

Testing the hypothesis:

Baseline

Consumption

200 days TOP

$p = 0.005$

$p = 0.2736$

$p = 0.3383$
Recap

No long-term persistence of AH1206: “non-persisters”

Long-term persistence of AH1206: “persisters”

In persisters:
- Underrepresentation of the species *B. longum*
- Underrepresentation of genes for carbohydrates utilization
- Higher resource availability

Species

Genes

Resources
Does *B. longum* AH1206 consumption change the taxonomic composition of the fecal microbial?

**MANOVA** $p = 0.999$

**MANOVA** $p = 0.005$
Conclusions and implications

• An autochthonous strain can be stably established in a subset of humans if an open niche is available in the microbiome.

  Should be tested with additional strains.
  Could be used to introduce ‘missing strains’.
  Could be used as a criterion to select strains?
  Regulatory implications.

• Engraftment depends on individualized features of the pre-treatment microbiome linked to a niche opportunity.

  Microbiome data could be used to personalize probiotic applications.

• Even a strain that stably engrafts into the microbiome does not change gut microbiota composition.

  Might be different for other strains, and more studies are needed.
  An ecological perspective is necessary to understand the impact of a probiotic on the overall community.
Characteristics of microbe(s) that influence engraftment

- Dose, frequency, and activity
- Traits to overcome habitat filters and facilitate competition
- Genetic and species diversity
- Phenotypic plasticity
- Adaptability

(A) Introduction into the gastrointestinal tract

(B) Establishment of a viable population

(C) Growth and persistence upon the acquisition of resources

(D) Impact

Host related mechanisms
- Habitat filtering (stomach pH, bile acids and defensins)
- Epithelial selection

Microbiome related mechanisms
- Competition
- Antagonism (inhibition) or facilitation
- Predation

Effects on the microbiome
- Shift and displacement of resident taxa
- Alteration of community function

Walter et al, in preparation, based on Mallon et al., Trends in Microbiology, 2015
Acknowledgments

María X. Maldonado-Gómez
Robert Hutkins
Amanda Ramer-Tait
Andrew Benson
Daniel Peterson
Joao Carlos Gomes Neto
Hatem Kittana

Inés Martínez

Selena Healy
Eileen Murphy
Jenny Roper
Barry Kiely

Francesca Bottacini
Amy O’Callaghan
Douwe van Sinderen

Rosemary Pauley-Hunter
Jon Vanderhoof

Dr. Dan Knights
Benjamin Hillmann
Pajau Vangay
Thank you!