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Does Breastmilk Influence the Development of Bronchopulmonary Dysplasia?

Juliane Spiegler, MD[1], Michael Preuß, PhD[2], Corinna Gebauer, MD[3], Meike Bendiks, MD[1], Egbert Herting, PhD[1], and Wolfgang Göpel, MD[1], on behalf of the German Neonatal Network (GNN)*

Advances in Nutrition An International Review Journal

ASN 2014 ANNUAL MEETING SYMPOSIUM SUMMARY

It's Alive: Microbes and Cells in Human Milk and Their Potential Benefits to Mother and Infant[1–3]

Lars Bode,[4]* Mark McGuire,[5] Juan M. Rodriguez,[6] Donna T. Geddes,[7] Foteini Hassiotou,[7] Peter E. Hartmann,[7] and Michelle K. McGuire[5]

[4]Department of Pediatrics, University of California, San Diego, La Jolla, CA; [5]Department of Animal and Veterinary Sciences, University of Idaho, Moscow, ID; [6]Department of Nutrition, Food Science, and Food Technology, Complutense University of Madrid, Madrid, Spain; [7]School of Chemistry and Biochemistry, Faculty of Science, University of Western Australia, Crawley, WA, Australia; and [8]School of Biological Sciences, Washington State University, Pullman, WA

ABSTRACT

Human milk is the optimal source of nutrition for the nursing infant. Classically, the nutrients (water, protein, lipid, carbohydrate, vitamins, and minerals) were studied as the critical components of milk serving the growth needs of the infant for optimum growth. However, human milk contains factors other than the classically defined nutrients for which researchers are investigating potential roles in infant and maternal health, development, and well-being. The symposium addressed some of the exciting factors being studied, including microbes and maternal cells found within milk. Drs. Michelle McGuire and Juan M. Rodriguez addressed the presence of a bacterial community in human milk produced by healthy and mastitic mothers, potential sources of those bacteria, and the impact of milk-derived bacteria on the nursing infant. Drs. Donna Geddes, Peter Hartmann, and Foteini Hassiotou discussed the potential importance of maternal cells. For years, immune cells were known to be present in human milk, but recent evidence suggests that their impact is as much on the infant as on the health of the lactating mammary gland. Finally, the existence of highly plastic stem cells in human milk opens doors for previously unforeseen developmental "training" of the nursing infant. *Adv Nutr* 5: 571–573, 2014.

Introduction

Human milk is "alive" and contains cells of both bacterial and host origin. Typically, the presence of bacteria in milk was considered an indication of infection. However, this notion may need revision because culture-dependent and -independent techniques more recently revealed that human milk produced by healthy women contains a diverse microbial community including >200 phylotypes. A core milk microbiome with a limited number of operational taxonomic units represents ~50% of the relative abundance. The other 50% of bacteria in human milk appears to be very person-alized, suggesting that their community structure can be modified by the mother's environmental exposure. How these bacteria reach the mammary gland and are incorporated into the milk remains unclear. Combined results of various studies suggest that some bacteria present in the maternal gastrointestinal tract could reach the mammary gland during late pregnancy and lactation through a mechanism involving intestinal immune cells. Thus, modulation of the maternal gastrointestinal microbiota during pregnancy and lactation could have a direct effect on infant health via their incorporation into milk. Conversely, some factors may cause a mammary dysbiosis, leading to infectious mastitis, a condition that represents 1 of the primary medical causes for early undesired weaning. Thus, altering human milk microbiota may offer novel ways to improve the health of the breast-fed infant and potentially the breastfeeding mother. The symposium presentations by Drs. Michelle McGuire and Juan M. Rodriguez focused on bacterial cells and shared emerging research data from metagenomic, transcriptomic, and metabolomic studies focused on the role of human milk bacteria in health and disease. Dr. Donna Geddes' symposium presentation focused on maternal immune cells because their numbers and composition rapidly respond to infections in the mammary gland and other maternal infections but also to infant infections. Although the maternal response to infant infection is not fully understood, it was

*This article is a summary of the symposium "It's Alive: Microbes and Cells in Human Milk and Their Potential Benefits to Mother and Infant" held 29 April 2014 at the ASN Scientific Sessions and Annual Meeting at Experimental Biology 2014 in San Diego, CA. The symposium was sponsored by the American Society for Nutrition (ASN), and the ASN Lactation Research Interest Section, and was supported by a nonrestricted educational grant from Medela.
†The organizer has indicated that retained reviews of this symposium will be submitted for publication in an upcoming issue of Advances in Nutrition.
‡Author disclosures: L. Bode, M. McGuire, J. M. Rodriguez, D. T. Geddes, F. Hassiotou, P. E. Hartmann, and M. K. McGuire, no conflicts of interest.
*To whom correspondence should be addressed. E-mail: lbode@ucsd.edu.

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Formula versus donor breast milk for feeding preterm or low birth weight infants (Review)

Quigley M, McGuire W

Quigley M, McGuire W.
Formula versus donor breast milk for feeding preterm or low birth weight infants.
Cochrane Database of Systematic Reviews 2014, Issue 4. Art. No.: CD002971.
DOI: 10.1002/14651858.CD002971.pub3.

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Formula versus donor breast milk for feeding preterm or low birth weight infants (Review)

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Human Milk Feeding as a Protective Factor for Retinopathy of Prematurity: A Meta-analysis

Jianjun Zhou, MD*, Vivek V. Shukla, MD†, Danny John, MBA, MPH†, Chao Chen, MD, PhD*

abstract

OBJECTIVE: Studies have suggested that human milk feeding decreases the incidence of retinopathy of prematurity (ROP); however, conflicting results have been reported.

OBJECTIVE: The aim of this meta-analysis was to pool currently available data on incidence of ROP in infants fed human milk versus formula.

DATA SOURCES: Medline, PubMed, and EBSCO were searched for articles published through February 2015.

STUDY SELECTION: Longitudinal studies comparing the incidence of ROP in infants who were fed human milk and formula were selected. Studies involving donor milk were not included.

DATA EXTRACTION: Two independent reviewers conducted the searches and extracted data. Meta-analysis used odds ratios (ORs), and subgroup analyses were performed.

RESULTS: Five studies with 2208 preterm infants were included. Searches including various proportions of human milk versus formula, any-stage ROP, and severe ROP were defined to pool data for analyses. For any-stage ROP, the ORs (95% confidence intervals [CIs]) were as follows: exclusive human milk versus any formula, 0.29 (0.12 to 0.72); mainly human milk versus mainly formula, 0.51 (0.26 to 1.03); any human milk versus exclusive formula, 0.54 (0.15 to 1.96); and exclusive human milk versus exclusive formula, 0.25 (0.13 to 0.49). For severe ROP, they were 0.11 (0.04 to 0.30), 0.16 (0.06 to 0.43), 0.42 (0.08 to 2.18), and 0.10 (0.04 to 0.29), respectively.

LIMITATIONS: Prospective randomized studies being impossible because of ethical issues, we chose observational studies for analysis. A few studies involving subgroup analyses presented high heterogeneity.

CONCLUSIONS: Based on current limited evidence, in very preterm newborns, human milk feeding potentially plays a protective role in preventing any-stage ROP and severe ROP.

*Children's Hospital of Fudan University, Shanghai, China; †The Hospital for Sick Children, Toronto, Ontario, and ‡Hospital Space Access Education Initiative, Manchester, United Kingdom

Drs Zhou and Chen conceptualized the study; Drs Zhou, Shukla, and Chen planned the study; Drs Zhou and Shukla searched for articles and drafted the manuscript; Dr Chen supervised the progress of the study; Drs Zhou and Shukla and Mr John collected data; Mr John performed the meta-analysis and critically appraised the manuscript; and all authors revised the manuscript and consented to the final manuscript as submitted.

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Address correspondence to Chao Chen, Division of Neonatology, Children's Hospital of Fudan University, 399 Wanyuan Road, Minhang District, Shanghai, China 201102. E-mail: chenc0108@163.com
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REVIEW ARTICLE

ESPGHAN

SOCIETY COMMENTARY

Donor Human Milk for Preterm Infants: Current Evidence and Research Directions

*†Sertac Arslanoglu, ‡Willemijn Corpeleijn, *Guido Moro, §Christian Braegger, ||Cristina Campoy, ¶Virginie Colomb, #Tamas Decsi, **Magnus Domellöf, ††Mary Fewtrell, ‡‡Iva Hojsak, §§Walter Mihatsch, ||||Christian Mølgaard, ¶¶Raanan Shamir, ##Dominique Turck, and ‡Johannes van Goudoever, ESPGHAN Committee on Nutrition

ABSTRACT

The Committee on Nutrition of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition aims to document the existing evidence of the benefits and common concerns deriving from the use of donor human milk (DHM) in preterm infants. The comment also outlines gaps in knowledge and gives recommendations for practice and suggestions for future research directions. Protection against necrotizing enterocolitis is the major clinical benefit deriving from the use of DHM when compared with formula. Limited data also suggest unfortified DHM to be associated with improved feeding tolerance and with reduced cardiovascular risk factors during adolescence. Presence of a human milk bank (HMB) does not decrease breast-feeding rates at discharge, but decreases the use of formula during the first weeks of life. This commentary emphasizes that fresh own mother's milk (OMM) is the first choice in preterm infant feeding and strong efforts should be made to promote lactation. When OMM is not guidelines. Storage and processing of human milk reduces some biological components, which may diminish its health benefits. From a nutritional point of view, DHM, like HM, does not meet the requirements of preterm infants, necessitating a specific fortification regimen to optimize growth. Future research should focus on the improvement of milk processing in HMB, particularly of heat treatment; on the optimization of HM fortification; and on further evaluation of the potential clinical benefits of processed and fortified DHM.

Key Words: donor milk, human milk, human milk banking, pasteurization, preterm infant

(*JPGN* 2013;57: 535–542)

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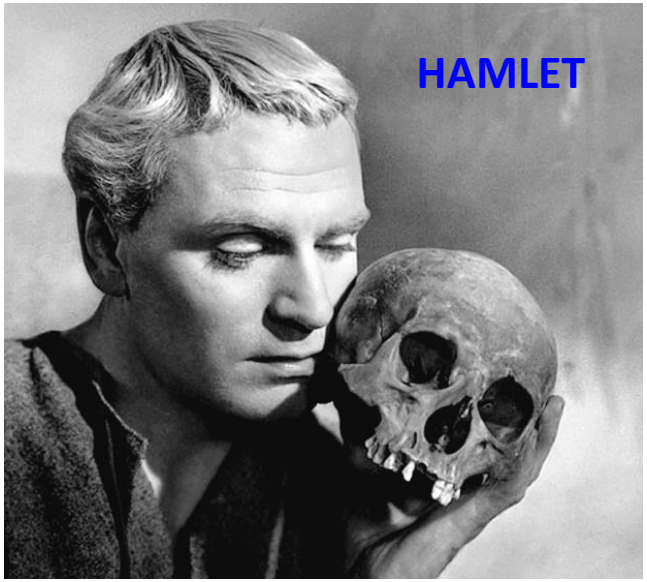


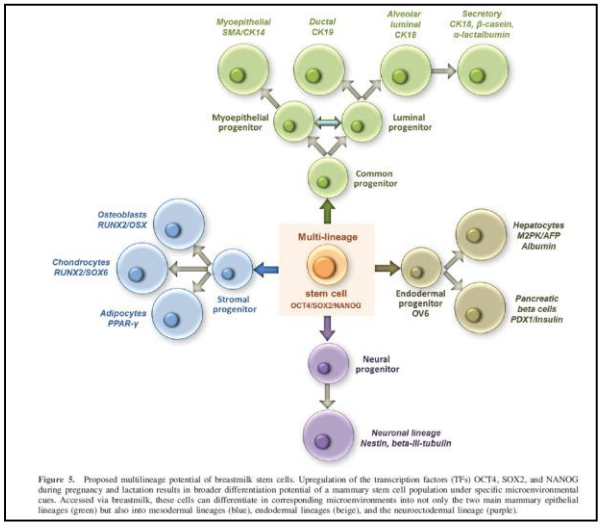
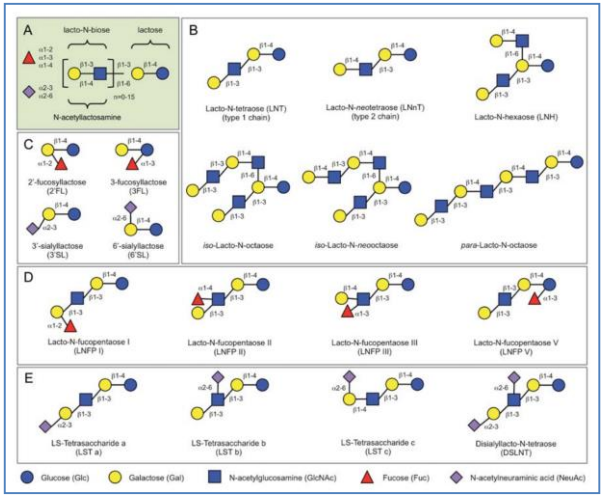
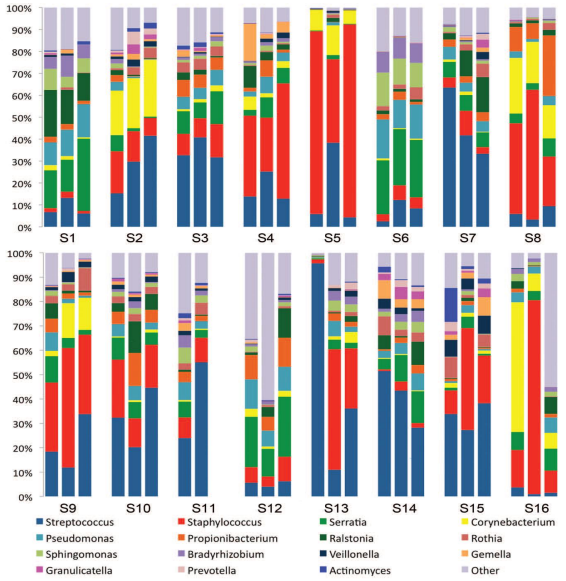


Figure 5. Proposed multilineage potential of breastmilk stem cells. Upregulation of the transcription factors (TFs) OCT4, SOX2, and NANOG during pregnancy and lactation results in broader differentiation potential of a mammary stem cell population under specific microenvironmental cues. Accessed via breastmilk, these cells can differentiate in corresponding microenvironments into not only the two main mammary epithelial lineages (green) but also into mesodermal lineages (blue), endodermal lineages (beige), and the neuroectodermal lineage (purple).





Neler Oldu?

- Paradigma değişikliği
- İhmallerin, gözden kaçırmaların itirafları
- Elimizde zaten var olan bulgulara yeni gözlüklerle bakma

  Yeni Nesil Teknikler

  Biyoinformatiğin $\geq$ emekleme dönemi
- Biz ve diğerleri .....
- Diğerleri nereden geldi?

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Current Opinion in Biotechnology

Got bacteria? The astounding, yet not-so-surprising, microbiome of human milk

Michelle K McGuire[1] and Mark A McGuire[2]

mucosa (the maternal gastrointestinal tract (enteric entero-mammary pathway) and through bacterial exposure of the breast during nursing. Currently, almost nothing is known about whether variation in microbe consumption by the infant via human milk and that of the mammary gland, itself, impacts short-term and/or long-term infant and maternal health although several studies suggest this is likely. We urge the clinical and public health communities to be patient, however, in order to allow human milk and lactation researchers to first understand what constitutes 'normal' in terms of the milk microbiome (as well as factors that impact microbial community structure) prior to jumping the gun to investigate if and how this important source of microbes impacts maternal and infant health.

Addresses
[1]School of Biological Sciences and Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA 99164, United States
[2]Department of Animal and Veterinary Science, University of Idaho, Moscow, ID, United States

Corresponding author: McGuire, Michelle K (amcguire@wsu.edu)

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This review comes from a themed issue on **Food biotechnology**

Edited by **Patrick Stover** and **Saurabh Mehta**

For a complete overview see the Issue and the Editorial

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Introduction

Since the completion of the Human Microbiome Project (HMP), much has been learned about the complex microbial communities that live in and on the human body. Indeed, even prior to its commencement in 2007, the core team involved in this NIH-funded project posited that, considering the estimated 10-fold greater abundance of

impact of the microbiota on disease predisposition and pathogenesis.

The initial idea for the HMP is often attributed to Relman and Falkow [3] who called for a 'second human genome project: A large-scale genomic sequence survey of the four major microbial niches within the human body: the mouth, intestinal tract, vagina and skin.' Little did they know that they were missing what might be one of the most important niches for bacterial inhabitation within the human-microbe supraorganism: the lactating mammary gland. Indeed, as we have previously argued [4], human milk is likely mother nature's prototypical probiotic food — safely supplying a cocktail of environmentally-determined microbes and their associated genes and antigens to the newborn infant during a critical period of growth and development. How did these world-class researchers (as well as those who worked to design the HMP) miss this important detail?

The answer to this question lies in the paradigm shift that has taken place over the last decade concerning the sterility (or lack, thereof) of human milk. Indeed, although there have existed reports of viable bacteria in milk produced by healthy women for over four decades (e.g. [5–9]), because of long-standing dogma that blood-derived human fluids are sterile, the origin of these bacteria was thought to be from the skin or other environmental sources. A classic example of the influence of prevailing dogma on interpretation of results was a study conducted Wyatt and Mata [8] designed to analyze milk produced by poor Guatemalan women; they concluded, 'The presence of Enterobacteriaceae in human colostrum and milk reflects the low levels of personal hygiene and environmental sanitation in the population studied.' It now appears that these bacteria are neither a consequence of poor sanitation nor environmental contaminations, but instead are ubiquitously present in milk produced by healthy breastfeeding women.

Indeed, most human lactation experts now concur that milk is not sterile. This astounding, yet not-so-surprising,

The meaning and impact of the human genome sequence for microbiology

David A. Relman and Stanley Falkow

The characterization of life is immeasurably enhanced by determination of complete genome sequences. For organisms that engage in intimate interactions with others, the genome sequence from one participant, and associated tools, provide unique insight into its partner. We discuss how the human genome sequence will further our understanding of microbial pathogens and commensals, and vice versa. We also propose criteria for implicating a host gene in microbial pathogenesis, and urge consideration of a 'second human genome project'.

The publication of a draft of the human genome sequence by two research teams surely represents one of the major scientific milestones in history[1,2]. It is a remarkable technological achievement that 'came in ahead of schedule and under budget'. Microbiologists might have become relatively accustomed to complete genome sequences, but this is an extraordinary event as viewed in terms of science, technology, sociology and ethics. The human genome will be looked upon by many scientists, law and policy makers, lay people and the press as the genomic sequence against which all others will be measured. More than ever, the driving force of research funding will be programmatic, with a disproportionate emphasis on human disease. For much too long, science policy decisions have reflected a human-disease-oriented perspective, and an unspoken philosophy of human 'genomic supremacy'. As part of this belief system, the size of one's genome and the number of predicted genes determine one's relative importance in the biosphere[3], and form the basis of relationships with species that are 'challenged' with many fewer genes and smaller genomes. To the surprise of some, recent findings suggest that microorganisms are extremely effective instructors about the natural world.

The discoveries of unimagined functional diversity, genomic plasticity and lateral gene transfer in the microbial world significantly expanded our understanding of biological systems far beyond the boundaries defined by studies in the mammalian world[4,5]. Microorganisms are also wonderful teachers about the workings of the human organism. The field of cellular microbiology capitalizes on the use of pathogens to reveal important features of host cell biology and biochemistry[6]. With the primary sequence of the complete complement of human expressed genes in hand, this host–microorganism cross-instructional process will mature even further. The recent discovery of at least 233 human genes with homologs found only among bacteria[7] and the earlier recognition of numerous endogenous retroviruses in our genome[3] emphasize the importance of microorganisms and viruses in shaping our evolutionary history and defining the very essence of who we are. Apropos of the preceding discussion of anthropocentrism, some have proposed a 'retrograde' evolutionary process by which bacteria might have acquired these genes from humans!

Microbiologists have learned in recent years that the evolution of microbial specialization, such as pathogenicity and nitrogen fixation, is a reflection of horizontal gene transfer and that these events are written in the sequence of microbial DNA. In microorganisms, the seeds of change have been found as often, or more often, in mobile genetic elements like viruses and transposons, than in the accumulation of adaptive mutations. To what extent is the same story written in the human genome and to what extent did viruses and mobile genetic elements contribute to this evolution? Is it not a touch of déjà vu for the microbiologist to learn that the capacity of fish and animals to develop an adaptive immune response is linked to the sudden appearance during evolution of a gene that has intrinsic transposase activity? Are there more examples of the fictional 'Darwin's Radio'?

There are other important insights to be gained from the use of the human genome sequence. By listening to the conversation between host and pathogen at a genome-wide transcriptional level, using high-density human and microbial DNA microarrays, we can now describe in detail the molecular events that accompany recognition of non-self, perturbation of host signaling pathways, and general features of innate immunity[8–11].

Complete genome sequences: every one tells an important story

We have become accustomed to this arbitrary concept of a representative genome. Many eukaryotic biologists and even some microbiologists consider the *Escherichia coli* genome to be the bacterial paradigm, despite the extreme variability between *E. coli* strains[12]. By default, for the immediate future, *Saccharomyces cerevisiae* represents the fungi, *Caenorhabditis elegans* the nematodes, *Drosophila melanogaster* the insects, *Methanococcus jannaschii* the Archaea, and *Mus musculus*, for many, will be representative of all other animals and perhaps the most useful animal

David A. Relman
Dept of Microbiology & Immunology, Stanford University School of Medicine, Stanford, CA 94305-5124, USA, Veterans Affairs Health Care System, Palo Alto, CA 94304-1207, USA.
e-mail: relman@cmgm.stanford.edu

Stanley Falkow
Depts of Microbiology & Immunology, and Medicine, Stanford University School of Medicine, Stanford, CA 94305-5124, USA.

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A second human genome project

The human body is host to a myriad of microorganisms. We are still woefully ignorant of the composition and variability of our endogenous microflora[23]. Many of these microorganisms depend on humans for their survival, and yet we still do not fully appreciate to what extent human life is dependent on its microflora. In the spirit of the recent 'human genome project' and in the hopes of capturing the imagination of the broad scientific community, it is time to embark on a comprehensive genomic inventory of the large portion of cellular life within the human body that has been ignored so far, the endogenous microflora. A large-scale genomic sequence survey of the four major microbial niches within the human body, the mouth, intestinal tract, vagina and skin would help to fill crucial gaps in our understanding of human evolution, development, immune system function and disease. It should

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The human microbiome project: exploring the microbial part of ourselves in a changing world

Peter J. Turnbaugh[1], **Ruth E. Ley**[1], **Micah Hamady**[2], **Claire Fraser-Liggett**[3], **Rob Knight**[4], and **Jeffrey I. Gordon**[1]

[1]Center for Genome Sciences, Washington University School of Medicine, St. Louis, MO 63108

[2]Department of Computer Science, University of Colorado at Boulder, Boulder, CO 80309

[3]Institute of Genome Sciences, University of Maryland School of Medicine, Baltimore, MD 21201

[4]Department of Chemistry and Biochemistry, University of Colorado at Boulder, Boulder, CO 80309

Abstract

The human microbiome project (HMP) reflects the fact that we are supraorganisms composed of human and microbial components. This international effort emanates from a confluence of ongoing technical and computational advances in the genome sciences, an evolving focus of microbiology on the properties and operations of microbial communities, and the notion that rapid, and marked, transformations in human lifestyles are not only affecting the health of the biosphere, but possibly our own health as a result of changes in our microbial ecology. HMP is designed to understand the microbial components of our genetic and metabolic landscape, and how they contribute to our normal physiology and disease predisposition. It is a global and interdisciplinary project that promises to break down the artificial barriers between medical and environmental microbiology. Here, we discuss some the challenges that HMP faces and options for addressing them.

Introduction

Prior to completion of the human genome sequencing project, some predicted that we would find ~100,000 genes. For many, feelings of surprise and perhaps humility were associated with the announcement that *our* genome only contains ~20,000 protein-coding genes, a number not greatly different from that of the fruit fly. However, by expanding our view of ourselves, we can see that the number 100,000 is likely an *under*estimate. The microbes that live inside and on us (the microbiota) outnumber our somatic and germ cells by an estimated 10-fold. The collective genomes of our microbial symbionts (the microbiome) provide us with traits we have not had to evolve on our own[1]. If we consider ourselves to be a composite of microbial and human species, our genetic landscape a summation of the genes embedded in our human genome and microbiome, and our metabolic features a coalescence of human and microbial traits, the self-portrait that emerges is one of a 'human supraorganism'. Thus, understanding the range of human genetic and physiologic diversity means that we must characterize our microbiome and the factors that influence the distribution and evolution of our microbial partners. The outcome may provide an additional perspective about contemporary human evolution, as we assess whether and how our rapidly

Correspondence and requests for materials should be addressed to J.I.G. (jgordon@wustl.edu).
The authors declare no competing financial interests.

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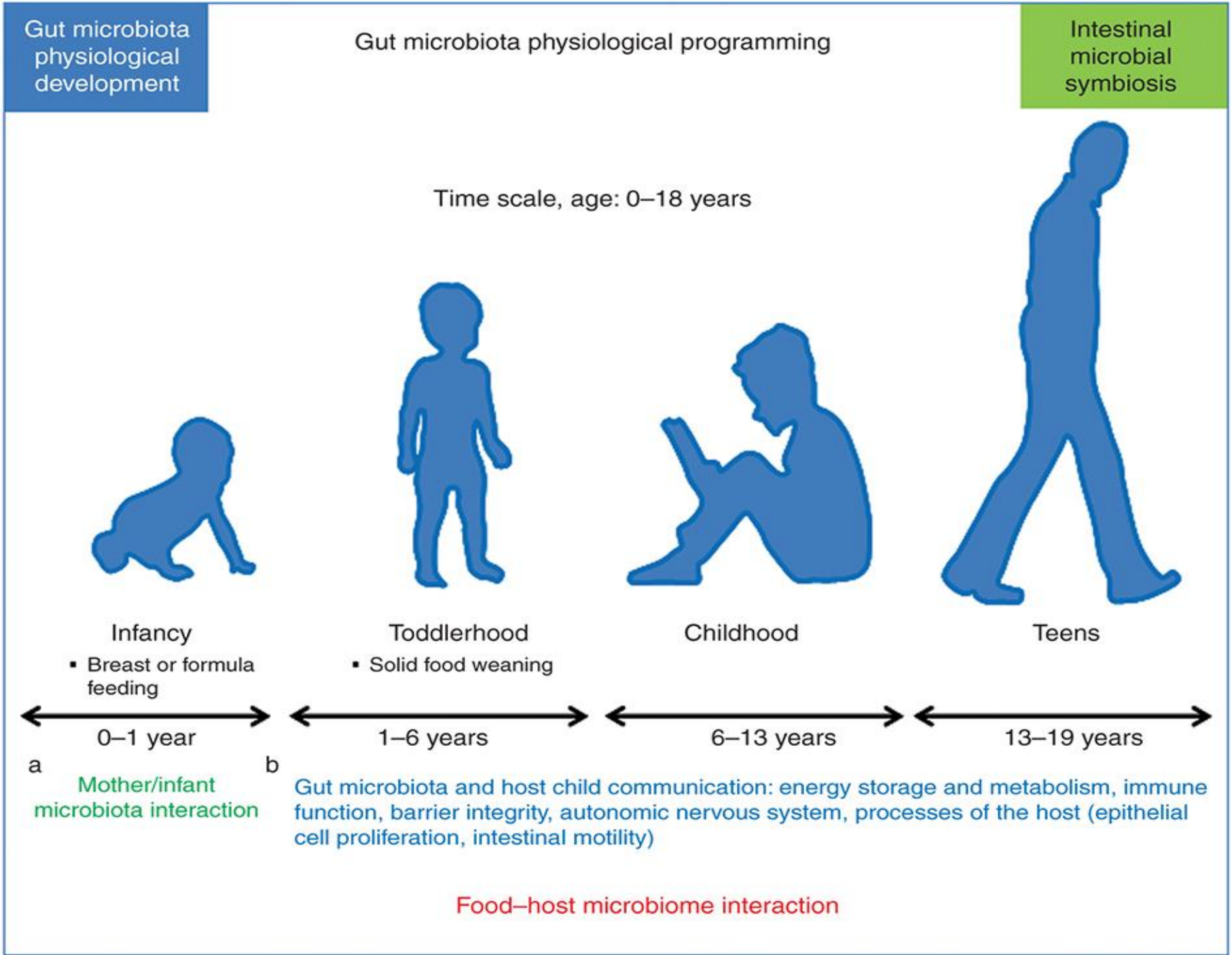
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Gut microbiota physiological programming
Intestinal microbial symbiosis
Time scale, age: 0–18 years
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Toddlerhood
▪ Solid food weaning
Childhood
Teens
0–1 year
1–6 years
6–13 years
13–19 years
a
b
Mother/infant microbiota interaction
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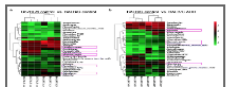

Neler Oldu?

- İhmallerin, gözden kaçırmaların itirafları
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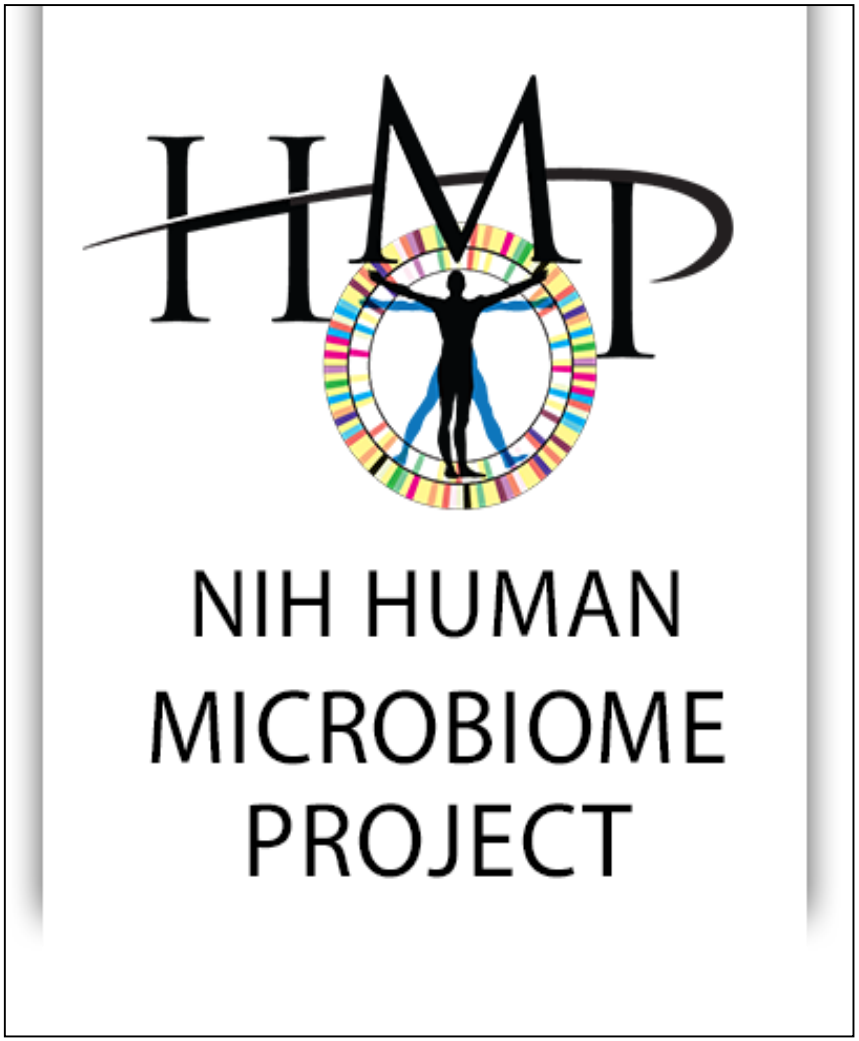

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  - Deri
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Paradigma: Anne Sütü Sterildir!

- Çok yakın bir geçmişte anne sütünün steril olduğuna kesin gözüyle bakıyor
- Ancak sağma/saklama sırasında kontamine olduğunda ya da mastit varlığında bakteri barındırdığı düşünüyorduk

Osterman KL, Rahm V-A. Lactation mastitis: bacterial cultivation of breast milk, symptoms, treatment, and outcomes. J Hum Lact 2000;16:297–302.
Thomsen AC, Hansen KB, Moller BR. Leukocyte counts and microbiological cultivation in the diagnosis of puerperal mastitis. Am J Obstet Gynecol 1983;146:938–41.
Thomsen AC, Espersen T, Maigaard S. Course and treatment of milk stasis, noninfectious inflammation of the breast, and infectious mastitis in nursing women. Am J Obstet Gynecol 1984;149:492–5.

2003 Ezber Bozma Yılı

- Anne sütü sterildir dogmasını sarsan 2 önemli çalışma da 2003'de yayınlandı

  Camputense Ü, İspanya (Martin 2003)

  Helsinki Ün (Heikkila 2003)

- Anne sütünün bebeğin bağırsağı için devamlı ve mükemmel bir kommensal ve probiyotik bakteri kaynağı olduğunu gösterdiler: Stafilokok, Streptokok ve LAB


KEEP
CALM
THERE'S A
PARADIGM
SHIFT
GOING ON

Kültüre dayalı yöntemlerin yalnızca patolojik olarak bilinen bakterileri saptayabilmesi

“Anne sütü sterildir” dogmasını sarsan öncü çalışma

HUMAN MILK IS A SOURCE OF LACTIC ACID BACTERIA FOR THE INFANT GUT

Rocío Martín, MSc, Susana Langa, MSc, Carlota Reviriego, MSc, Esther Jiménez, MSc, María L. Marín, PhD, Jordi Xaus, PhD, Leonides Fernández, PhD, and Juan M. Rodríguez, PhD

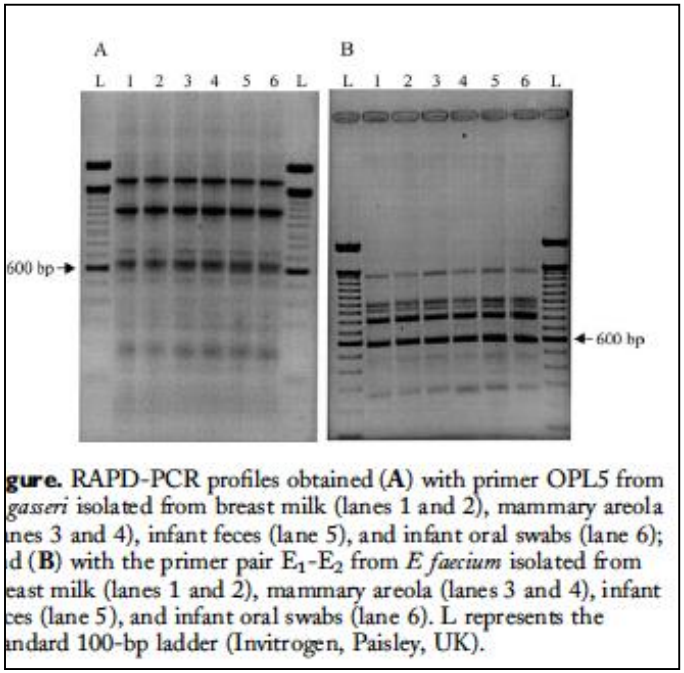
Objectives To investigate whether human breast milk contains potentially probiotic lactic acid bacteria, and therefore, whether it can be considered a synbiotic food.

Study design Lactic acid bacteria were isolated from milk, mammary areola, and breast skin of eight healthy mothers and oral swabs and feces of their respective breast-fed infants. Some isolates (178 from each mother and newborn pair) were randomly selected and submitted to randomly amplified polymorphic DNA (RAPD) polymerase chain reaction analysis, and those that displayed identical RAPD patterns were identified by 16S rDNA sequencing.

Results Within each mother and newborn pair, some rod-shaped lactic acid bacteria isolated from mammary areola, breast milk, and infant oral swabs and feces displayed identical RAPD profiles. All of them, independently from the mother and child pair, were identified as *Lactobacillus gasseri*. Similarly, among coccoid lactic acid bacteria from these different sources, some shared an identical RAPD pattern and were identified as *Enterococcus faecium*. In contrast, none of the lactic acid bacteria isolated from breast skin shared RAPD profiles with lactic acid bacteria of the other sources.

Conclusions Breast-feeding can be a significant source of lactic acid bacteria to the infant gut. Lactic acid bacteria present in milk may have an endogenous origin and may not be the result of contamination from the surrounding breast skin. *(J Pediatr 2003;143:754-8)*

- Kültür bazlı ve kültür bağımsız tanı yöntemlerinin kullanıldığı bu araştırmada sağlıklı annelerin sütünde, areolada, bebeğin ağzında ve dışkısında **Lactobacillus gasseri ve Enterococcus faecium** üretildi.
- Sütte ve göğüs derisinde üremiş olan laktik asid bakterileri birbirinden farklıydı.



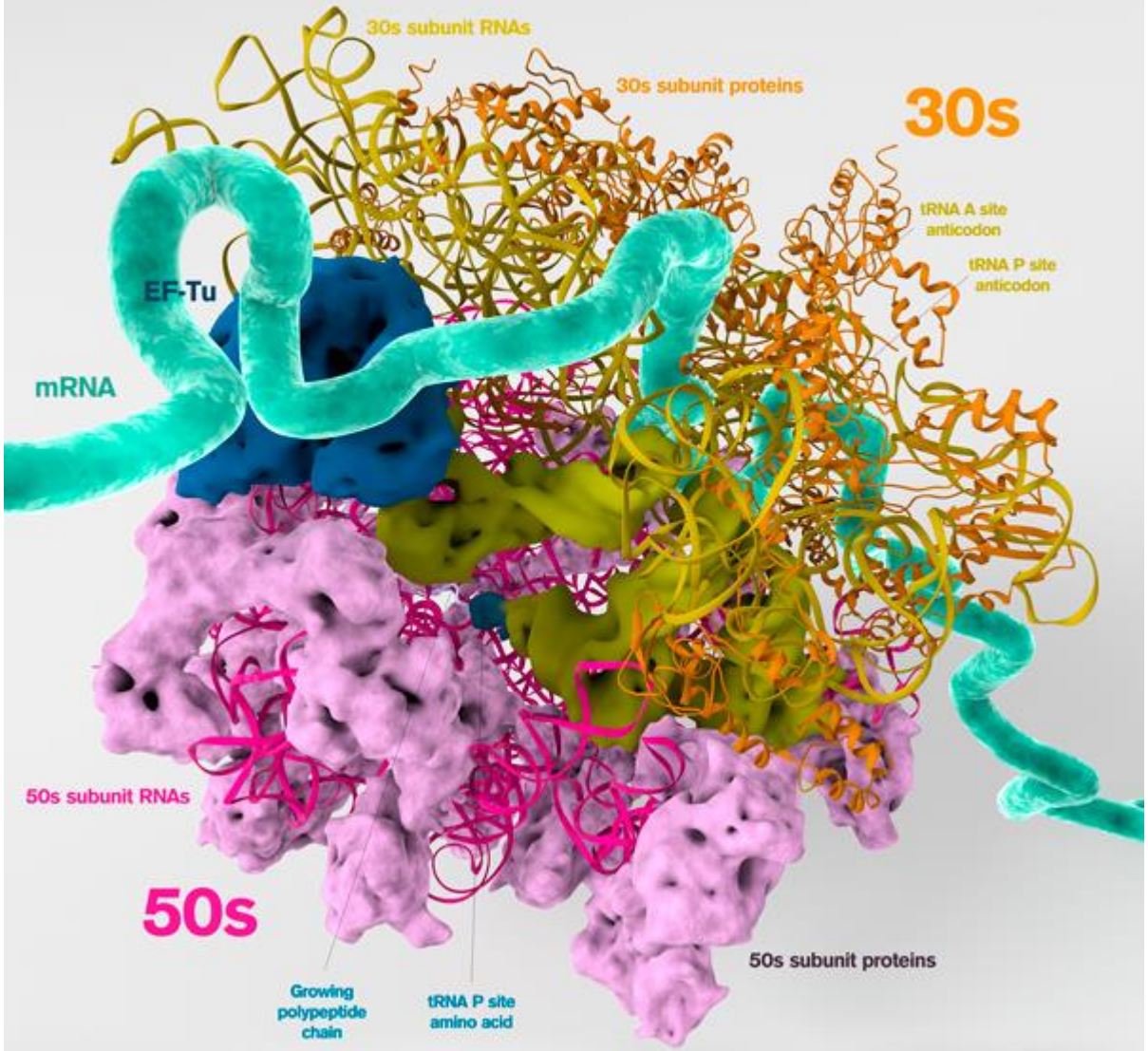
gure. RAPD-PCR profiles obtained (**A**) with primer OPL5 from *gasseri* isolated from breast milk (lanes 1 and 2), mammary areola nes 3 and 4), infant feces (lane 5), and infant oral swabs (lane 6); d (**B**) with the primer pair E_1-E_2 from *E faecium* isolated from east milk (lanes 1 and 2), mammary areola (lanes 3 and 4), infant ces (lane 5), and infant oral swabs (lane 6). L represents the ndard 100-bp ladder (Invitrogen, Paisley, UK).

High-throughput Methods
(Yüksek Verimli) Yöntemler

- Anne sütündeki bakteri topluluklarının bütününü saptayabilen yöntemler

- Burada kastedilen kullanılan sistemin aynı anda büyük bir veri yoğunluğunu işleyebiliyor olmasıdır.

BACTERIAL RIBOSOME



OPEN ACCESS Freely available online

PLoS one

Characterization of the Diversity and Temporal Stability of Bacterial Communities in Human Milk

Katherine M. Hunt[1,6], James A. Foster[2,6], Larry J. Forney[2,6], Ursel M. E. Schütte[2], Daniel L. Beck[2,6], Zaid Abdo[3,6], Lawrence K. Fox[4], Janet E. Williams[1], Michelle K. McGuire[5], Mark A. McGuire[1,6]*

1 Department of Animal and Veterinary Science, University of Idaho, Moscow, Idaho, United States of America, 2 Department of Biological Science, University of Idaho, Moscow, Idaho, United States of America, 3 Department of Statistics, Department of Mathematics, University of Idaho, Moscow, Idaho, United States of America, 4 Department of Veterinary Clinical Science, Washington State University, Pullman, Washington, United States of America, 5 School of Biological Sciences, Washington State University, Pullman, Washington, United States of America, 6 Initiative for Bioinformatics and Evolutionary Studies (IBEST), University of Idaho, Moscow, Idaho, United States of America

Abstract

Recent investigations have demonstrated that human milk contains a variety of bacterial genera; however, as of yet very little work has been done to characterize the full diversity of these milk bacterial communities and their relative stability over time. To more thoroughly investigate the human milk microbiome, we utilized microbial identification techniques based on pyrosequencing of the 16S ribosomal RNA gene. Specifically, we characterized the bacterial communities present in milk samples collected from 16 women at three time-points over four weeks. Results indicated that milk bacterial communities were generally complex; several genera represented greater than 5% of the relative community abundance, and the community was often, yet not always, stable over time within an individual. These results support the conclusion that human milk, which is recommended as the optimal nutrition source for almost all healthy infants, contains a collection of bacteria more diverse than previously reported. This finding begs the question as to what role this community plays in colonization of the infant gastrointestinal tract and maintaining mammary health.

Citation: Hunt KM, Foster JA, Forney LJ, Schütte UME, Beck DL, et al. (2011) Characterization of the Diversity and Temporal Stability of Bacterial Communities in Human Milk. PLoS ONE 6(6): e21313. doi:10.1371/journal.pone.0021313

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Competing Interests: The authors have declared that no competing interests exist.

* E-mail: mmcguire@uidaho.edu

Introduction

Due to the considerable health benefits it confers, human milk is universally considered the optimal source of nutrition for almost all healthy infants. For instance, breastfeeding provides infants with critical protection from diarrheal [1] and respiratory diseases [2], especially in developing countries, and is associated with reduced long-term risk of obesity [3,4]. Past research [5,6] has extensively investigated the presence and health implications of the traditional nutrients in milk, such as fatty acids, vitamins, and minerals; however, recent work has shown that human milk also contains communities of bacteria [7,8,9,10,11,12] that may have health implications.

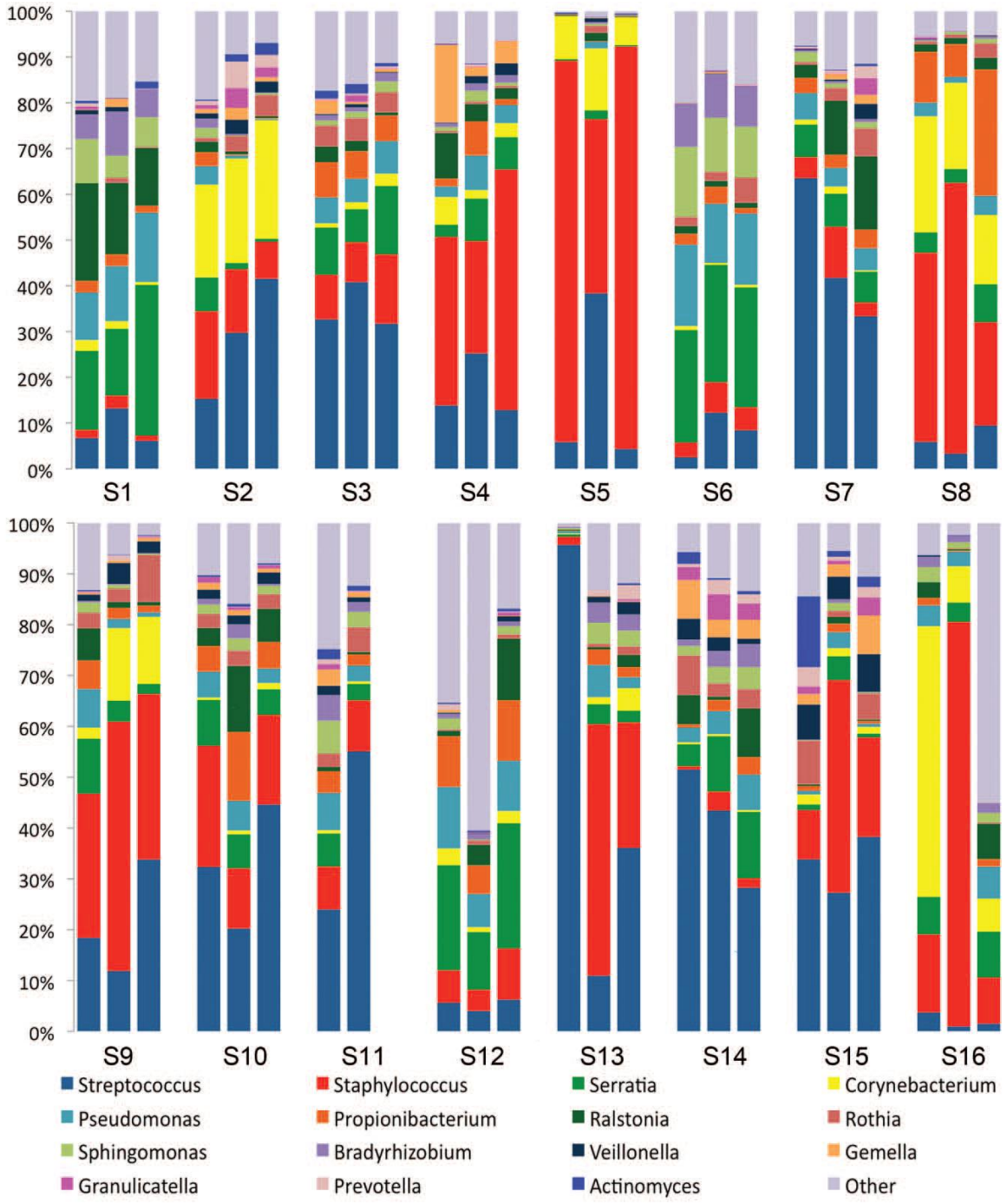
Culture-dependant methods have long confirmed the presence of bacteria in aseptically collected milk including *Staphylococcus* and *Streptococcus* species [7], whereas culture-independent studies utilizing microbial characterization techniques based on the amplification of bacterial 16S rRNA have shown that human milk contains several additional genera of bacteria including *Lactobacillus* and *Bifidobacterium* [8,9,10]. While these studies provide clear evidence that aseptically collected milk contains bacteria, very little work has examined the possibility that a core milk microbiome exists among lactating women, or investigated the stability of these communities within an individual over time. These types of analyses are critical because they make it possible to determine the roles these communities may play in maintaining mammary gland health, bacterial colonization of the infant's gastrointestinal tract, and other indices of short- and long-term maternal and infant health. Consequently, the present study was designed to probe more deeply into the stability and diversity of human milk bacterial communities over time. We hypothesized that human milk contains a greater diversity of bacterial phylotypes than previously noted, and that these communities would be stable over time within each individual lactating woman.

Results

With the exception of milk collected from one participant who donated only 2 samples, bacterial genomic DNA was extracted from milk samples collected at 3 time points over a 4-wk interval from 16 lactating women self-described as healthy and free from lactational mastitis. Samples were collected using a method designed to reduce skin contamination. The V1-V2 region of the bacterial 16S rRNA gene was amplified from the DNA using universal primers, and barcoded pyrosequencing of the amplicons produced approximately 300,000 reads. Conservative quality control measures were employed to remove sequences with potential error such as those that had ambiguous bases, did not match the forward primer sequences, failed to align correctly to an established 16S rRNA sequence database, or were flagged as

Table 1. Genus assignments of the 9 OTUs identified in every sample ($n = 47$) and their relative abundance (%).

Core OTU Genera	Relative abundance of OTU in total community (%)
Staphylococcus	15.8
Streptococcus	8.2
Serratia	7.6
Pseudomonas	4.5
Corynebacterium	3.8
Ralstonia	3.7
Propionibacterium	3.6
Sphingomonas	2.4
Bradyrhizobiaceae	1.9
Sum of all "core" OTUs	51.5


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S11
S12
S13
S14
S15
S16
Streptococcus
Staphylococcus
Serratia
Corynebacterium
Pseudomonas
Propionibacterium
Ralstonia
Rothia
Sphingomonas
Bradyrhizobium
Veillonella
Gemella
Granulicatella
Prevotella
Actinomyces
Other

Urbaniak et al. Microbiome (2016) 4:1
DOI 10.1186/s40168-015-0145-y

Microbiome

RESEARCH Open Access

Human milk microbiota profiles in relation to birthing method, gestation and infant gender

Camilla Urbaniak[1,2], Michelle Angelini[3], Gregory B. Gloor[4] and Gregor Reid[1,2*]

Abstract

Background: Human milk is an important source of bacteria for the developing infant and has been shown to influence the bacterial composition of the neonate, which in turn can affect disease risk later in life. Very little is known about what factors shape the human milk microbiome. The goal of the present study was to examine the milk microbiota from a range of women who delivered vaginally or by caesarean (C) section, who gave birth to males or females, at term or preterm.

Methods: Milk was collected from 39 Caucasian Canadian women, and microbial profiles were analyzed by 16S ribosomal RNA (rRNA) sequencing using the Illumina platform.

Results: A diverse community of milk bacteria was found with the most dominant phyla being Proteobacteria and Firmicutes and at the genus level, *Staphylococcus*, *Pseudomonas*, *Streptococcus* and *Lactobacillus*. Comparison of bacterial profiles between preterm and term births, C section (elective and non-elective) and vaginal deliveries, and male and female infants showed no statistically significant differences.

Conclusions: The study revealed the diverse bacterial types transferred to newborns. We postulate that there may be a fail-safe mechanism whereby the mother is "ready" to pass along her bacterial imprint irrespective of when and how the baby is born.

Keywords: Human milk, Milk microbiota, Factors affecting the milk microbiota

Background

With the incidence of various non-infectious diseases on the rise, there is much interest in the developmental origins of health and disease and the potential role of early life feeding practices in modulating these outcomes. Breast-fed infants have been shown to be better protected than formula-fed infants against necrotizing enterocolitis and diarrhoea, allergy and asthma, inflammatory bowel disease, type 1 and type II diabetes, obesity and cardiovascular disease [1, 2]. In addition to immune protection and bioactive compounds being conveyed through maternal milk, a possible protective role of bacteria has been suggested. Lower than average levels of *Bifidobacterium* in human milk correlate with low levels of *Bifidobacterium* in the neonatal gut [3], allowing for higher than normal levels of *Bacteroides* to be established [4]. These high levels of *Bacteroides* early in life have been associated with an increased risk of asthma and obesity later in life [5–7]. Indeed, efforts to manipulate the microbiota of formula-fed infants through probiotic supplementation have resulted in protection against some of the above diseases, comparable to that observed for breast-fed infants [8–10].

Differences exist in bioactive components, macronutrients, cytokines, enzymes, protains and immunological factors between preterm and term milk and milk from mothers giving birth by vaginal and caesarean deliveries [11–16]. As well, the energy content differs in milk depending on gender of the newborn, with breast milk from mothers who give birth to sons having more fat content than that of daughters [17, 18].

* Correspondence: greglor@uwo.ca
[1]Lawson Health Research Institute, 268 Grosvenor Street, London, ON N6A 4V2, Canada
[2]Department of Microbiology & Immunology, University of Western Ontario, London, ON N6A 5C1, Canada
Full list of author information is available at the end of the article

Results: A diverse community of milk bacteria was found with the most dominant phyla being Proteobacteria and Firmicutes and at the genus level, Staphylococcus, Pseudomonas, Streptococcus and Lactobacillus. Comparison of bacterial profiles between preterm and term births, C section (elective and non-elective) and vaginal deliveries, and male and female infants showed no statistically differences

Praveen et al. Microbiome (2015) 3:41
DOI 10.1186/s40168-015-0104-7

Microbiome

RESEARCH Open Access

The role of breast-feeding in infant immune system: a systems perspective on the intestinal microbiome

Paurush Praveen[1*], Ferenc Jordan[1], Corrado Priami[1,2] and Melissa J. Morine[1,2]

- Metagenomik sekanslama
- Konakta transkriptomik analiz
- Anne sütü ile beslenen bebeklerde intestinal mikrobiyota çeşitliliği daha az

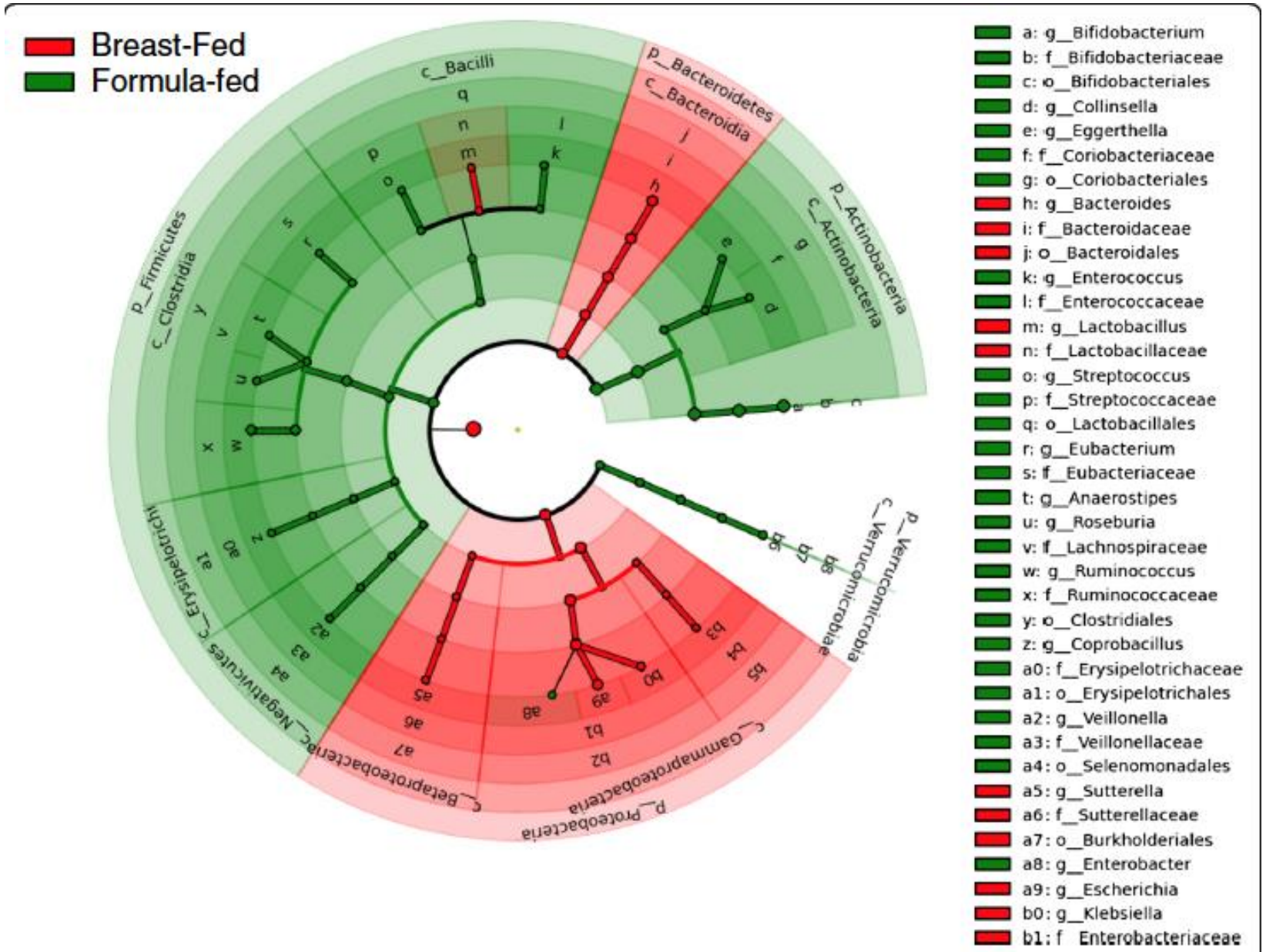


Fig. 1 The LEfSe plot for clades of the microbiota under breast-fed (BF) and formula-fed (FF) conditions. The cladograms report the taxa (highlighted by *small circles* and by *shading*) showing different abundance values (according to LEfSe). Colors of circle and shading indicate the microbial lineages that are enriched within corresponding samples. LEfSe highlights several genus-level clades, e.g., the class *Bacilli* is under-abundant in BF samples with an otherwise over-abundant *Lactobacillus* lineage (indicated with a *red shade over green* for indices *m* and *n* (see adjacent legend)). A contrary example can be seen in case of *Enterobacter* (indexed as *a8*)

Praveen et al. Microbiome (2015) 3:41
DOI 10.1186/s40168-015-0104-7

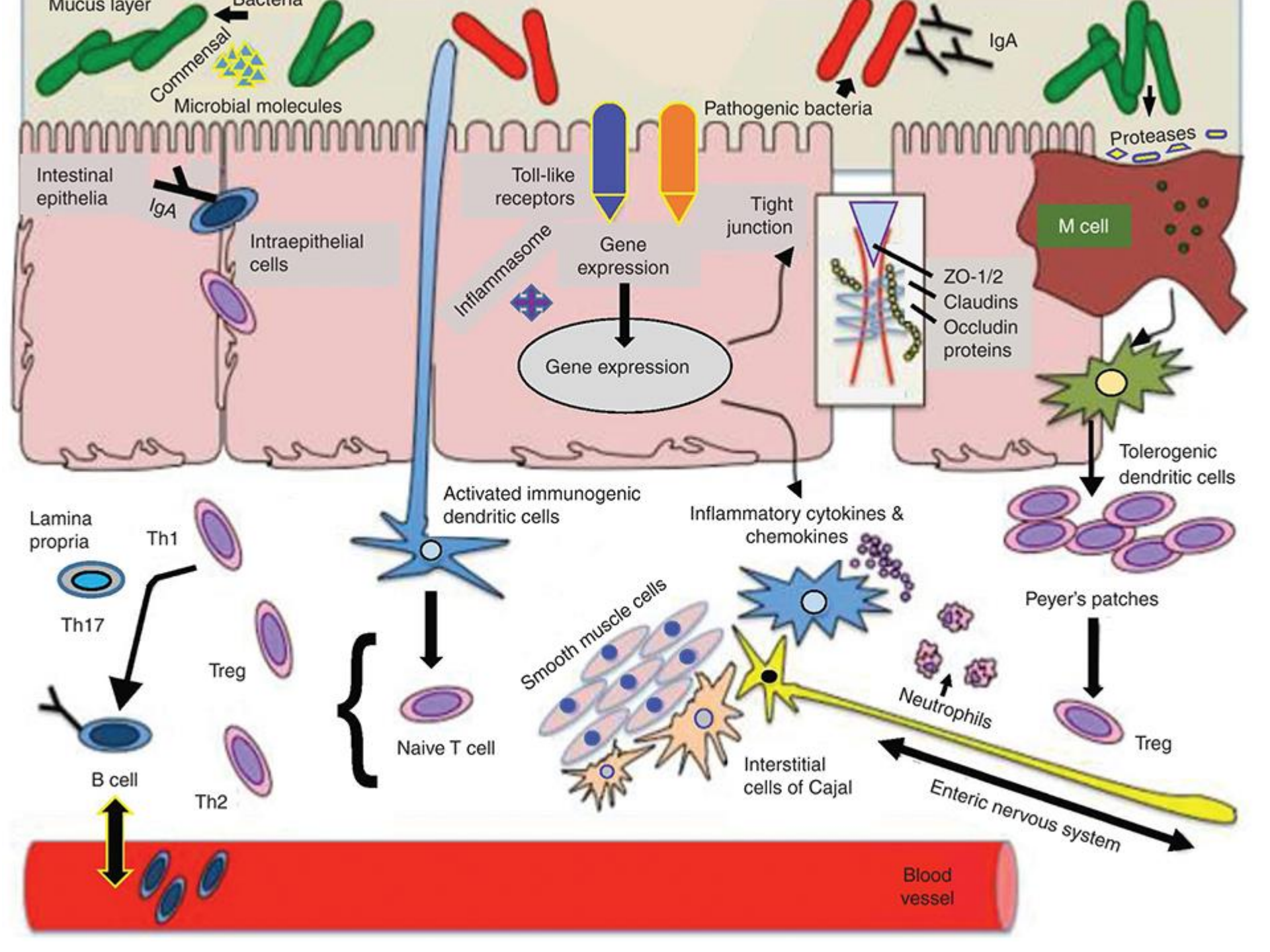
Microbiome

RESEARCH Open Access

The role of breast-feeding in infant immune system: a systems perspective on the intestinal microbiome

Paurush Praveen[1*], Ferenc Jordan[1], Corrado Priami[1,2] and Melissa J. Morine[1,2]

- Ama……bu bakterilerin genleri konağın bazı özel genleriyle iki kat daha fazla etkileşim iletişim içinde
- Bakteri genlerinin etkileştiği bu genler
  - immunolojik
  - metabolik
  - biyosentetik aktivitelerle ilişkili genler


Mucus layer
Bacteria
Commensal
Microbial molecules
IgA
Pathogenic bacteria
Proteases
Intestinal epithelia
IgA
Intraepithelial cells
Toll-like receptors
Inflammasome
Gene expression
Gene expression
Tight junction
ZO-1/2
Claudins
Occludin proteins
M cell
Tolerogenic dendritic cells
Activated immunogenic dendritic cells
Inflammatory cytokines & chemokines
Lamina propria
Th1
Th17
Treg
Th2
B cell
Naive T cell
Smooth muscle cells
Neutrophils
Interstitial cells of Cajal
Peyer's patches
Treg
Enteric nervous system
Blood vessel

MEKANİSTİK BULUŞ

The Origin of Human Milk Bacteria: Is There a Bacterial Entero-Mammary Pathway during Late Pregnancy and Lactation?[1–4]

Juan M. Rodríguez*

Department of Nutrition, Food Science and Food Technology, Complutense University of Madrid, Madrid, Spain

ABSTRACT

Human milk is a source of bacteria to the infant gut; however, the origin of milk bacteria, as well as their impact on neonatal gut microbiota establishment, remains largely unknown. In the past years, results provided by different research groups suggest that certain bacteria from the maternal gastrointestinal tract could translocate through a mechanism involving mononuclear immune cells, migrate to the mammary glands via an endogenous cellular route (the bacterial entero-mammary pathway), and subsequently colonize the gastrointestinal tract of the breast-fed neonate. If such findings are confirmed in the future, we could exert a positive influence on infant health by modulating the maternal gut microbiota. *Adv Nutr 2014;5:779–784.*

GELENEKSEL DÜŞÜNCE - KONTAMİNASYON

- Annenin derisinden ya da
- Bebeğinden ağzından

HİPOTEZ - GİS'DEN BAKTERİYEL TRANSLOKASYON

Gebeliğin geç dönemlerinde ve laktasyon sürecinde – GİS'ten meme dokusuna

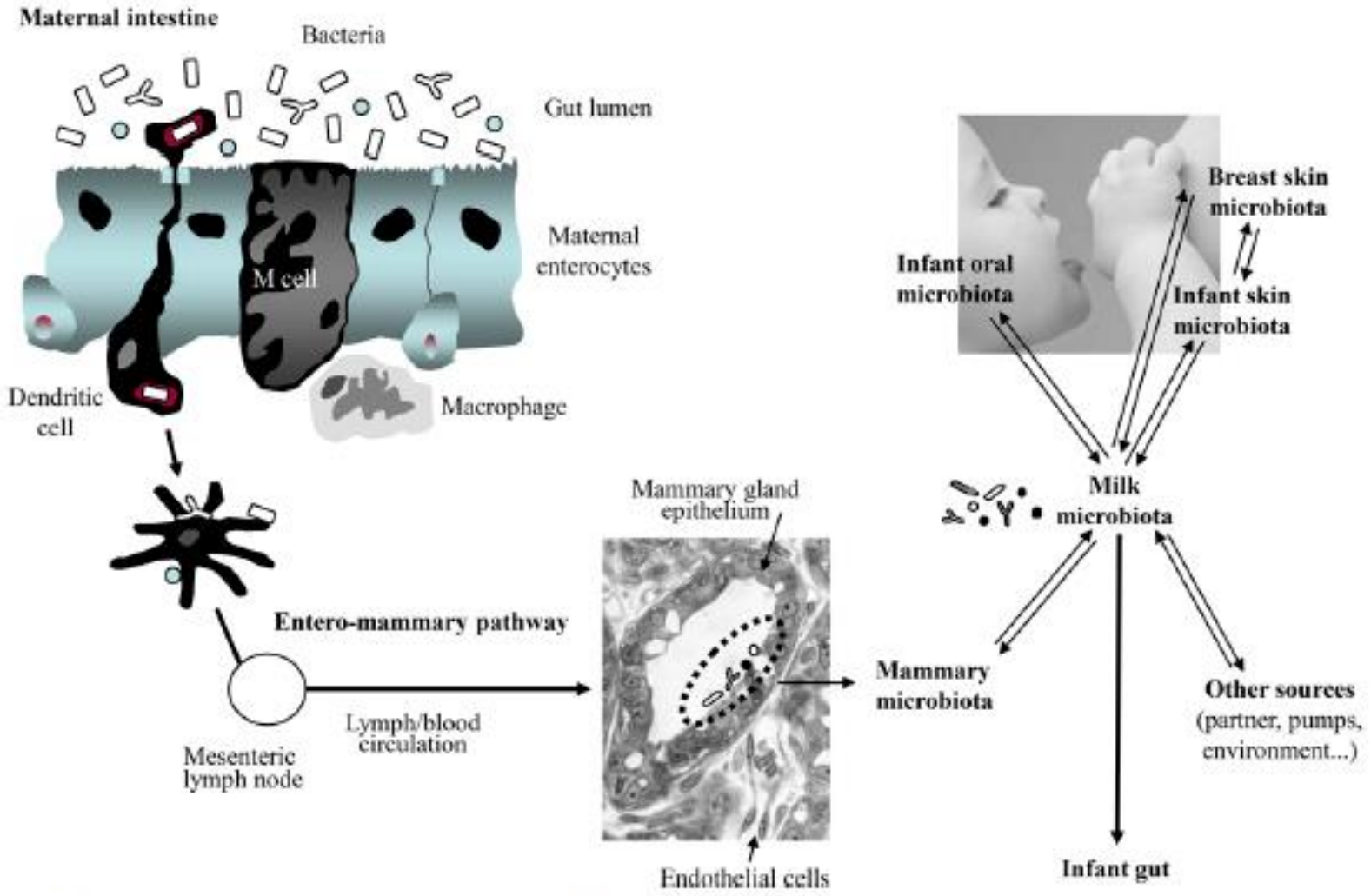


FIGURE 1 Sources of the bacteria present in human milk, including a model to explain how some maternal bacterial strains could be transferred to the infant gut through an entero-mammary pathway.

Ancak mekanistik bir çalışma yoktu

nutrients

MDPI

Article

Physiological Translocation of Lactic Acid Bacteria during Pregnancy Contributes to the Composition of the Milk Microbiota in Mice

Javier de Andrés [1,†], Esther Jiménez [1,*,†,‡], Isabel Chico-Calero [2], Manuel Fresno [2], Leónides Fernández [1] and Juan Miguel Rodríguez [1,*]

1 Department of Nutrition, Food Science and Food Technology, Complutense University of Madrid, 28040 Madrid, Spain; javierdeandres.vet@gmail.com (J.d.A.); leonides@ucm.es (L.F.)
2 Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas (CSIC), Universidad Autónoma de Madrid (UAM), and Instituto Sanitario de Investigación Princesa, 28049 Madrid, Spain; iccalero@gmail.com (I.C.-C.); mfresno@cbm.uam.es (M.F.)
* Correspondence: esjimene@ucm.es (E.J.); jmrodrig@vet.ucm.es (J.M.R.)
† These two authors share the first authorship.
‡ Current address: Probisearch S.L.U., Tres Cantos, 28760 Madrid, Spain.

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Abstract: The human milk microbiota is a complex and diverse ecosystem that seems to play a relevant role in the mother-to-infant transmission of microorganisms during early life. Bacteria present in human milk may arise from different sources, and recent studies suggest that at least some of them may be originally present in the maternal digestive tract and may reach the mammary gland through an endogenous route during pregnancy and lactation. The objective of this work was to elucidate whether some lactic acid bacteria are able to translocate and colonize the mammary gland and milk. For this purpose, two lactic acid bacteria strains (*Lactococcus lactis* MG1614 and *Lactobacillus salivarius* PS2) were transformed with a plasmid containing the *lux* genes; subsequently, the transformed strains were orally administered to pregnant mice. The murine model allowed the visualization, isolation, and Polymerase Chain Reaction (PCR)-detection of the transformed bacteria in different body locations, including mammary tissue and milk, reinforcing the hypothesis that physiological translocation of maternal bacteria during pregnancy and lactation may contribute to the composition of the mammary and milk microbiota.

Keywords: human milk; translocation; *Lactobacillus salivarius*; *lux*; bioluminescence; pregnancy; lactation

Biyolüminesan L.lactis transformasyonu başarılı

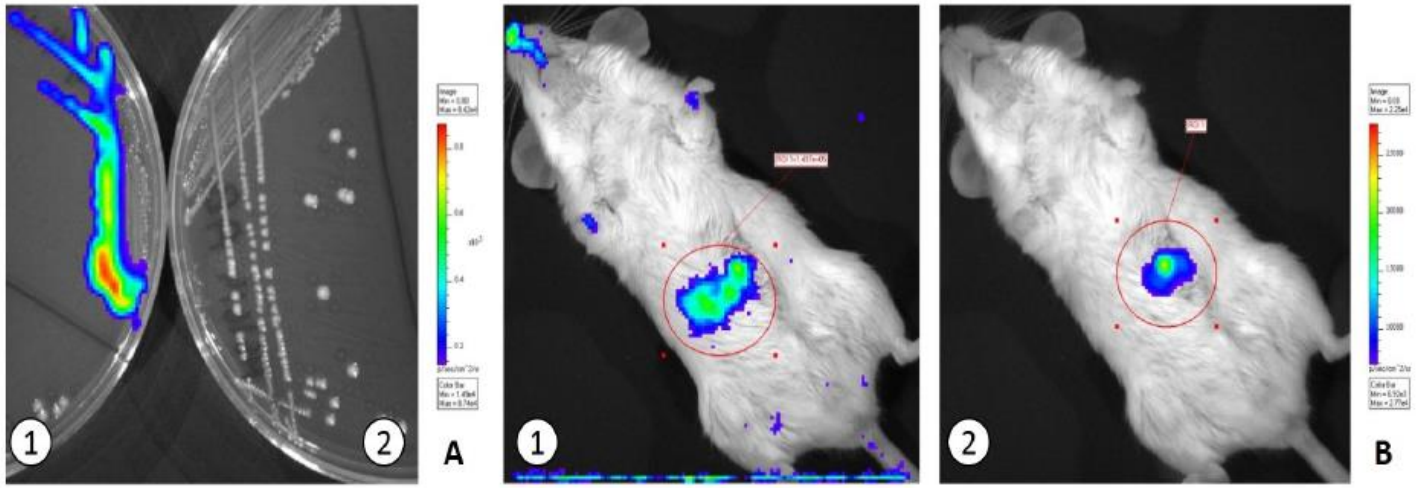


Figure 2. In vitro and in vivo detection of *L. lactis* MG1614 transformed with pMG36::*luxABCDE*. (**A**) GM17 agar plate with transformed (left) and non-transformed (right) *L. lactis* MG1614 cells. (**B**) Mouse immediately (left) and 20 min (right) after being fed with *L. lactis* pMG36e::*luxABCDE*.

Biyolüminesan Bütün Vücut Görüntüleme

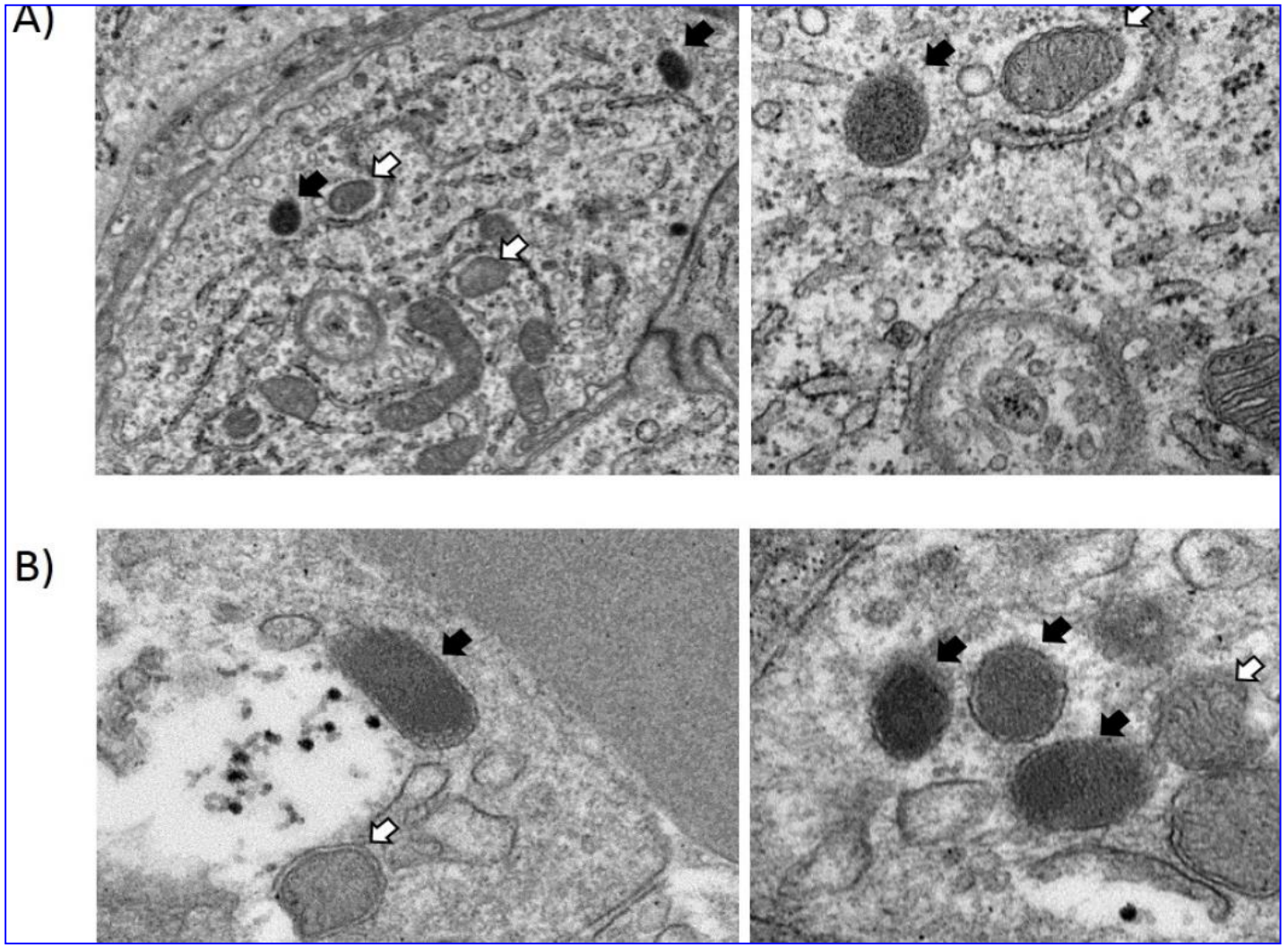
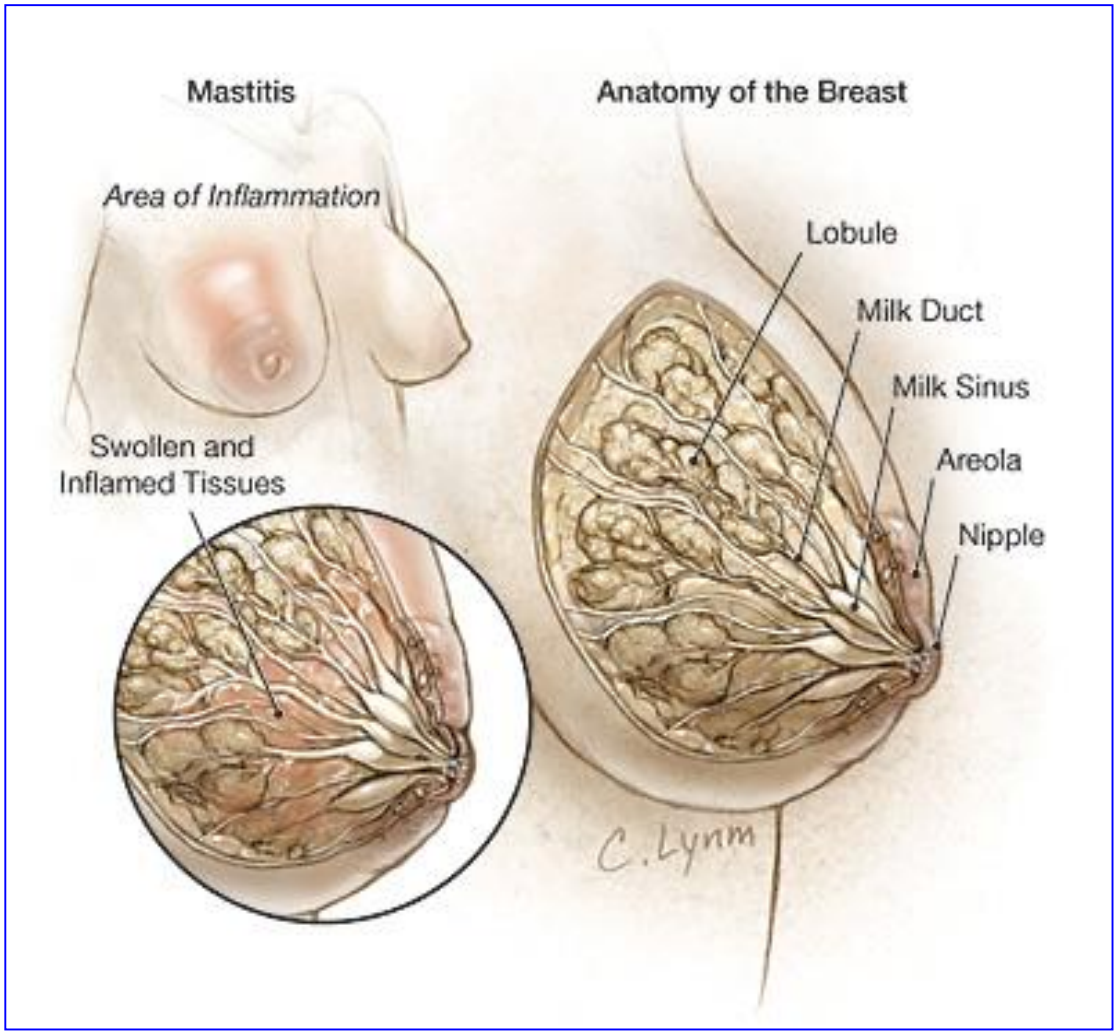


Figure 4. Transmission electron microscopy (TEM) images. Bacteria (black arrows) and mitochondria (white arrows) present in samples from a mesenteric lymph node (A) and spleen (B).

MASTITIS
(Devitalizing Disease)



Medical Microbiology and Immunology
https://doi.org/10.1007/s00430-017-0532-z

2018

REVIEW

The microbiology and treatment of human mastitis

Angeliki Angelopoulou[1,2] · Des Field[1,2] · C. Anthony Ryan[1,3] · Catherine Stanton[1,4] · Colin Hill[1,2] · R. Paul Ross[1,2]

Received: 23 October 2017 / Accepted: 15 December 2017

Abstract
Mastitis, which is generally described as an inflammation of breast tissue, is a common and debilitating disease which frequently results in the cessation of exclusive breastfeeding and affects up to 33% of lactating women. The condition is a primary cause of decreased milk production and results in organoleptic and nutritional alterations in milk quality. Recent studies employing culture-independent techniques, including metagenomic sequencing, have revealed a loss of bacterial diversity in the microbiome of mastitic milk samples compared to healthy milk samples. In those infected, the pathogens *Staphylococcus aureus, Staphylococcus epidermidis* and members of corynebacteria have been identified as the predominant etiological agents in acute, subacute and granulomatous mastitis, respectively. The increased incidence of antibiotic resistance in the causative species is also a key cause of concern for treatment of the disease, thus leading to the need to develop novel therapies. In this respect, probiotics and bacteriocins have revealed potential as alternative treatments.

Keywords Human mastitis · Microbiota · Antibiotics · Probiotics · Bacteriocins

Introduction

Over the last decade, breastfeeding has been the subject of renewed attention in developed countries because of the demonstrated health benefits to the mother–child dyad [1, 2]. Consequently, international and national health organizations encourage exclusive breastfeeding during the first 6 months of life [3–5]. However, in certain cases, exclusive breastfeeding may not be an option for mothers owing to many reasons [6], with mastitis considered as the greatest cause of undesired weaning.

Mastitis, an inflammation of breast tissue, is an acute, devitalizing condition and a potentially serious illness that may lead to breast abscess and septic fever [7]. The main causes of mastitis are milk stasis and infection [3, 8]. Milk stasis is usually the primary cause and occurs when milk is not removed properly from the breast duct due to poor attachment of the infant, fruitless suckling and blockage of the ducts [3, 9]. It is widely accepted that most mastitic cases are related to changes in the microbiome of the mammary gland and that most mastitis-causing bacteria have the ability to form biofilms in the milk ducts which are quite narrow; this results in the impairment of milk flow and the retention of milk [10] (Fig. 1a, b). The incidence of lactational mastitis varies between 2 and 33% of lactating mothers [11, 12] and most episodes occur in the first 6 weeks postpartum [13]. According to epidemiologic studies, there are many factors which might be implicated in its occurrence [13–16]. Risk factors include age, with mothers under 21 and over 35 years having a decreased incidence [17], mastitis with a previous child [16], cracked or sore nipples, use of ointments, incorrect breastfeeding practices and peripartum antibiotherapy [8, 18, 19].

The aim of this review is to provide an overview of the latest findings in terms of the microbiological load involved in human mastitis, particularly at a time when the advances in sequencing technologies have provided an excellent platform to study both cultivable and non-cultivable microorganisms, giving a more accurate view of the microbiological dysbiosis which shapes this disease. The review also describes the available therapies to treat mastitis, the most

✉ R. Paul Ross
p.ross@ucc.ie

1 APC Microbiome Ireland, University College Cork, Cork, Ireland

2 School of Microbiology, University College Cork, Cork, Ireland

3 Department of Neonatology, Cork University Maternity Hospital, Cork, Ireland

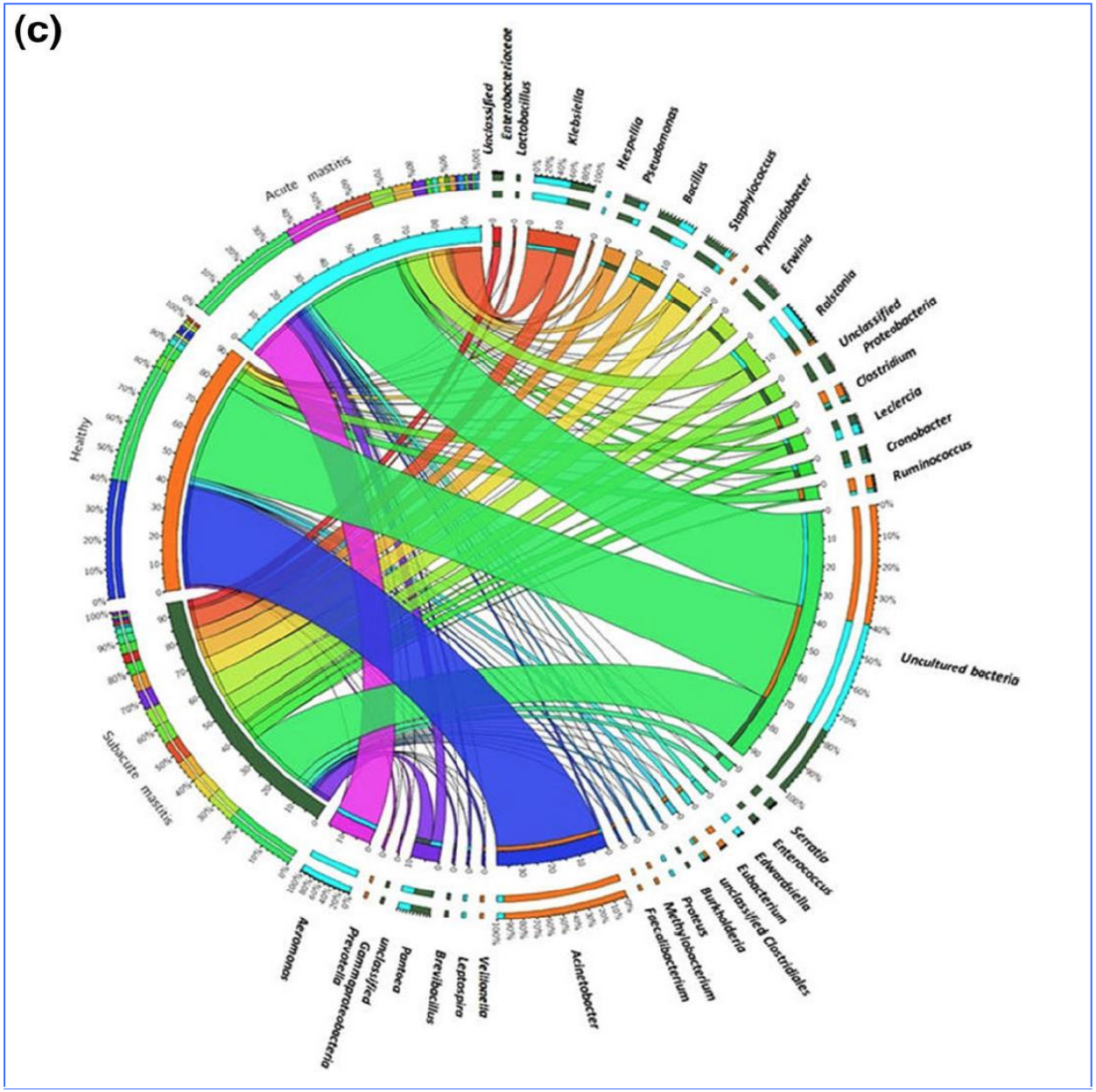
4 Food Biosciences, Teagasc Food Research Centre, Fermoy, Co Cork, Ireland

Published online: 19 January 2018

- Laktasyonel mastitte mikrobiyal yükü araştıran yayın sayısı sınırlı

- Mastitli annenin sütüne metagenomik analiz uygulayan bir çalışma bakteriyel çeşitliliğin kaybolduğunu gösteriyor Jimenez 2015

- *Staphylococcus* en dominant cins, *S. aureus* akut mastitt, *S. epidermidis* subakut mastit, *Corynobacterium* türleri ise granülomatöz mastit ile ilişkili



Circos representation of the top most abundant bacterial genera from healthy milk samples and from subacute and acute mastitis milk samples is reproduced from Patel et al.

- Probiyotikler: Profilaktik, terapötik (antibiyotiklerle eş, bazen üstün etki)
- Meme dokusunun geç gebelik ve laktasyon döneminde kendine has bir mikrobiyotası olduğu kabul ediliyor
- Sütten izole edilen ve meme dokusunun kendi mikrobiyotası olduğu düşünülen suşlar kullanılmış ve etkili
- Belki de yakın gelecekte mastit riski olan annelerde (öykü) “kişisel probiyotikler ” üretilebilecek

YALNIZCA BAKTERİ TOPLULUĞU MU?

SCIENTIFIC REPORTS

OPEN

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Multiple Approaches Detect the Presence of Fungi in Human Breastmilk Samples from Healthy Mothers

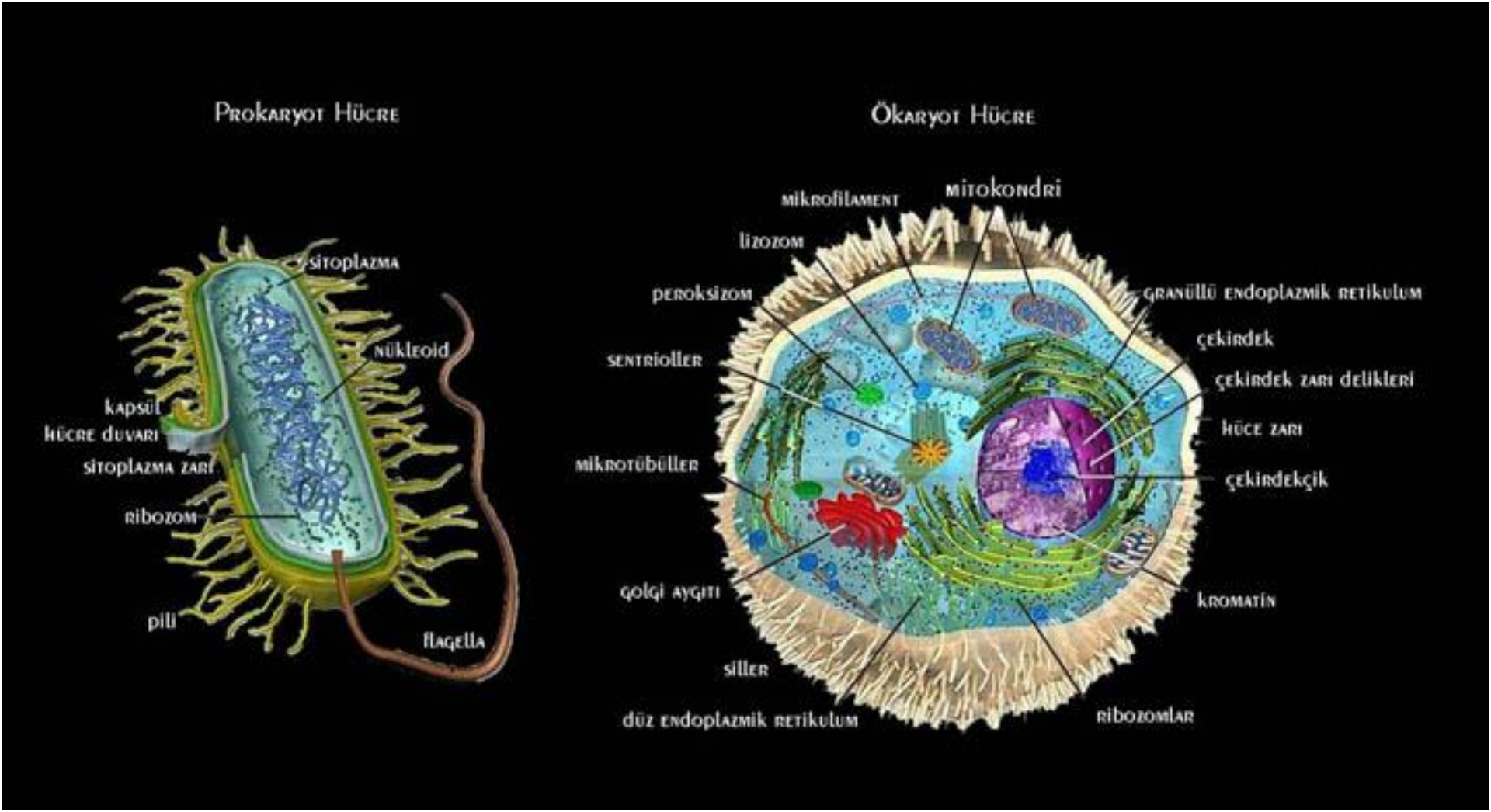
Alba Boix-Amorós[1,2], Cecilia Martinez-Costa[3], Amparo Querol[1], Maria Carmen Collado[1] & Alex Mira[2]

Human breastmilk contains a variety of bacteria that are transmitted to the infant and have been suggested to contribute to gut microbiota development and immune maturation. However, the characterization of fungal organisms in milk from healthy mothers is currently unknown although their presence has been reported in the infant gut and also in milk from other mammals. Breastmilk samples from healthy lactating mothers (n = 65) within 1 month after birth were analyzed. Fungal presence was assessed by different techniques, including microscopy, growth and identification of cultured isolates, fungal load estimation by qPCR, and fungal composition using 28S rRNA gene high-throughput sequencing. In addition, milk macronutrients and human somatic cells were quantified by spectrophotometry and cytometry. qPCR data showed that 89% of samples had detectable levels of fungal DNA, at an estimated median load of $3{,}5 \times 10^5$ cells/ml, potentially including both viable and non-viable fungi. Using different culture media, 33 strains were isolated and identified, confirming the presence of viable fungal species. Pyrosequencing results showed that the most common genera were *Malassezia* (44%), followed by *Candida* (19%) and *Saccharomyces* (12%). Yeast cells were observed by fluorescence microscopy. Future work should study the origin of these fungi and their potential contribution to infant health.

Microbiome development in the newborn is a stepwise and crucial process, contributing at the physiological level and influencing the development and maturation of the immune system[1,2]. During delivery, the neonate is exposed to maternal microbes, first from the mother's reproductive system, rapidly after from the maternal skin and the environment, and later influenced by diet, including breastfeeding. Breastmilk plays an important role in the microbial supply as it contains a variety of potential beneficial bacteria, as well as a wide source of nutrients and essential protective substances that makes it the optimal nutrition for the infant[1,2]. Those bacteria residing in breastmilk are transmitted to the infant during breastfeeding, getting to the intestine and contributing to the settlement of the gut microbiota and acquired immunity[3]. Although bacteria in human milk have been widely assessed, information about the natural presence of fungal species is generally lacking, and it is limited to a few studies focused on mammary infections describing breast candidiasis[4,5] and a recent metagenomic study on human breastmilk from mothers suffering from mastitis, which confirmed the presence of fungal sequences, in addition to the dominant bacterial fraction[6]. However, fungal presence in the milk of other mammals has been widely described in several studies[7–12], which supports the idea that human breastmilk could also contain fungi under normal, healthy conditions.

Furthermore, there is evidence that fungal species (yeast-like mainly) can be found in the infant gut early in life[13–17]. The importance of the fungal component -mycobiome- in the human gut has received increased attention

[1]Institute of Agrochemistry and Food Technology, Spanish National Research Council (IATA-CSIC), Department of Biotechnology, Av. Agustin Escardino 7, 46980, Valencia, Spain. [2]Department of Health and Genomics, Center for Advanced Research in Public Health, FISABIO Foundation, Valencia, Spain. [3]Department of Paediatrics. University of Valencia, Paediatric Gastroenterology and Nutrition Section, Hospital Clínico Universitario de Valencia (Spain), Blasco Ibáñez Av., 17, 46010, Valencia, Spain. Maria Carmen Collado and Alex Mira contributed equally to this work. Correspondence and requests for materials should be addressed to M.C.C. (email: mcolam@iata.csic.es) or A.M. (email: mira_ale@gva.es)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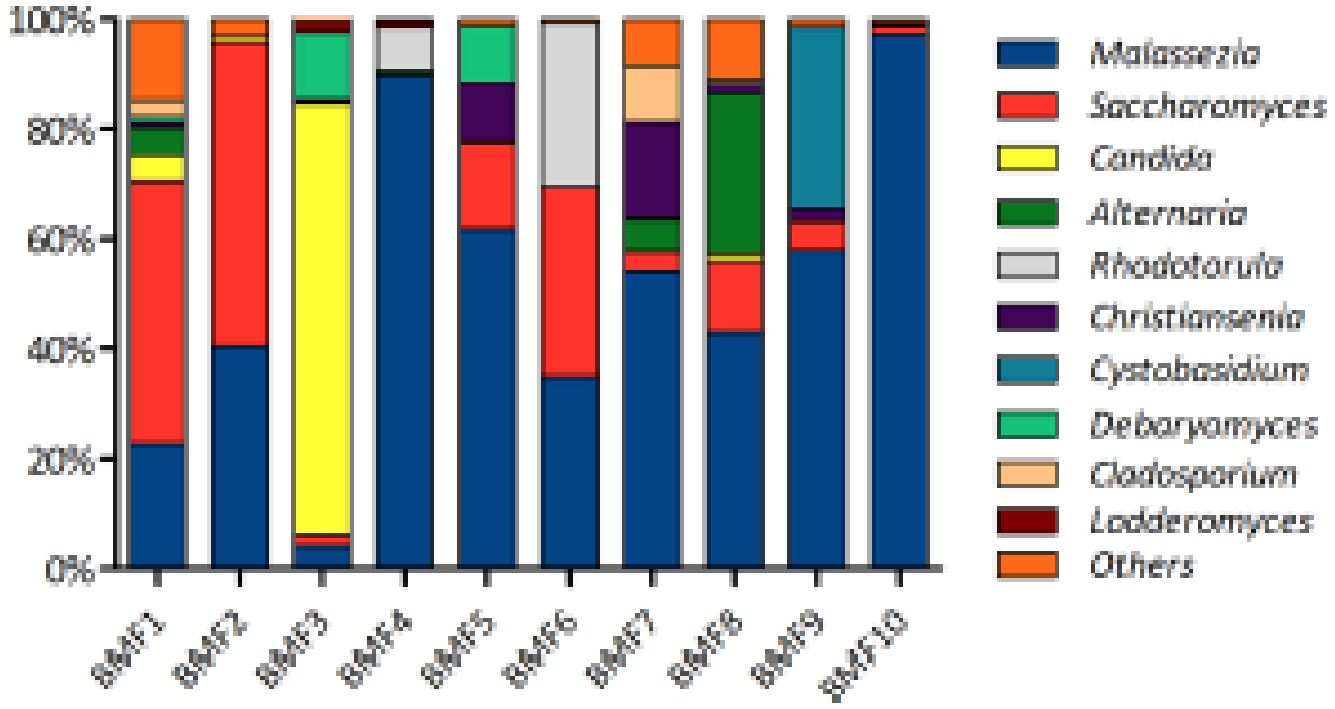
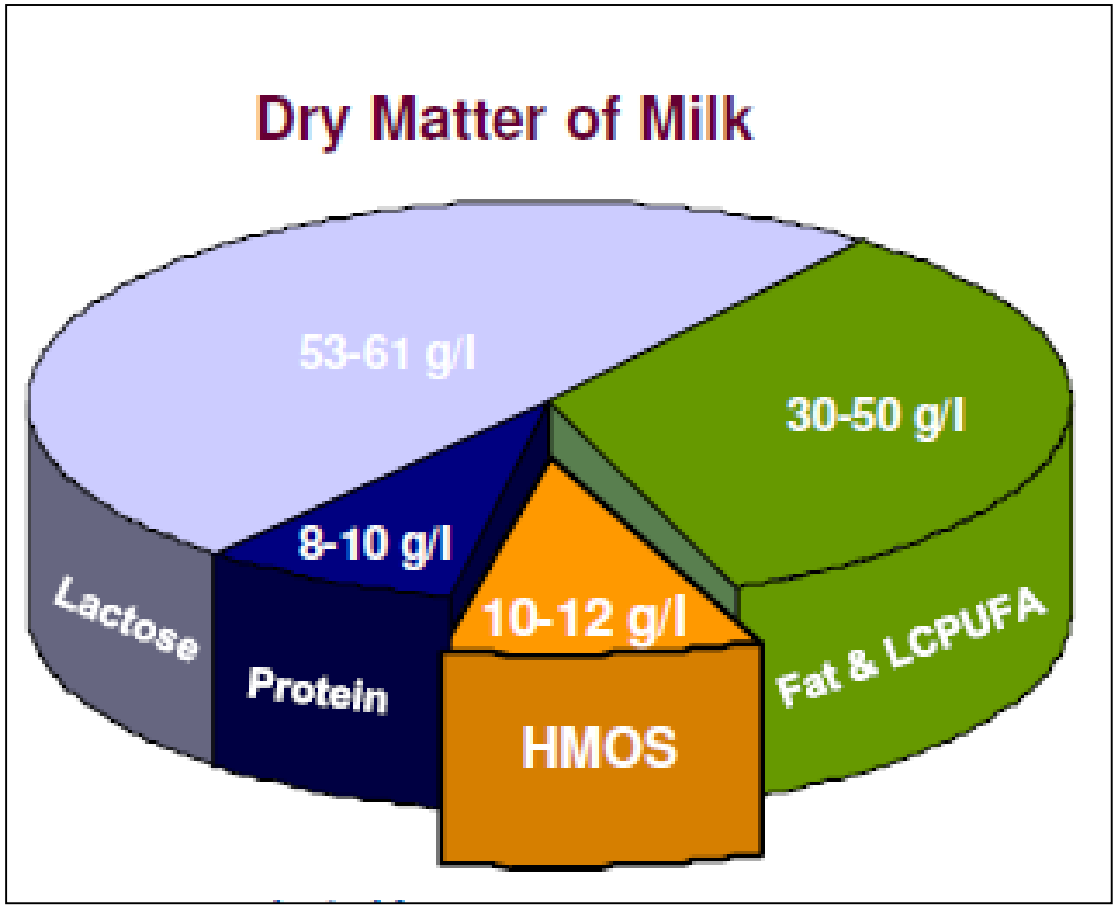
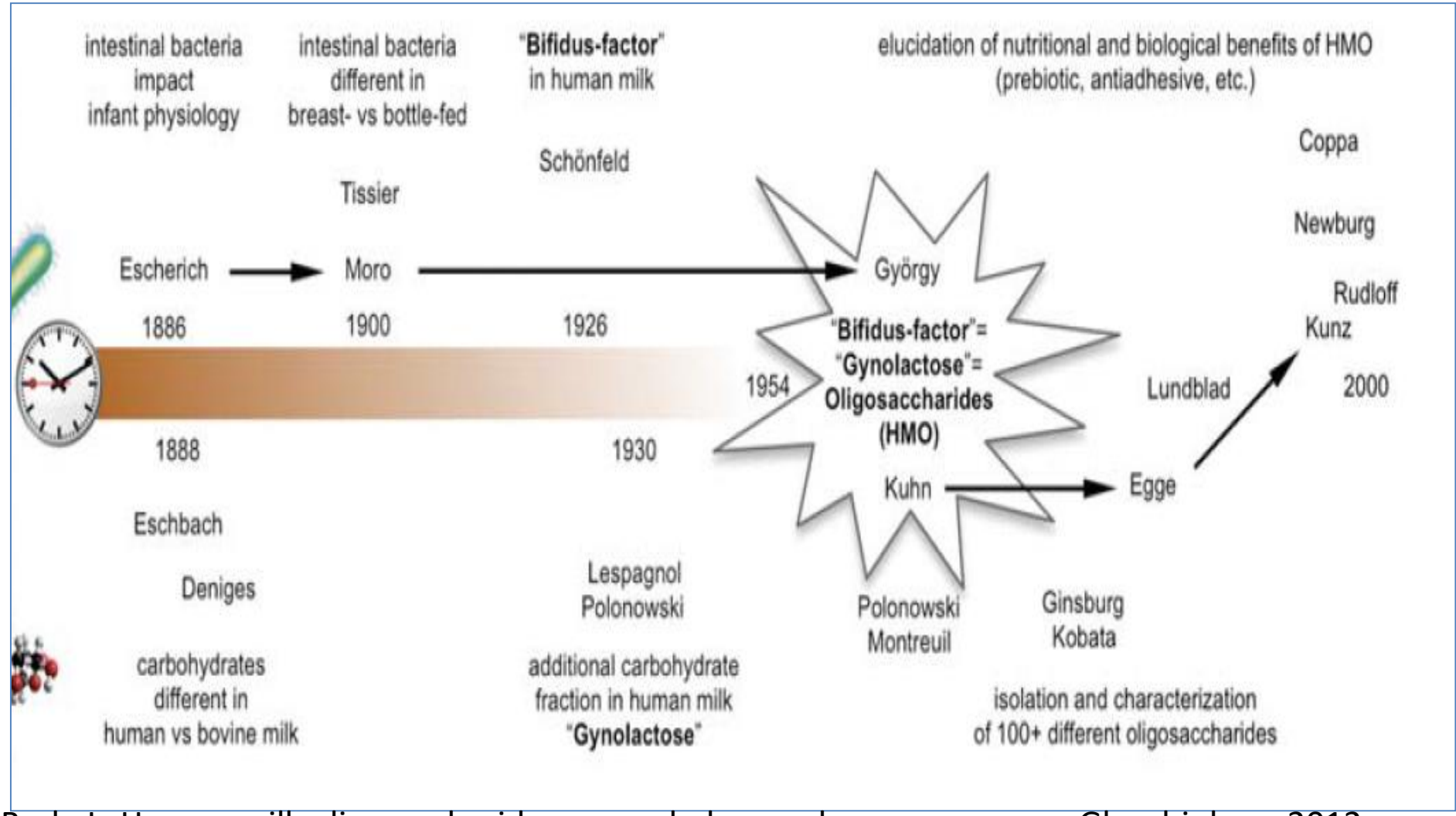


Figure 3. Fungal taxonomic composition of human breastmilk. Bars show the proportion of fungal genera as inferred by PCR amplification and pyrosequencing of the 28S rRNA gene in healthy mothers (n = 10). Each code in the X axis corresponded to a donor. Fungal genera that were under 1% were grouped in the "Others" category. The majority of the samples presented correspond to mature milk samples, except for BMF5 and BMF8 (colostrum) and BMF9 (transitional milk).

Prebiyotik-
Bifidus Faktörü



Timeline with Key Events in HMO Discovery



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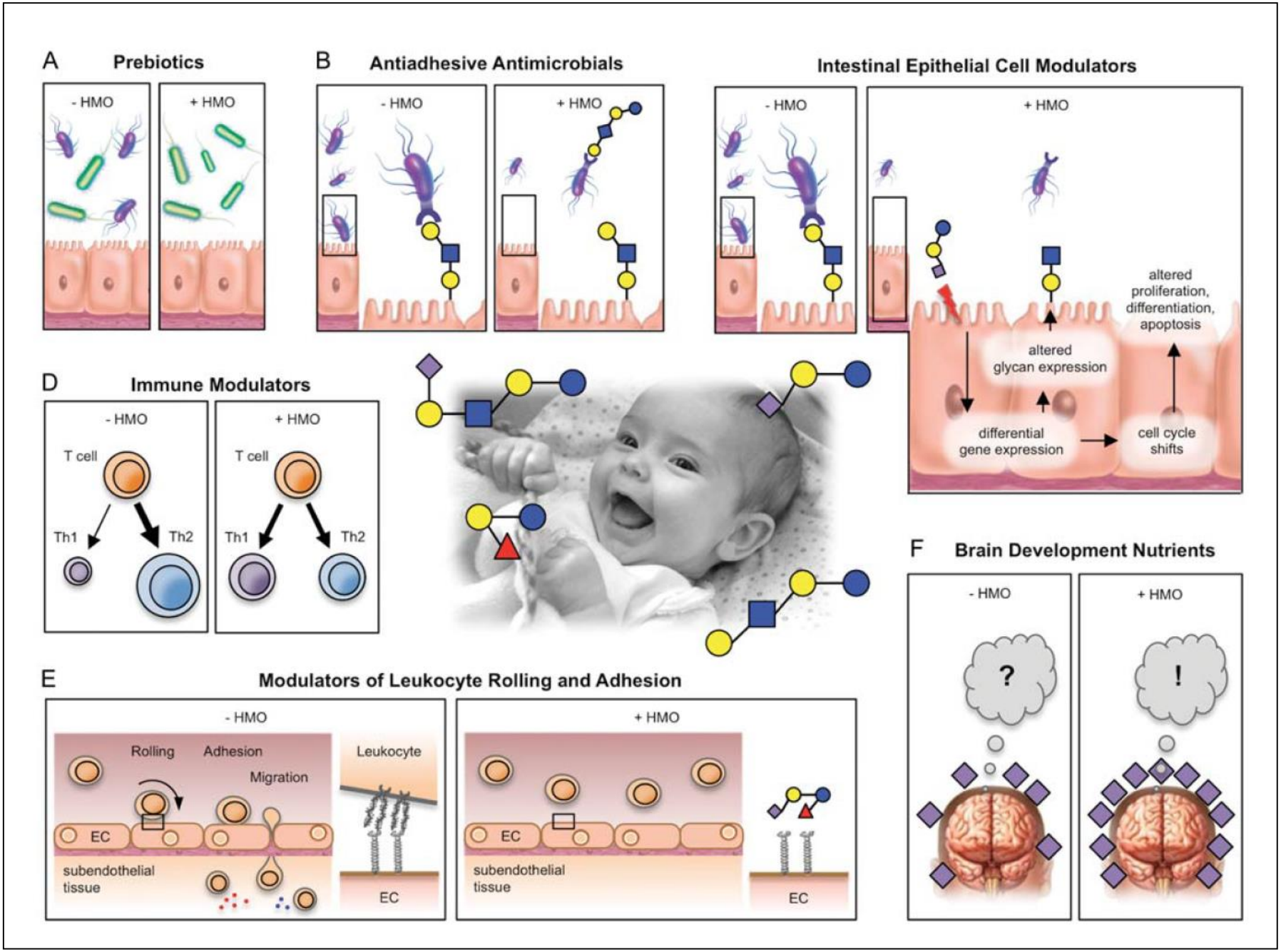

Bode L. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology. 2012 Sep;22(9):1147-62.

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Bifidobacterium longum subspecies *infantis*: champion colonizer of the infant gut

Mark A. Underwood[1,2], **J. Bruce German**[2,3], **Carlito B. Lebrilla**[2,4], and **David A. Mills**[2,3,5]

[1]Department of Pediatrics, University of California, Davis, Sacramento, California

[2]Foods for Health Institute, University of California, Davis, Davis, California

[3]Department of Food Science and Technology, University of California, Davis, Davis, California

[4]Department of Chemistry, University of California, Davis, Davis, California

[5]Department of Viticulture and Enology, University of California, Davis, Davis, California

Abstract

Oligosaccharides are abundant in human milk. Production of these highly diverse structures requires significant energy expenditure by the mother and yet these human milk oligosaccharides offer no direct nutritive value to her infant. A primary function of human milk oligosaccharides is to shape the infant's intestinal microbiota with life-long consequences. *Bifidobacterium longum* subspecies *infantis* (*B. infantis*) is unique among gut bacteria in its prodigious capacity to digest and consume any human milk oligosaccharide structure, the result of a large repertoire of bacterial genes encoding an array of glycosidases and oligosaccharide transporters not found in other bacterial species. *In vitro*, *B. infantis* grows better than other bacterial strains in the presence of human milk oligosaccharides, displays anti-inflammatory activity in premature intestinal cells, and decreases intestinal permeability. In premature infants, *B. infantis* given in combination with human milk increases *B. infantis* and decreases Enterobacteriaceae in the feces. Probiotics containing *B. infantis* decrease the risk of necrotizing enterocolitis in premature infants. Colonization with *B. infantis* is also associated with increased vaccine responses. Probiotic organisms have historically been selected based on ease of production and stability. The advantages of *B. infantis*, selected through coevolution with human milk glycans, present an opportunity for focused manipulation of the infant intestinal microbiota.

The colonization of the fetal gut begins *in utero* with swallowing of amniotic fluid. At that point, infants begin a lifelong relationship with their gut microbiota. Major shifts in the community of microbes inhabiting the intestinal tract (the gut microbiota) and the genes expressed by these microbes (the gut microbiome) and presumably the health consequences of the phenotype of the gut microbiota occur with rupture of the fetal membranes, birth, initiation of feeding, addition of solid foods, weaning, and interventions such as antibiotics,

Correspondence: Mark A. Underwood (mark.underwood@ucdmc.ucdavis.edu).

Disclosure: Three of the authors (J.B.G., C.B.L., D.A.M.) are the cofounders of Evolve Biosystems, a company focused on diet-based manipulation of the gut microbiota.

In Infants With Necrotizing Enterocolitis, Gut Dysbiosis Precedes Disease

Julie A. Jacob, MA

When Edward McCabe, MD, PhD, was a pediatric resident in the mid-1970s, he often treated preterm infants with necrotizing enterocolitis (NEC). "It's a horrible disease," he said. Forty years later, when he retired from clinical practice in 2012, few strides had been made in prevention, treatment, or mortality. The lack of significant advances to prevent or treat NEC in fragile preterm infants is frustrating to clinicians who care for them, McCabe said.

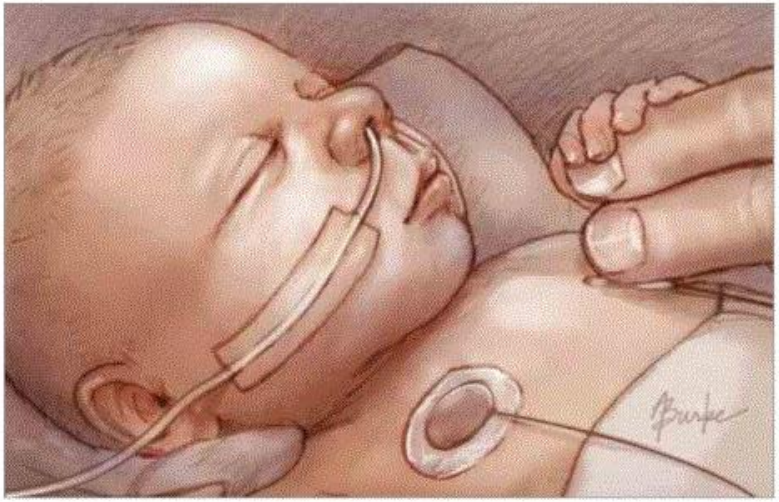
"There have been lots of studies on [causes and treatments] with essentially no change in mortality," said McCabe, the senior vice president and chief medical officer for the March of Dimes. Currently, about 12% of preterm infants weighing less than 1500 g develop NEC, and about one-third die from sepsis or other complications (Gephart SM et al. *Adv Neonatal Care*. 2012;12[2]:77-87; http://1.usa.gov/21IRhiH).

However, a new prospective case-control study by researchers at Washington University School of Medicine in St Louis provides a preliminary road map for additional investigation into causes and potential treatments (Warner BB et al. *Lancet*. doi: 10.1016/S0140-6736(16)00081-7 [published online March 8, 2016]). The research team sequenced DNA extracted from 3586 stool samples retrieved from 166 preterm infants who were hospitalized in neonatal intensive care units at 3 hospitals: St Louis (Missouri) Children's Hospital; Kosair Children's Hospital in Louisville, Kentucky; and Children's Hospital at Oklahoma University in Oklahoma City. All babies weighing less than 1500 g without congenital heart disease or intestinal perforations who were expected to survive more than 1 week were eligible for the study. The babies' stool samples were analyzed from neonatal admission to 60 days of age or until a NEC diagnosis, whichever occurred first.

Investigators discovered that the gastrointestinal bacterial microbiome of 46 preterm babies who developed NEC contained significantly more gram-negative gammaproteobacteria, such as *Escherichia coli*, and less anaerobic bacteria, particularly *Negativicutes*, compared with preterm babies who did not develop the disease.

"Neonatologists have long believed that gut bacteria could have a bearing on developing or being protected from necrotizing enterocolitis," said Phillip I. Tarr, MD, the study's senior author and a professor of pediatrics and microbiology at the Washington University School of Medicine in St Louis. That hypothesis, he explained, is based on several factors, including the association between greater antibiotic use and NEC and the protective factor of breastfeeding. "However, the identity of the risk-conferring microbes had not been clarified," Tarr added.

It was the study's scope and methodology, however, that enabled the researchers to demonstrate that the gut microbiome transition occurs before infants develop NEC, noted Scott Lorch, MD, a neonatologist and director of the Neonatal-Perinatal Medicine Fellowship at the Children's Hospital of Philadelphia, who was not involved in the study. Because thousands of stool samples were sequenced from the time the infants were admitted to neonatal intensive care—before any were diagnosed with NEC—researchers were able to study how the infants' gut microbiomes evolved over several



July 2017

PAEDIATRICS

Are human milk oligosaccharides the magic bullet for necrotizing enterocolitis?

Michael S. Caplan

There have been no major improvements in the prevention or treatment of necrotizing enterocolitis (NEC) over the past several decades, and therefore a 'magic bullet' is urgently needed. However, new data demonstrate that disialyllacto-*N*-tetraose levels in breast milk can predict the risk of NEC, and these findings might provide a strategy for successful intervention.

Refers to Autran, C. A. *et al.* Human milk oligosaccharide compostion predicts risk of necrotizing enterocolitis in preterm infants. *Gut* http://dx.doi.org/10.1136/gutjnl-2016-312819 (2017)

Necrotizing enterocolitis (NEC) is an acute, inflammatory necrosis of the intestine that primarily affects premature infants and continues to account for substantial morbidity and mortality in neonatal intensive care units worldwide. Despite >30 years of intensive research, the precise aetiology of this disease remains unknown, although studies suggest that intestinal dysbiosis, unbalanced inflammatory responses and accentuated cell death contribute to the development of this unique disease (FIG. 1). Unfortunately, fully effective preventive and treatment approaches are unavailable[1]. Nonetheless, human milk has long been known to reduce the risk of NEC compared with infant formula, yet the specific factor(s) responsible for this effect are not well delineated[2]. Now, in new research published in *Gut*, Autran *et al.*[3] demonstrate that one specific human milk oligosaccharide (HMO), disialyllacto-*N*-tetraose (DSLNT), had lower levels in breast milk fed to babies who developed NEC than in breast milk fed to age-matched healthy controls. The implication is that milk DSLNT levels might be an effective biomarker to identify infants at high-risk of NEC and that DSLNT supplementation could ultimately prove to be an effective preventive strategy.

HMOs are a diverse group of complex glycans with multiple effects and are the third largest component of human milk[4]. HMOs stimulate the growth of beneficial intestinal commensal bacteria such as *Bifidobacteria* spp., and many randomized trials and meta-analyses have demonstrated that probiotic supplementation can reduce the risk of NEC in preterm infants[5]. In addition, specific isotypes of HMOs, of which >150 exist, bind to various microbial pathogens in a specific manner and this process might reduce the inflammatory response to bacteria at the mucosal surface. A previous study from authors of the new research demonstrated that DSLNT supplementation reduced the risk of NEC in a neonatal rat model, whereas all other HMOs had much less or no effect[6]. The latest study demonstrating that low milk DSLNT levels are associated with NEC in humans supports the animal observations and has provocative implications.

An urgent need exists to identify a reliable biomarker for NEC that can be measured before the onset of clinical symptoms and signs. Many representative molecules have been evaluated, including faecal calprotectin, intestinal fatty acid binding protein, platelet activating factor, among others[7]. However, the specificity, sensitivity and, more importantly, the positive and negative predictive values have not been robust enough to reliably identify clinically significant cases of NEC[7]. Furthermore, biomarker development has been hampered by imprecise definitions and categorization of NEC, which currently depends on the modified Bell Staging system and is not a reliable differentiator between NEC and a variety of acquired intestinal pathologies of the neonate, such as spontaneous intestinal perforation, cow's milk protein allergy or feeding intolerance[8]. Notably, of the cases included in Autran *et al.*[3], 3 of 10 patients might not be 'true' NEC at all, and might represent feeding intolerance or dysmotility.

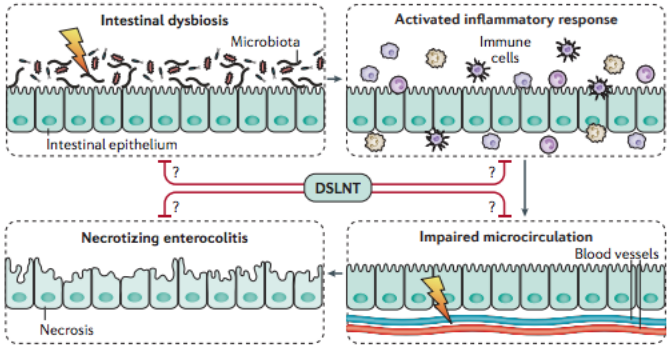
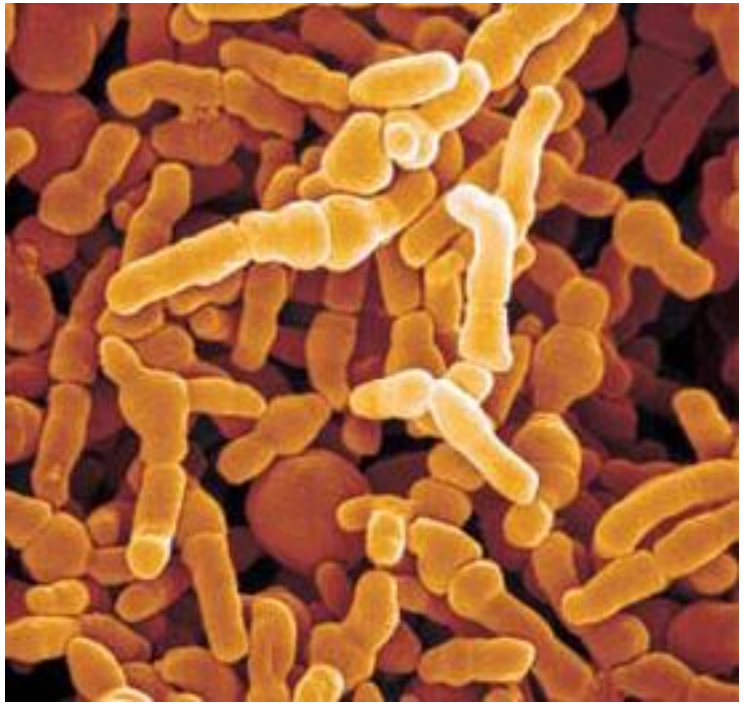


Figure 1 | **Proposed pathophysiology and inhibition of necrotizing enterocolitis.** Possible mechanisms for how disialyllacto-*N*-tetraose (DSLNT) might inhibit necrotizing enterocolitis are shown, including via the gut microbiota, inflammatory responses and impaired microcirculation.

Selektivite- Disbiyozisin Önlenmesi

Selektif olarak Bifidobakterileri bağırsağımıza yerleştiriyor

İlk kolonize olanlar çok önemli



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Fucosylated oligosaccharides in mother's milk alleviate the effects of caesarean birth on infant gut microbiota

Katri Korpela[1,2], Anne Salonen[1], Brandon Hickman[1], Clemens Kunz[3], Norbert Sprenger[4], Kaarina Kukkonen[5], Erkki Savilahti[6], Mikael Kuitunen[6] & Willem M. de Vos[1,7]

One of the most abundant components in human milk is formed by oligosaccharides, which are poorly digested by the infant. The oligosaccharide composition of breast milk varies between mothers, and is dependent on maternal secretor (FUT2) genotype. Secretor mothers produce milk containing α1-2 fucosylated human milk oligosaccharides, which are absent in the milk of non-secretor mothers. Several strains of bacteria in the infant gut have the capacity to utilise human milk oligosaccharides (HMOs). Here we investigate the differences in infant gut microbiota composition between secretor (N = 76) and non-secretor (N = 15) mothers, taking into account birth mode. In the vaginally born infants, maternal secretor status was not associated with microbiota composition. In the caesarean-born, however, many of the caesarean-associated microbiota patterns were more pronounced among the infants of non-secretor mothers compared to those of secretor mothers. Particularly bifidobacteria were strongly depleted and enterococci increased among the caesarean-born infants of non-secretor mothers. Furthermore, *Akkermansia* was increased in the section-born infants of secretor mothers, supporting the suggestion that this organism may degrade HMOs. The results indicate that maternal secretor status may be particularly influential in infants with compromised microbiota development, and that these infants could benefit from corrective supplementation.

Infants are adapted to obtaining all of their nutrition from human milk during the first months of life. In addition to nutrients for the infant, breast milk contains a diverse mixture of complex oligosaccharides, termed human milk oligosaccharides (HMOs), at an abundance of approximately 10 g/l[1]. These oligosaccharides are poorly digested by the infant, but are favoured growth substrates for intestinal bacteria that have the appropriate enzymatic degradation capacity. The oligosaccharide composition and abundance in breast milk is dependent on maternal genetics, particularly the FUT2 gene, which encodes an enzyme responsible for the addition of fucose at the α1-2 position on a backbone of abundant glycans containing galactose[1]. The breast milk of mothers with a functional FUT2 allele, the so-called secretors, contains a large amount of α1-2 fucosylated HMOs, most abundantly 2′fucosyllactose (2′FL), and in lesser amounts lactodifucotetraose (LDFT), lacto-N-difucohexaose I (LNDFH I) and lacto-*N*-fucopentaose I (LNFP I)[1,2]. The breast milk of non-secretor mothers lacks or has only traces of these α1-2 fucosylated oligosaccharides, thus containing a lower total amount of HMOs[1,2], although this lack may be partly compensated by higher abundances of lacto-N-tetraose (LNT), LNFP II, and III and LNDFH II[1]. The abundance of 2′FL in breast milk has been shown to be a reliable indicator of secretor status[2].

Maternal secretor phenotype has been recently linked with reduced risk of atopic dermatitis in a cohort of caesarean-born infants[3], and individual HMOs were related to reduced risk of cow's milk allergy[4]. Although HMOs are reported to have immunomodulatory effects, these are mainly restricted to sialylated HMOs[5,6], which

[1]Immunobiology Research Programme, Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland. [2]European Molecular Laboratory, Heidelberg, Germany. [3]Institute of Nutritional Sciences, Justus-Liebig University Giessen, 35392, Giessen, Germany. [4]Nestlé Research Center, Nestec S.A., Vers-Chez-Les-Blanc, 26, Lausanne, 1000, Switzerland. [5]Skin and Allergy Hospital, Department of Paediatrics, Helsinki University Central Hospital, Helsinki, Finland. [6]Children's Hospital, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland. [7]Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands. Correspondence and requests for materials should be addressed to K. Korpela (email: katri.korpela@helsinki.fi)

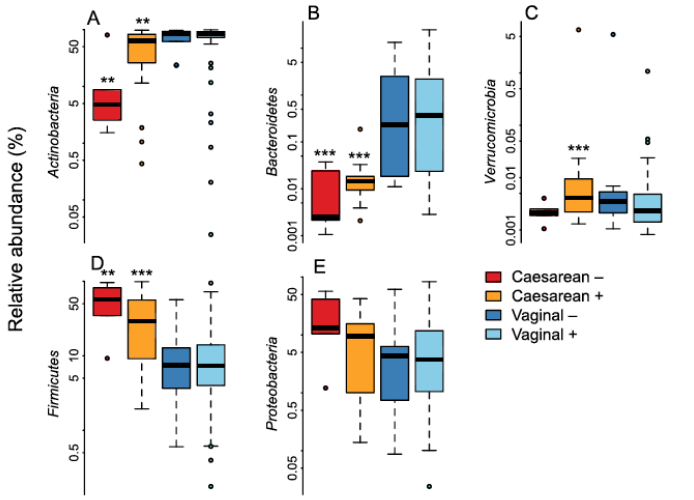
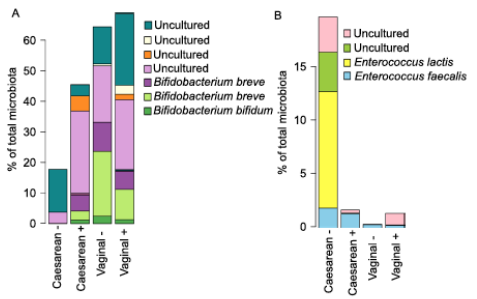


Figure 2. Relative abundance of the bacterial phyla by birth mode and maternal secretor status. The asterisks indicate the significance of the difference to the vaginally born infants of secretor mothers: $^{**}p < 0.01$, $^{***}p < 0.001$.



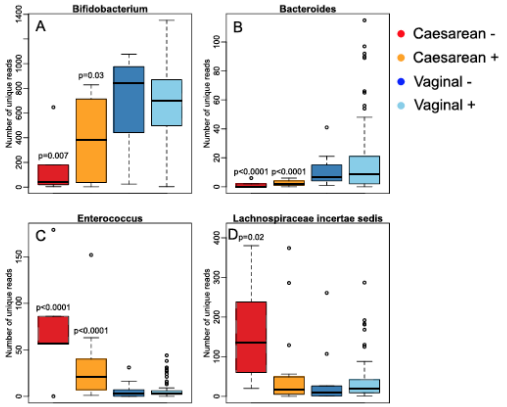
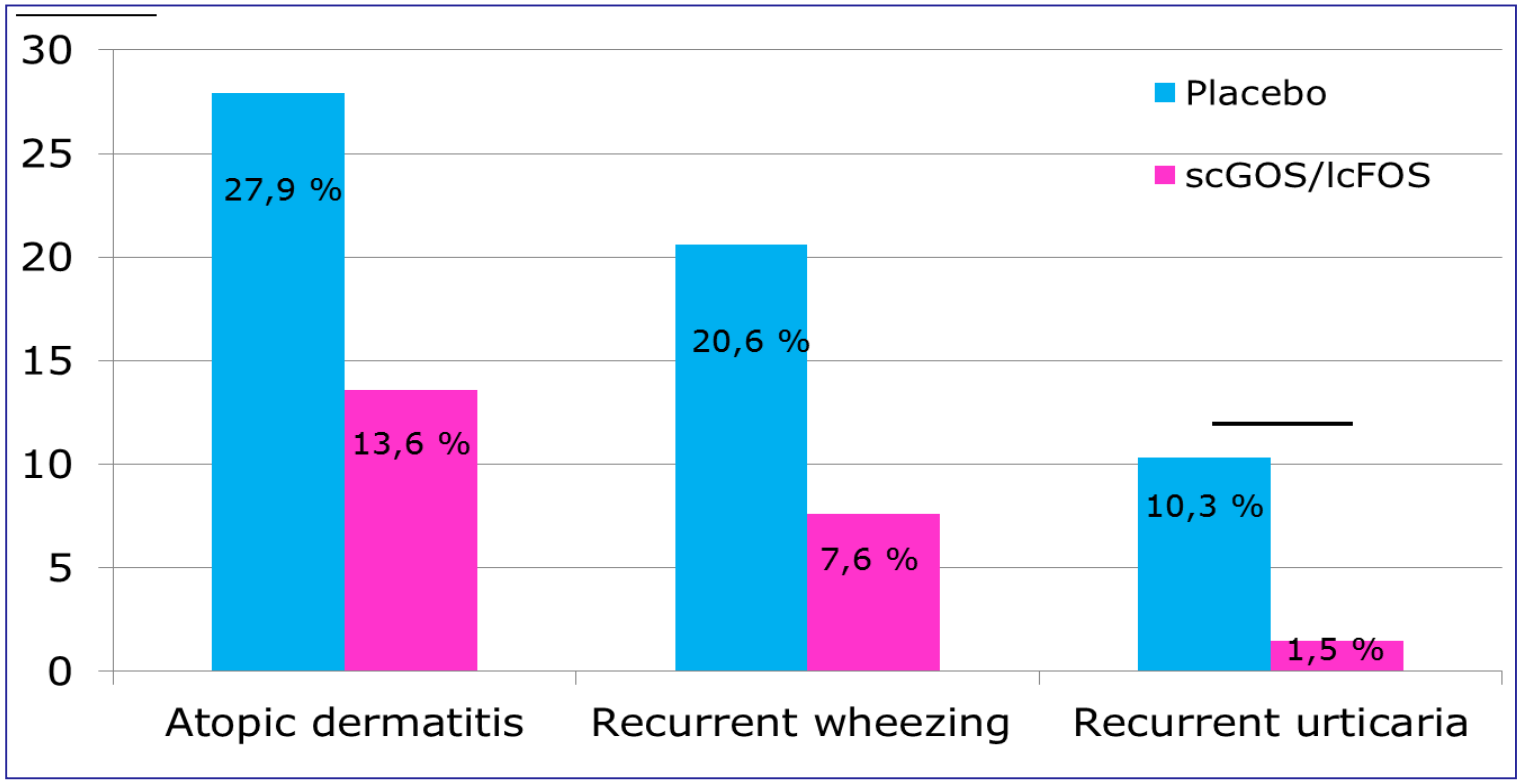


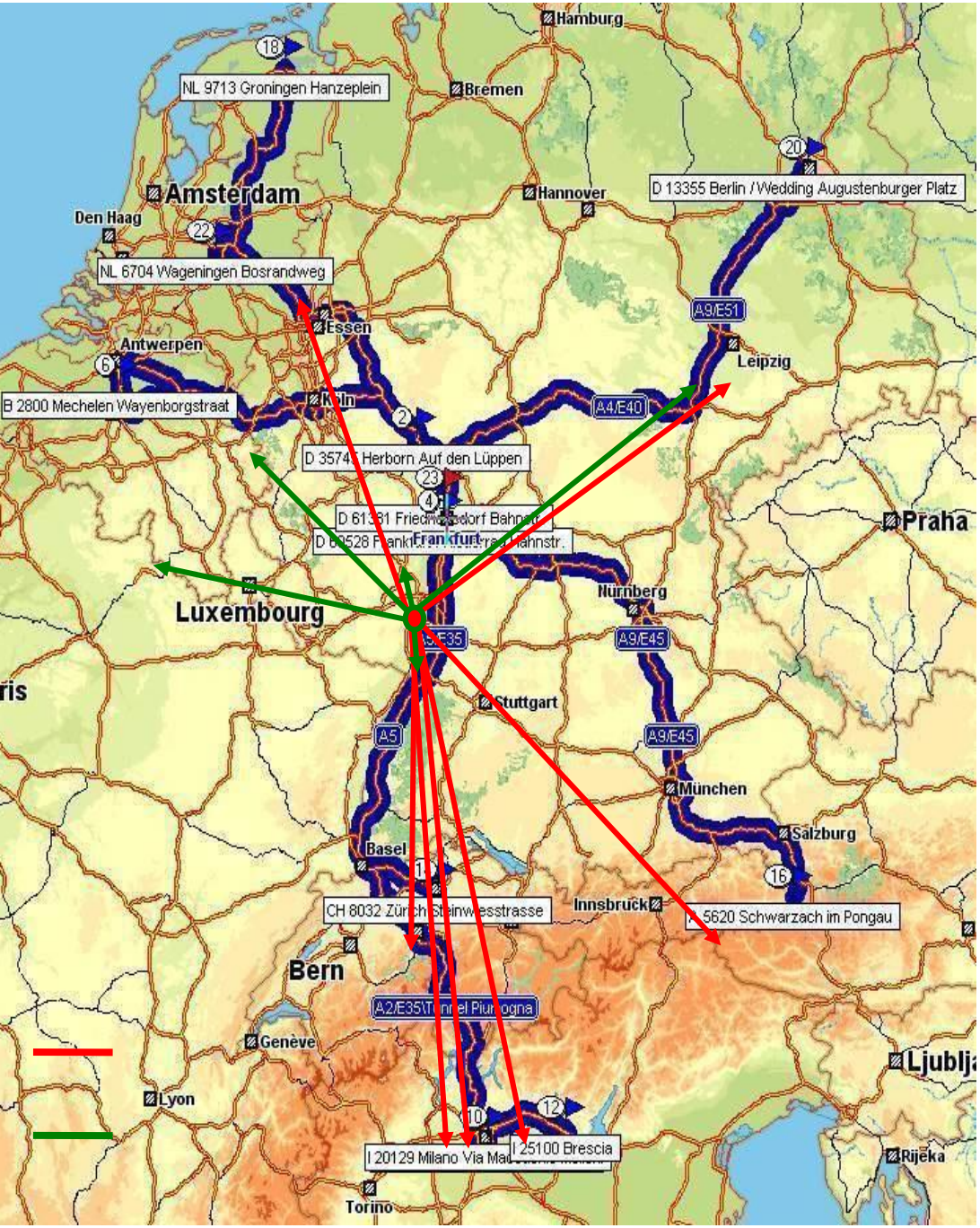
Figure 4. Sequence-level diversity (richness) within selected genera. The p-values represent the significance of the difference to the vaginally born infants of secretor mothers ("Vaginal +"), from negative binomial regression.

Early Dietary Intervention with a Mixture of Prebiotic Oligosaccharides Reduces the Incidence of Allergic Manifestations and Infections during the First Two Years of Life[1,2]

Sertac Arslanoglu,[3]* Guido E. Moro,[3] Joachim Schmitt,[4] Laura Tandoi,[3] Silvia Rizzardi,[3] and Gunther Boehm[4,5]

J. Nutr. 138: 1091–1095, 2008.





7 MERKEZ

5 BATI AVRUPA ÜLKESİ

Multicentre Immuno Programming Study

Study site
Berlin (Germany)
Brescia (Italy)
Groningen (The Netherlands)
Milan I (Italy)
Milan II (Italy)
Schwarzach (Austria)
Zurich (Switzerland)


emba
4th International Congress
European Milk Bank Association (EMBA)
GLASGOW
5th-6th October 2017
tp://europeanmilkbanking.com

SONUÇ-YORUM

- Anne sütü ile beslenme bebeğin mikrobiyomunun ve dolayısı ile ileri dönem sağlığının oluşumunda çok önemli
- Son 10 yılda anne sütü sterildir dogması yıkıldı
- Yüksek Verim Teknolojileri anne sütünde total içeriğe bakmamızı sağlıyor
- Yöntemler çok pahalı, biyoinformatik emekleme dönemini biraz geçti

- **Yeni:** Sütün bakteriyel topluluğu yanısıra "mikobiyomu" var
- **Perçinlendi:** Mastitte temel neden disbiyozis
- Anne sütü probiyotikleri mastitte etkili
- **Yeni gözlükle bir daha:** Lantibiyotik üreten bakteriler içeriyor (antibakteriyel)
- **Kanıtlandı:** "Anne bağırsağından meme dokusuna ve süte yol var (fizyolojik translokasyon)"

Biraz Sabır ve Temkin Gerekiyor

- Bu bakterilerin anne ve bebek sağlığını kısa ve uzun dönemde nasıl etkilediği ise çok daha geniş ve kompleks bir konudur
- Ama önce sağlıklı bir annenin sütünün “normal” mikrobiyomunun ne olduğunu ve bu normali etkileyen faktörleri bilmeliyiz.
- Prebiyotiklerin ve özellikle anne sütü oligosakkaridleri önemli

Anne Sütü ile beslenmenin sağlanması prematüre bebekler için ayrı önem taşıyor!

Donor Human Milk for Preterm Infants: Current Evidence and Research Directions

*†Sertac Arslanoglu, ‡Willemijn Corpeleijn, *Guido Moro, §Christian Braegger, ||Cristina Campoy, ¶Virginie Colomb, #Tamas Decsi, **Magnus Domellöf, ††Mary Fewtrell, ‡‡Iva Hojsak, §§Walter Mihatsch, ||||Christian Mølgaard, ¶¶Raanan Shamir, ##Dominique Turck, and ‡Johannes van Goudoever, ESPGHAN Committee on Nutrition

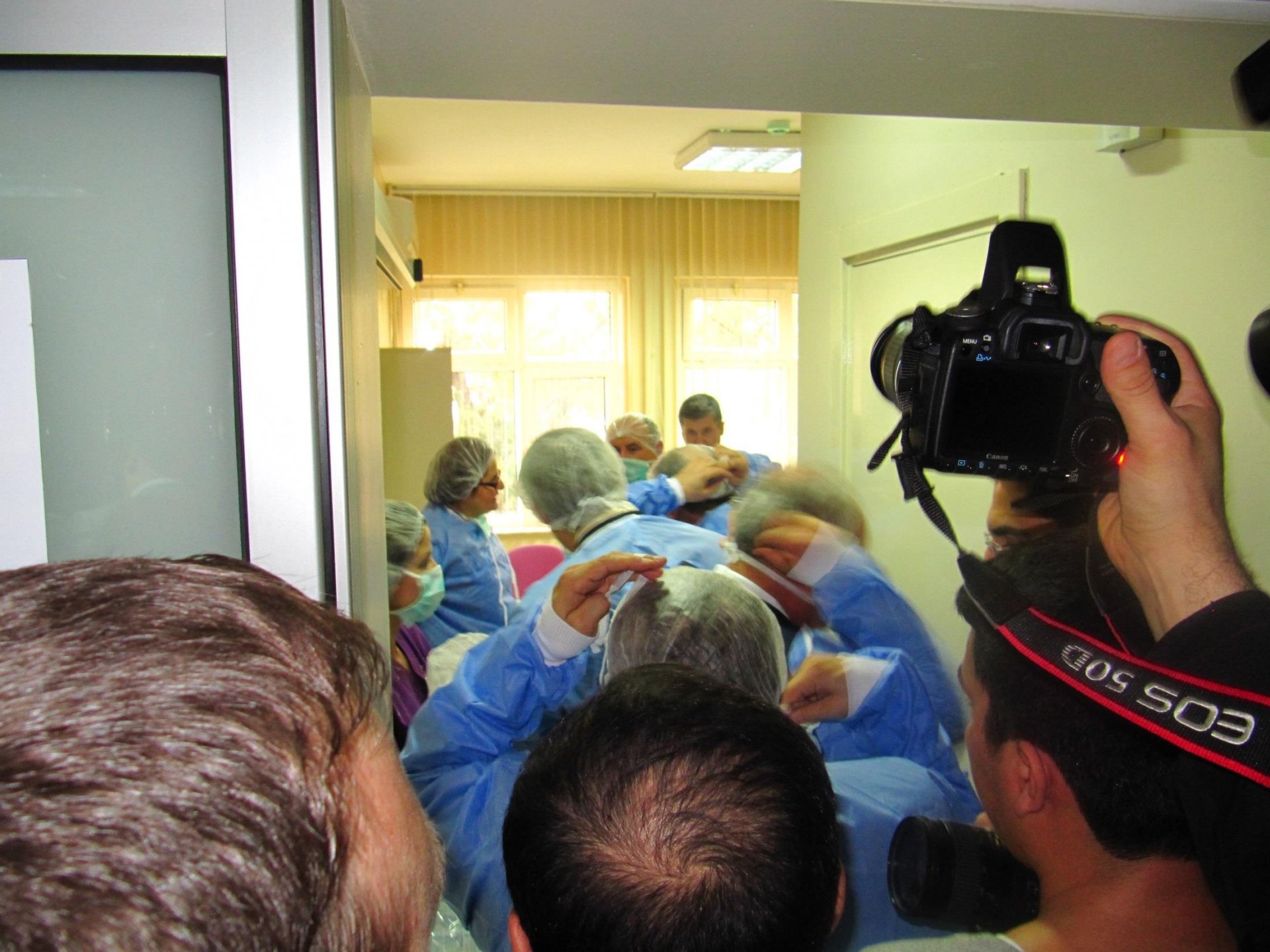
ABSTRACT

The Committee on Nutrition of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition aims to document the existing evidence of the benefits and common concerns deriving from the use of donor human milk (DHM) in preterm infants. The comment also outlines gaps in knowledge and gives recommendations for practice and suggestions for future research directions. Protection against necrotizing enterocolitis is the major clinical benefit deriving from the use of DHM when compared with formula. Limited data also suggest unfortified DHM to be associated with improved feeding tolerance and with reduced cardiovascular risk factors during adolescence. Presence of a human milk bank (HMB) does not decrease breast-feeding rates at discharge, but decreases the use of formula during the first weeks of life. This commentary emphasizes that fresh own mother's milk (OMM) is the first choice in preterm infant feeding and strong efforts should be made to promote lactation. When OMM is not available, DHM is the recommended alternative. When neither OMM nor guidelines. Storage and processing of human milk reduces some biological components, which may diminish its health benefits. From a nutritional point of view, DHM, like HM, does not meet the requirements of preterm infants, necessitating a specific fortification regimen to optimize growth. Future research should focus on the improvement of milk processing in HMB, particularly of heat treatment; on the optimization of HM fortification; and on further evaluation of the potential clinical benefits of processed and fortified DHM.

Key Words: donor milk, human milk, human milk banking, pasteurization, preterm infant

(*JPGN* 2013;57: 535–542)

8 Mart 2013


MENU
Canon
EOS 50D

……Az değil dedin
HER DAMLASI ALTIN
Anladım annemdi endişe gözlü güzel kadın.
Eğildi bana baktı
Sevgi önce gözlerinden sonra göğsünden aktı.
Bembeyaz damlacıklar yaşam iksirimdi.
Kendi varlığından bana en güzel hediyeydi.

Minik Cesur Kalpler
Sertaç Arslanoglu 2017

sertacarslanoglu@gmail.com