MICROBES IN THE BREAST
-CANCER, MASTITIS AND PROGRAMMING THE NEWBORN
Research on the human microbiome is booming, and scientists have moved from simply taking stock of gut flora to understanding the influence of microbes throughout the body.

By The Scientist Staff | August 1, 2014

Already in 1979, Carroll et al., reported than only 3% of milk samples were sterile!
Human milk is not sterile

“Human milk and breast tissue microbiome—Who is there and how do they get there?”

“Human milk and breast tissue microbiome—What is their role?”

“Human milk and breast tissue microbiome—Which factors influence its composition?”

“I’m not so much interested in obtaining new facts as to discovering new ways of thinking about them.”

They are causing infections

There is a normal milk microbiota

It may have a protective role

It is dependent of health status and environmental factors

“The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them.”
Human milk microbiota: Culture techniques

- Staphylococcus epidermidis
- Staphylococcus hominis
- Staphylococcus caprae
- Streptococcus salivarius
- Streptococcus mitis
- Streptococcus parasanguinis
- Streptococcus lactarius
- Lactobacillus crispatus
- Lactobacillus gasseri
- Lactobacillus fermentum
- Lactobacillus plantarum
- Lactobacillus reuteri
- Lactobacillus rhamnosus
- Lactobacillus salivarius
- Enterococcus faecium
- Enterococcus faecalis
- Lactococcus lactis
- Leuconostoc mesenteroides
- Leuconostoc garclicum
- Leuconostoc citreum
- Bifidobacterium adolescentis
- Bifidobacterium bifidum
- Bifidobacterium breve

Other Gram +
- Actinomyces spp.
- Corynebacterium spp.
- Kocuria spp.
- ...

Bacterias Gram -
- Escherichia coli
- Klebsiella milletis
- Klebsiella oxytoca
- Klebsiella pneumoniae
- Ralstonia spp.
- ...

50 genera
200 species
Non described species (*)

Heikkila and Saris, 2003
Martin et al., 2003, 2006, 2007a,b, 2009
Martin et al., 2010
Reviewed by Jost et al., 2015

10^3 cfu/mL
Human milk microbiota: 16S rRNA pyrosequencing

High inter-individual variability

>5%

9 core OTUs (50%)

16 women
3 samples each

Hunt et al, 2011
Human milk microbiota: 16S rRNA pyrosequencing + culture

Assessment of bacterial diversity in breast milk using culture-dependent and culture-independent approaches

Ted Jost¹, Christophe Lacroix¹, Christian Braegger² and Christophe Chassard¹
¹Laboratory of Food Biotechnology, Institute of Food, Nutrition and Health, ETH Zurich, Schmelzbergstrasse 7, 8092 Zurich, Switzerland
²Division of Gastroenterology and Nutrition, University Children’s Hospital Zurich, Steinwiesenstrasse 75, 8032, Zurich, Switzerland

(Submitted 30 August 2012 – Final revision received 28 January 2013 – Accepted 28 January 2013 – First published online 14 March 2013)

<table>
<thead>
<tr>
<th>ISOLATION FREQUENCY</th>
<th>%</th>
<th>PREVALENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ACTINOBACTERIA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium sp.</td>
<td>1.7</td>
<td>2 / 7</td>
</tr>
<tr>
<td>Propionibacterium spp.</td>
<td>13.7</td>
<td>7 / 7</td>
</tr>
<tr>
<td>Rothia sp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FIRMICUTES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>0.9</td>
<td>2 / 7</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>2.1</td>
<td>2 / 7</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>60.1</td>
<td>7 / 7</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>51.9</td>
<td>7 / 7</td>
</tr>
<tr>
<td>Veillonella sp.</td>
<td>1.7</td>
<td>2 / 7</td>
</tr>
<tr>
<td><strong>PROTEOBACTERIA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia/Shigella spp.</td>
<td>0.4</td>
<td>1 / 7</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>0.4</td>
<td>1 / 7</td>
</tr>
</tbody>
</table>

Jost et al, 2013
Human milk microbiota: 16S rRNA pyrosequencing

12 predominant genera
Detected in >90% of samples

16 subdominant genera

7 women
3 samples each

Jost et al, 2013
Breast tissue is not sterile

Locations within the breast of tissue samples collected for bacterial analysis

43 Canadian women
38 Irish women

Human milk microbiota: the origin

Human milk: a source of more life than we imagine

Phenotypic and genotypic analyses of lactic acid bacteria in local fermented food, breast milk and faeces of mothers and their babies
Human milk microbiota: the origin

Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria

Nature Immunology 2, 361 - 367 (2001)
doi:10.1038/86373

Maria Rescigno¹, Matteo Urbano¹, Barbara Valzasina¹, Maura Francolini³, Gianluca Rotta¹, Roberto Bonasio¹, Francesca Granucci¹, Jean-Pierre Kraehenbuhl³ & Paola Ricciardi-Castagnoli¹
Human milk microbiota: the origin

Bacterial gut translocation in vivo

CaMV35S  episp  NOS

10^8 cfu

Labelled strain MW 300bp 200bp 100bp

pTG262

CmR

Bacterial gut translocation in vivo

pTG262

CmR

10^8 cfu

MW 1 2 3 4 1 2 3 4 CN

MW 1 2 3 4

300bp 200bp 100bp
Human milk microbiota: the origin

Bacterial gut translocation \textit{in vivo}

Proportion of positive MLN (■) and mammary glands (□) Cultures

C: Control; TP1: Pregnant mice; TP2: lactating mice 0 to 1 day; TP3: Lactating mice 3 to 4 days; TP4: Lactating mice 14 to 15 days

Gram staining, showing bacteria (arrowshead) in the subepithelial dome (SED) and interfollicular region (IFR) of Peyer’s Patches, in mammary gland and in lamina propria (LP) of the distal small intestine
The DNA of *B. longum* is present in the mother’s blood, faecal sample and breast milk and in the infant faecal sample.

Identification of bacterial bodies in association with mononuclear cells.

Fig. 2. Intact bacterial structures in milk cells and peripheral blood mononuclear cells (PBMC) from lactating mothers stained with acridine orange.

Bifidobacterial surface-exopolysaccharide facilitates commensal-host interaction through immune modulation and pathogen protection.

*Donnet-Hughes et al., 2010*
Human milk microbiota: the origin

Bacterial gut translocation *in vivo*

Oral Administration of *Lactobacillus* Strains Isolated from Breast Milk as an Alternative for the Treatment of Infectious Mastitis during Lactation

E. Jiménez, L. Fernández, A. Maldonado, R. Martín, M. Olivares, J. Xaus, and J. M. Rodríguez

*Lb. gasseri* CECT5714
*Lb. salivarius* CECT5713

~ 10^{10} cfu/day
4 weeks
Human milk microbiota: the origin

Bacterial gut translocation *in vivo*

*Lb. salivarius* CECT5713

*Lb. fermentum* CECT5716

~ $10^9$ cfu / day

3 weeks

Treatment of Infectious Mastitis during Lactation: Antibiotics versus Oral Administration of Lactobacilli Isolated from Breast Milk

Rebeca Arrayo, Virginia Martín, Antoni and Juan Miguel Rodríguez
Departamento de Nutrición, Gerontología y Tecnología Nutricional
The differential composition of bacterial communities in the GIT, vagina and oral cavity has been related to the health of the host.
Human milk microbiota: their role

Infant colonization

Diversity of the *Lactobacillus* group in breast milk and vagina of healthy women and potential role in the colonization of the infant gut

R. Martin¹, G.H.J. Heilig², E.G. Zoetendal², H. Smidt² and J.M. Rodríguez¹

4 mother-infant pairs
Vaginally delivered
Human milk microbiota: their role

Infant colonization

BMC Microbiology

Research article

*Staphylococcus epidermidis*: A differential trait of the fecal microbiota of breast-fed infants

Esther Jiménez¹, Susana Delgado¹, Antonio Maldonado¹, Rebeca Arroyo¹, Mar Albuíjar², Natalia García², Manel Jariod², Leonides Fernández¹, Adolfo Gómez² and Juan M Rodríguez*¹

Table 4: Bacteria detected in the samples of breast milk and feces of the breast- and formula-fed infants and percentage of samples in which they were detected

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Milk</th>
<th>Breast-fed infants</th>
<th>Formula-fed infants</th>
<th>P-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>100.00%</td>
<td>86.05%</td>
<td>13.33%</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>16.28%</td>
<td>16.28%</td>
<td>13.33%</td>
<td>NS</td>
</tr>
<tr>
<td>Other <em>Staphylococcus</em> spp.</td>
<td>16.28%</td>
<td>6.98%</td>
<td>6.67%</td>
<td>NS</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>20.93%</td>
<td>53.49%</td>
<td>100.00%</td>
<td>0.0011</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em></td>
<td>ND c</td>
<td>2.33%</td>
<td>13.33%</td>
<td>NS</td>
</tr>
<tr>
<td>Other <em>Enterococcus</em> spp.</td>
<td>4.65%</td>
<td>9.30%</td>
<td>26.67%</td>
<td>NS</td>
</tr>
<tr>
<td><em>Streptococcus</em> spp.</td>
<td>27.91%</td>
<td>13.95%</td>
<td>ND</td>
<td>NS</td>
</tr>
<tr>
<td>Other Gram-positive bacteria</td>
<td>20.93%</td>
<td>69.77%</td>
<td>33.33%</td>
<td>0.0130</td>
</tr>
<tr>
<td>Gram-negative bacteria</td>
<td>46.51%</td>
<td>97.62%</td>
<td>66.67%</td>
<td>0.0008</td>
</tr>
</tbody>
</table>
Human milk microbiota: their role

Infant colonization

Transmission of Intestinal *Bifidobacterium longum* subsp. *longum* Strains from Mother to Infant, Determined by Multilocus Sequencing Typing and Amplified Fragment Length Polymorphism

Hiroshi Makino,1,2,* Akira Kusihiro,2 Eiji Ishikawa,2 Delphine Muylderm1, Hiroyuki Kubota,1,2 Takaumi Sakai,1,2 Kenji Oishi,1,2 Rocío Martín,4 Kaouthar Ben Amor,3 Raish Oozawa,3 Jan Knol,3 and Ruyihiro Tanaka1,2

Yakult Honsha European Research Center for Microbiology, ESV, Technologiepark 4, 9052 Ghent-Zwijnaarde, Belgium1; Yakult Central Institute for Microbiological Research, 1796 Yaho, Kamisachi, Tokyo 188-8550, Japan3; and Danone Research, Centre for Specialised Nutrition, Wageningen, The Netherlands2

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Family No.</th>
<th>Isolation source</th>
<th>Isolation period</th>
<th>Sequence type</th>
</tr>
</thead>
<tbody>
<tr>
<td>111</td>
<td>1</td>
<td>Mother feces</td>
<td>53 days before delivery</td>
<td>A*</td>
</tr>
<tr>
<td>112</td>
<td>1</td>
<td>Mother feces</td>
<td>37 days before delivery</td>
<td>A*</td>
</tr>
<tr>
<td>113</td>
<td>1</td>
<td>Infant feces</td>
<td>37 days before delivery</td>
<td>C*</td>
</tr>
<tr>
<td>114</td>
<td>1</td>
<td>Infant feces</td>
<td>37 days before delivery</td>
<td>C*</td>
</tr>
<tr>
<td>115</td>
<td>1</td>
<td>Infant feces</td>
<td>37 days before delivery</td>
<td>C*</td>
</tr>
<tr>
<td>116</td>
<td>1</td>
<td>Meconium</td>
<td>37 days before delivery</td>
<td>C*</td>
</tr>
<tr>
<td>117</td>
<td>1</td>
<td>Infant feces</td>
<td>37 days before delivery</td>
<td>C*</td>
</tr>
<tr>
<td>118</td>
<td>1</td>
<td>Infant feces</td>
<td>37 days before delivery</td>
<td>C*</td>
</tr>
<tr>
<td>119</td>
<td>1</td>
<td>Infant feces</td>
<td>37 days before delivery</td>
<td>C*</td>
</tr>
</tbody>
</table>

**Vertical mother–neonate transfer of maternal gut bacteria via breastfeeding**

Tedd Jost, Christoph Leenen,1,* Christian P. Bregger,1 Florence Hochleitner1 and Christophe Chevallier2

Laboratory of Food Biotechnology, Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland.

Division of Gastroenterology and Nutrition, University Children’s Hospital Zurich, Zurich, Switzerland.

Méridal Research Center, Lausanne, Switzerland.

[Image of a tree diagram illustrating the transmission of *Bifidobacterium longum* subsp. *longum* from mother to infant.]
Human milk microbiota: their role in protection?

Inhibition of Human Immunodeficiency Virus Type 1 by Lactic Acid Bacteria from Human Breastmilk

Virginia Martin, Antonio Maldonado, Leónides Fernández, Juan M. Rodríguez, and Ruth I. Connor

BREASTFEEDING MEDICINE
Volume 5, Number 4, 2010
© Mary Ann Liebert, Inc.
DOI: 10.1089/bfm.2010.0001

Table 6. PCR detected bacteriocin genes in the DNA of colostrum and respective MRS consortia and sequence similarity values in the NCBI database using BLAST.

<table>
<thead>
<tr>
<th>Bacteriocin genes (detected)</th>
<th>Fig 1 sample mark</th>
<th>Colostrum</th>
<th>Consortia (MRS)</th>
<th>Query coverage</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytolysin</td>
<td>□</td>
<td>-</td>
<td>+</td>
<td>98%</td>
<td>100%</td>
</tr>
<tr>
<td>Salivaricin A</td>
<td>#</td>
<td>+</td>
<td>-</td>
<td>99%</td>
<td>100%</td>
</tr>
<tr>
<td>Salivaricin B</td>
<td>*</td>
<td>+/-</td>
<td>+</td>
<td>100%; 99%; 99%</td>
<td>98%</td>
</tr>
<tr>
<td>Streptin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>97%; 79%; 82%</td>
<td>78%</td>
</tr>
</tbody>
</table>

* = PCR product of the expected size was amplified and the identity was confirmed by sequence analysis; 
+/- = PCR product of the expected size was weakly amplified and the identity was not confirmed by sequence analysis; = PCR product of the expected size was not detected.

doi:10.1371/journal.pone.0123324.t006

Colostrum of Healthy Slovenian Mothers: Microbiota Composition and Bacteriocin Gene Prevalence

Tanja Obermajer, Luka Lipoglavšek, Gorazd Tompa, Primož Treven, Petra Mohar Lorbeg, Bojana Bogovič Matijašič, Irena Rogelj

RESEARCH ARTICLE

DANONE NUTRICIA
Early Life Nutrition
Human milk microbiota: their role

Human milk metagenome: a functional capacity analysis

Tonya L Ward1, Sergey Hosid1,2, Ilya Ioshkhes1,2 and Ilmar Altosaar1,2

Figure 3 Functional categorization of open reading frames within human milk. The percent of ORFs assigned to each functional category is shown. Using the “Hierarchical Classification” tool within MG-RAST, 41,352 ORFs were submitted, 33,793 were annotated and assigned to a functional category (minimum e-value of 1×10^-5, minimum identity of 60%, and minimum alignment length of 15 aa). Three categories of genes (stress, virulence, carbohydrates) are expanded on the right to demonstrate the diverse capabilities of milk-derived DNA sequences.
The differential composition of bacterial communities in the GIT, vagina and oral cavity has been related to the health of the host.

**Human milk microbiota: the role**

- Important role in maternal health
- Important role in infant gut colonization
  - Short-term and long-term benefits
Human milk microbiota: their role

Microbial dysbiosis associated with breast cancer

**Microbial Dysbiosis Is Associated with Human Breast Cancer**

Caiyun Xuan¹, Jaime M. Shamonki², Alice Chung³, Maggie L. DiNome⁴, Maureen Chung⁴, Peter A. Sieling¹, Delphine J. Lee¹×

Breast microbiota in paired normal adjacent tissue ("paired normal") and tumor tissue from 20 patients with estrogen receptor (ER)-positive breast cancer
Human milk microbiota: their role

Microbial dysbiosis associated with breast cancer

11 / 1614 OTUs differentially abundant;
8 more abundant in paired normal tissue → 50% belonged to the genus *Sphingomonas*
3 more abundant in tumor tissue → 66.7% belonged to the genus *Methylobacterium*

Reduction of bacterial load

In advanced stage breast tumor
Human milk microbiota: their role

Lactational mastitis

Human breast milk is nutrition for infants but it also contains a large number of bacterial species, including some opportunistic pathogens of lactational mastitis.

The latter is not only interested in by theoretical community ecologist clinical significance for understanding the health and disease implications. For example, mastitis is inflammation of the breast with or without infection, *Staphylococcus aureus* has traditionally been believed to be the pathogen that is typically infectious mastitis.

Ma et al (2014 submitted manuscript) recently postulated that the opportunistic *Staphylococcus aureus*, which has been confirmed to exist in the human gut normally suppressed by a network of beneficial bacteria coexisted in the breast milk microbiome, but may become source of infectious mastitis when the host environment permits.

Therefore, the breast milk microbiome, similar to the microbiome in the other human body sites such as gut microbiota and perhaps the macrobiome in nature such as lakes, oceans, forests, must maintain their ecosystem (dynamic) balances to stay healthy. Revealing the mechanism of microbiome assembly, succession and maintenance should offer important insights for understanding the health and disease implications of the break milk microbiome.

A study conducted by a group of scientists led by the University of Idaho Professor Mark McGuire...
Human milk microbiota: their role

Infectious lactational mastitis

Network analysis suggests a potentially 'evil' alliance of opportunistic pathogens inhibited by a cooperative network in human milk bacterial communities

Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus spp.
Factors affecting the composition of the human milk microbiome

- Diet
- Medical conditions (antibiotics, health status)
- Stress (ie., emergency CS)
- Diet, maternal weight
Factors affecting the composition of the human milk microbiome

Antibiotic therapy

Lactobacilli and Bifidobacteria in Human Breast Milk: Influence of Antibiotherapy and Other Host and Clinical Factors

*Ana Soto, †Virginia Martín, ‡Esther Jiménez, §Isabelle Mader, ‡Juan M. Rodríguez, and §Leonides Fernández

N = 160

**p<0.001

* p<0.03

No Antibiotics

Antibiotic

Prevalence (%)

0 20 40 60 80 100

Lactobacillus

Bifidobacterium

NO (n = 95)

YES (n = 65)
Effect of chemotherapy on the microbiota and metabolome of human milk, a case report

Camilla Urbanik1,2, Amy McMillan1,2, Michelle Angelini3, Gregory B Gloo4, Mark Sumarah1,6, Jeremy P Burton1,7 and Gregor Reid1,5

Factors affecting the composition of the human milk microbiome

Chemotherapy?
Factors affecting the composition of the human milk microbiome

Lactational stage

Protein content in HM

Human milk oligosaccharides content

Microbiome?

Total bacteria, *Bifidobacterium* and *Enterococcus* spp. counts

Bauer J et al. 2011

Khodayar-Pardo et al., 2014

Cabrera-Rubio et al., 2012

Thurl S, et al., 2010
Factors affecting the composition of the human milk microbiome

Birth: Gestational age and mode of delivery?

↑ Bifidobacterium
↓ Enterococcus

Bauer J et al. 2011

Khodayar-Pardo et al., 2014
Cabrera-Rubio et al., 2012
Factors affecting the composition of the human milk microbiome

Pre-pregnancy BMI and excessive weight gain

Milk from obese women had a more homogenous bacterial composition in comparison with human samples from women with normal weight.

High BMI and excessive weight gain
Higher number of *Lactobacillus* and *Staphylococcus* and lower numbers of *Bifidobacterium*.

Cabrera-Rubio et al., 2012

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DANONE NUTRICIA
NUTRICIA RESEARCH

Early Life Nutrition
Factors affecting the composition of the human milk microbiome

Probiotics

Treatment of Infectious Mastitis during Lactation: Antibiotics versus Oral Administration of Lactobacilli Isolated from Breast Milk

Rebeca Arroyo, Virginia Martín, Antonio Maldonado, Esther Jiménez, Leantides Fernández, and Juan Miguel Rodríguez
Departamentos de Nutrición, Bromatología y Tecnología de los Alimentos, Universidad Complutense de Madrid, Madrid, Spain

3

M
St
W

C

PL
CE
~2

N :
(35%)
(36%)
(29%)

Pain feeling

10 without pain

0 very painful

*** = P < 0.0001

*** = P < 0.0001; ** = P < 0.001; * = P < 0.01

Arroyo et al, 2010

Staphylococcus epidermidis
Staphylococcus aureus
Streptococcus mitis

Change (log10 cfu ml⁻¹)

N=96 N=88 N=77 N=67 N=55 N=30 N=37 N=36 N=35

Arroyo et al, 2010
Breast tissue and human milk contain bacteria

Human milk microbiome and bacteria in breast tissue contribute to infant and maternal health

There is a mother-infant transfer of bacteria through human milk

Future research is needed to better understand how bacteria reach the mammary gland

Take home message

- Breast tissue and human milk contain bacteria
- Human milk microbiome and bacteria in breast tissue contribute to infant and maternal health
- There is a mother-infant transfer of bacteria through human milk
- Future research is needed to better understand how bacteria reach the mammary gland
Species diversity and relative abundance of lactic acid bacteria in the milk of rhesus monkeys (Macaca mulatta)

L. Jin, K. Hinde, and L. Tao

1Department of Oral Biology, College of Dentistry, University of Illinois at Chicago, Chicago, IL, USA
2Brain, Mind, & Behavior Unit, California National Primate Research Center, University of California Davis, Davis, CA, USA
3Nutrition Laboratory, Smithsonian National Zoological Park, Washington, DC, USA