

# M. Carmen Collado

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Breast milk microbiota and factors influencing its composition.

La microbiota de la leche materna y los factores que influyen en su composición.





Instituto de Agroquímica  
y Tecnología de Alimentos



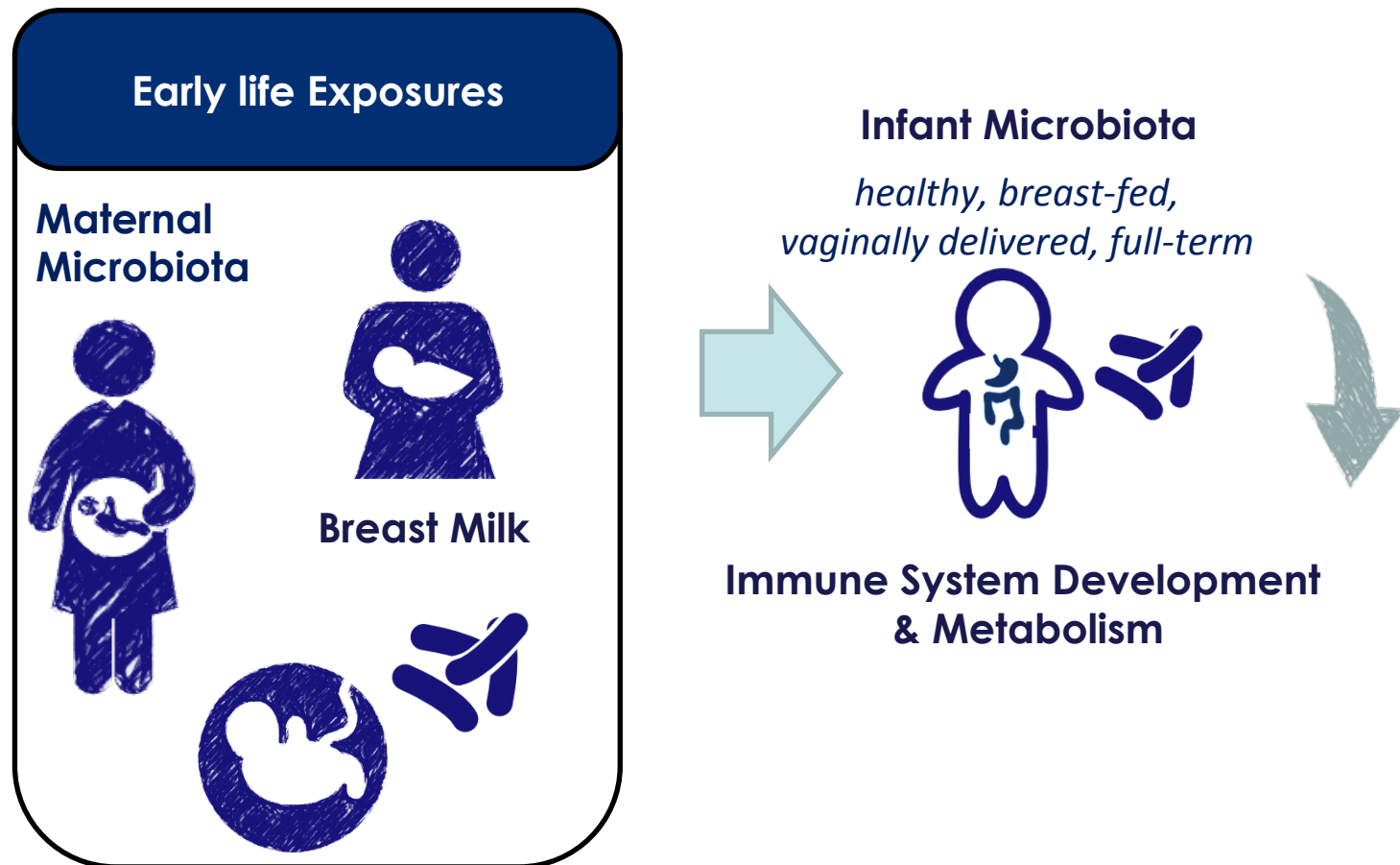
INTERNATIONAL  
SCIENTIFIC CONFERENCE  
ON RAW MILK

## Breast milk microbiota and factors influencing its composition.

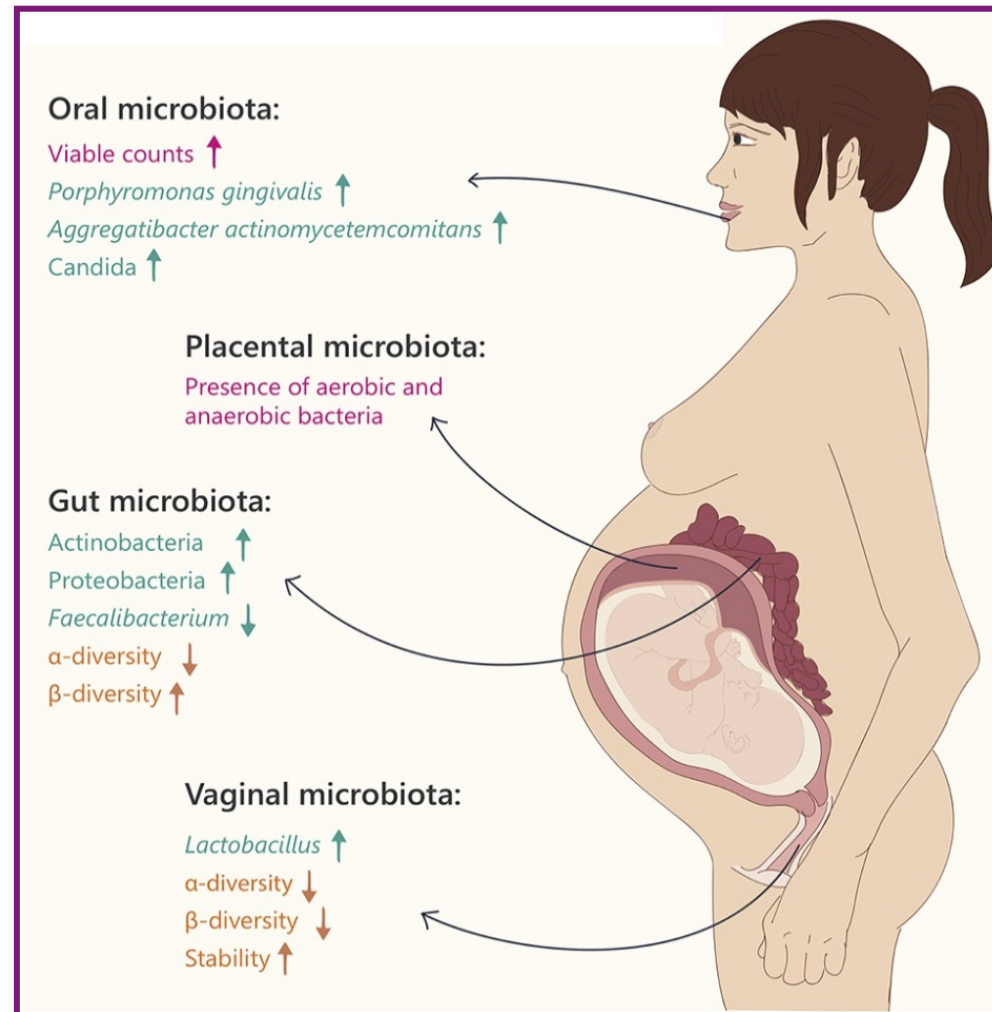
Maria Carmen Collado

6/11/19

**Maternal microbiota** is being recognized as one of the essential factors determining maternal-child health outcomes, which would also be affected by perinatal factors



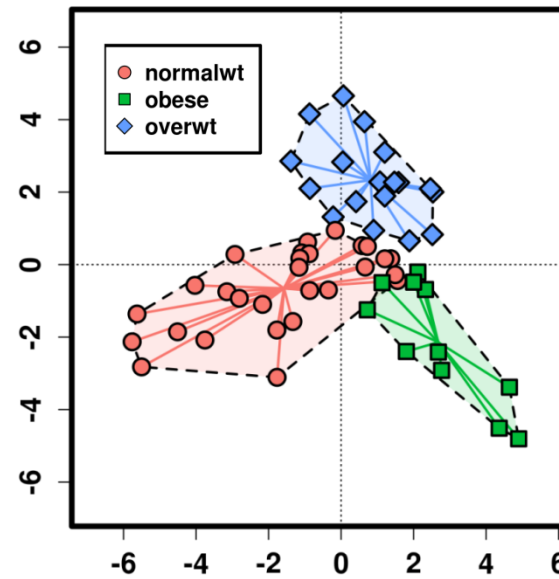
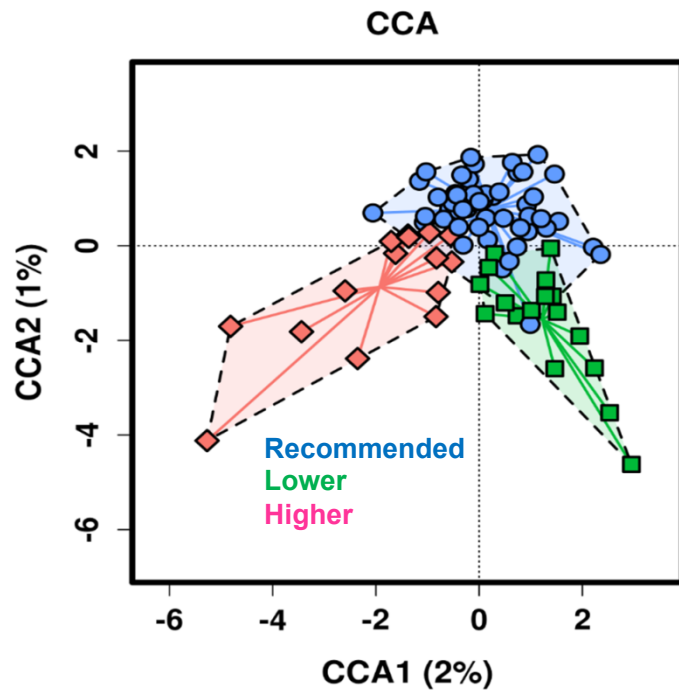
## Shifts in the maternal microbiome have been implicated in metabolic adaptations to pregnancy



## The maternal gut microbiota is modified by BMI and weight gain over pregnancy



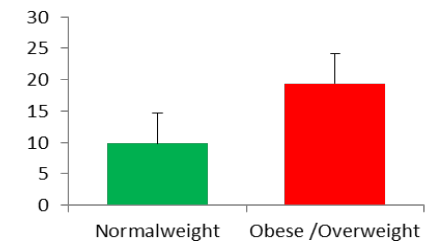
Marta Selma-Royo



Associations with pre-gestational BMI

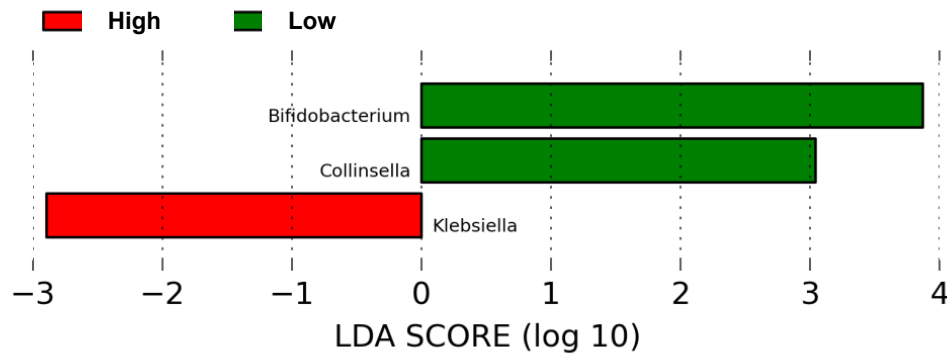
↓ Diversity and richness ↑ BMI and weight gain

Ratio Firmicutes to Barcteroides



## The maternal gut microbiota is influenced by diet

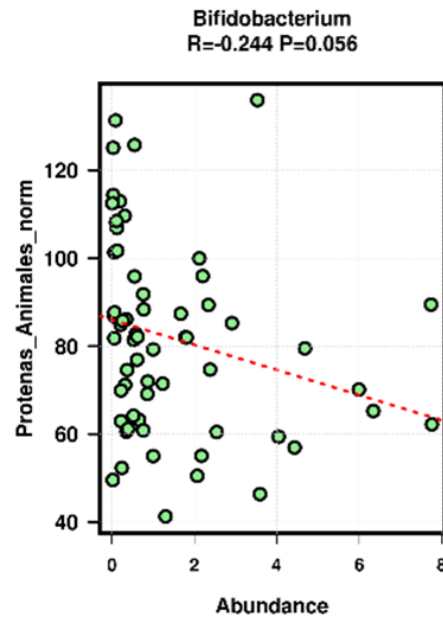
### Total animal protein intake



FFQ and  
3d recall

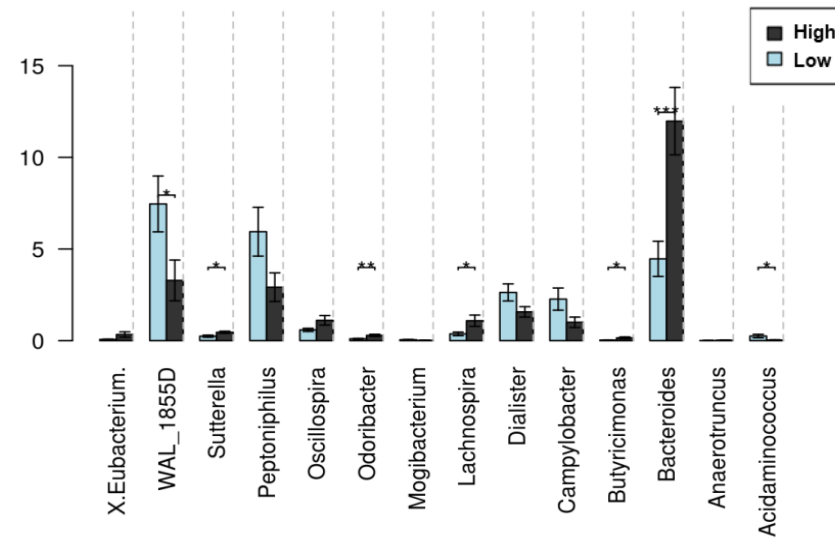


Izaekun  
Garcia-Mantrana



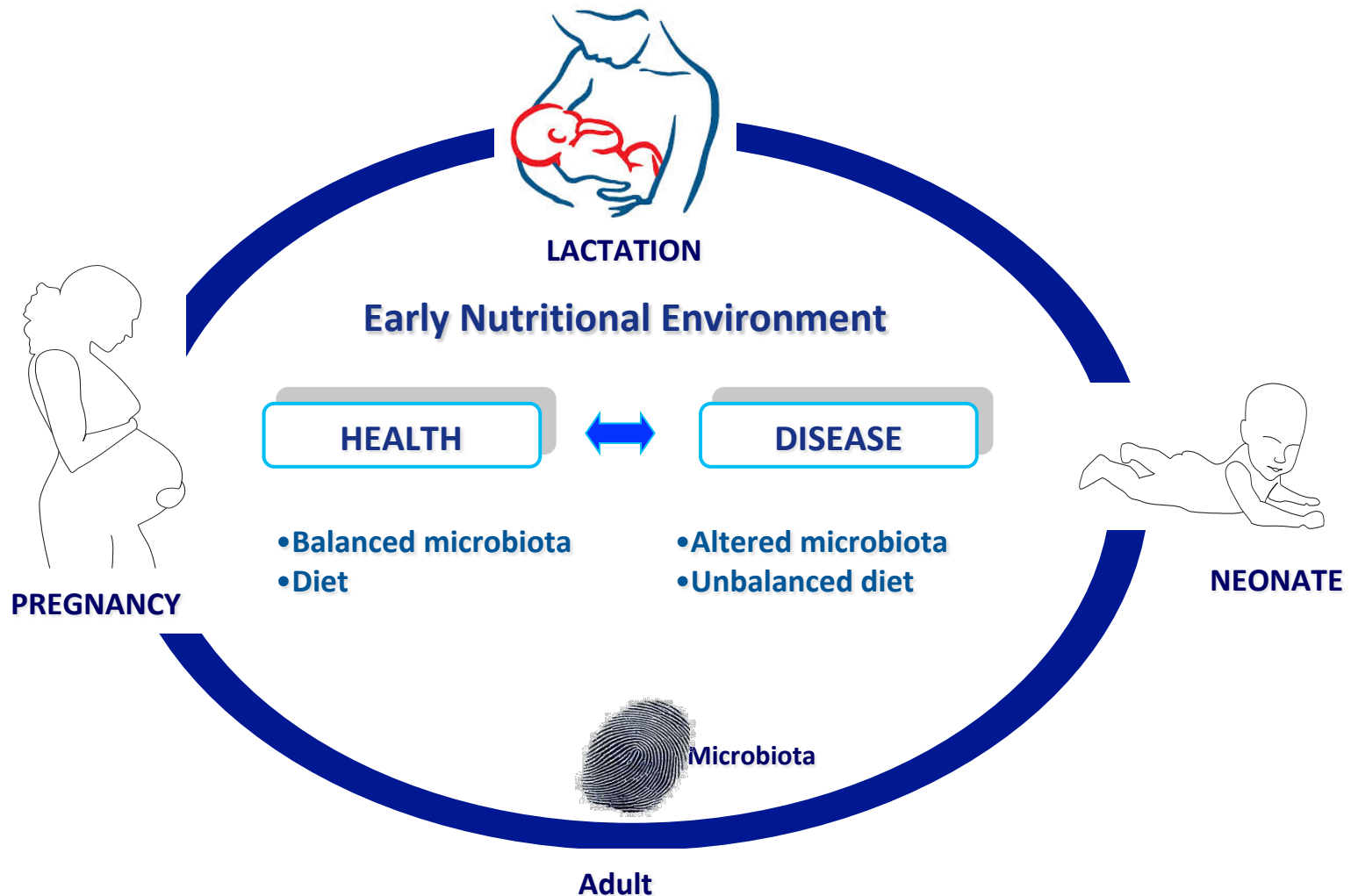
### Fiber Intake

genus (p<0.09, anova)

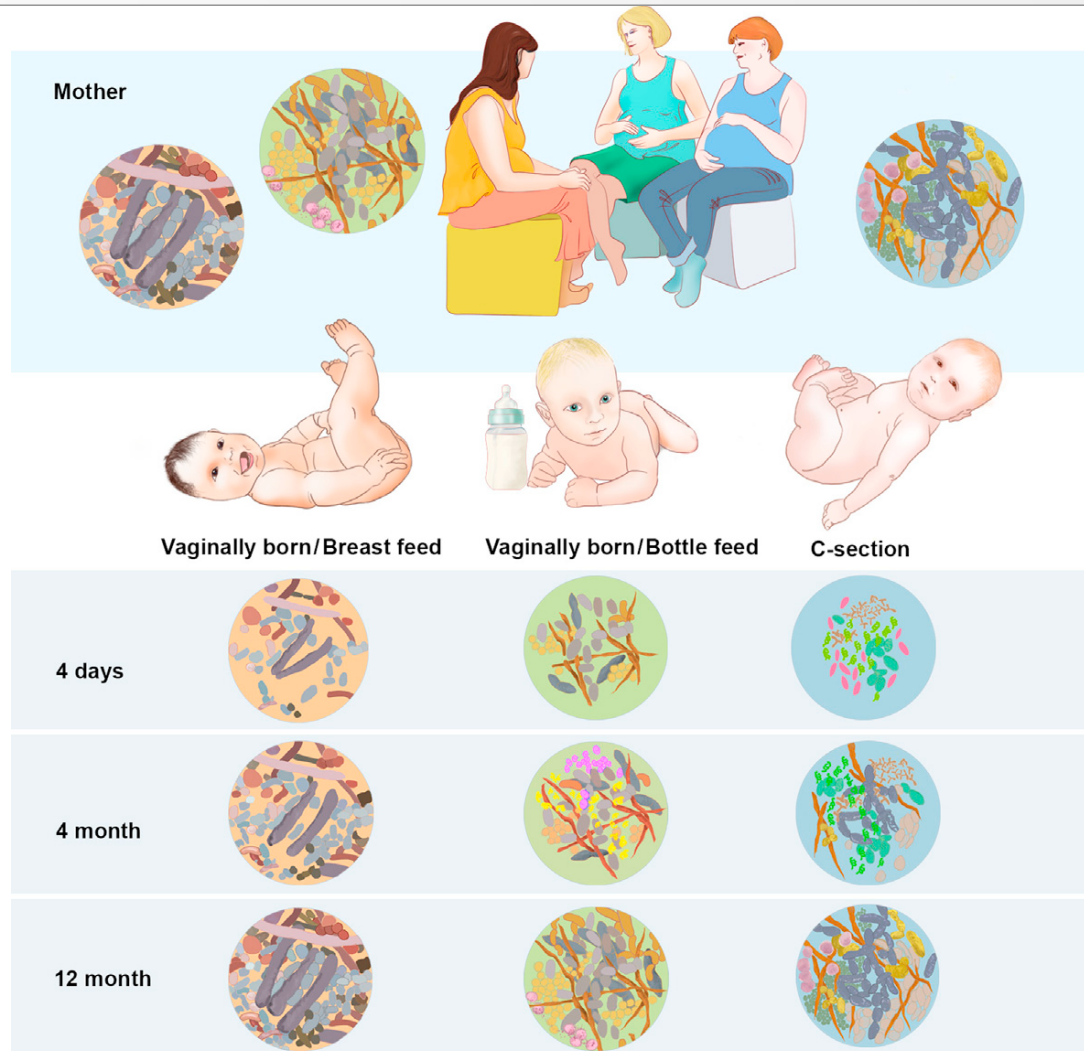


# Maternal Microbiota

What extend influence of maternal microbial and nutritional environment to infant health?.



Mode of delivery and Breastfeeding practices: role in neonatal microbiome



**Diet shapes infant gut microbial community**

*Nutrition had a major impact on early microbiota composition and function, with cessation of breast-feeding, rather than introduction of solid food, being required for maturation into an adult-like microbiota.*

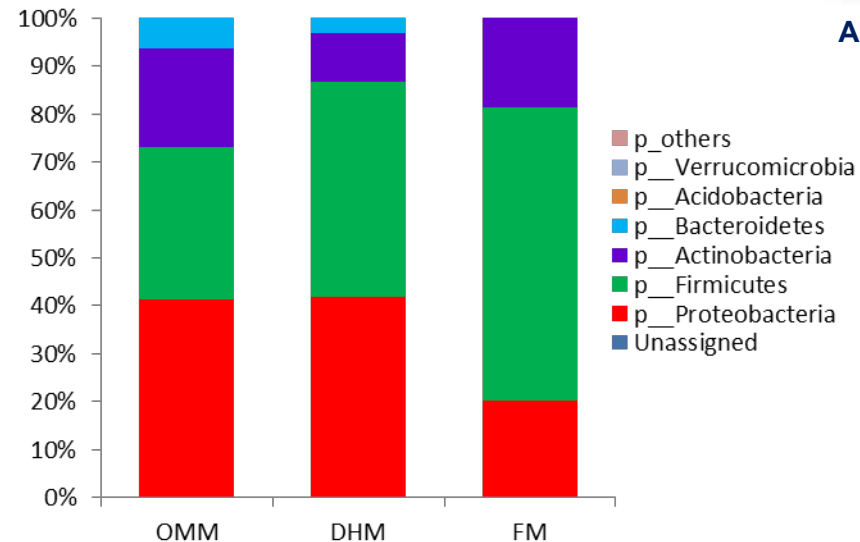
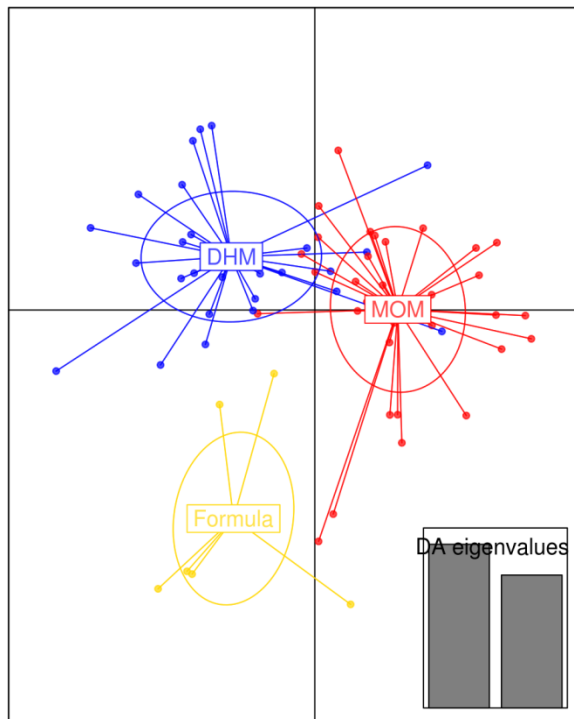


**PRETERM GUT MICROBIOME influenced by FEEDING TYPE**

A prospective observational cohort study, paired, in NICU >32 weeks and birth weight  $\leq 1.500$



Anna Parra



**Despite the higher variability, preterm microbiota composition and predictive functional profiles were significantly influenced by feeding type although no differences in microbial diversity and richness were found.**

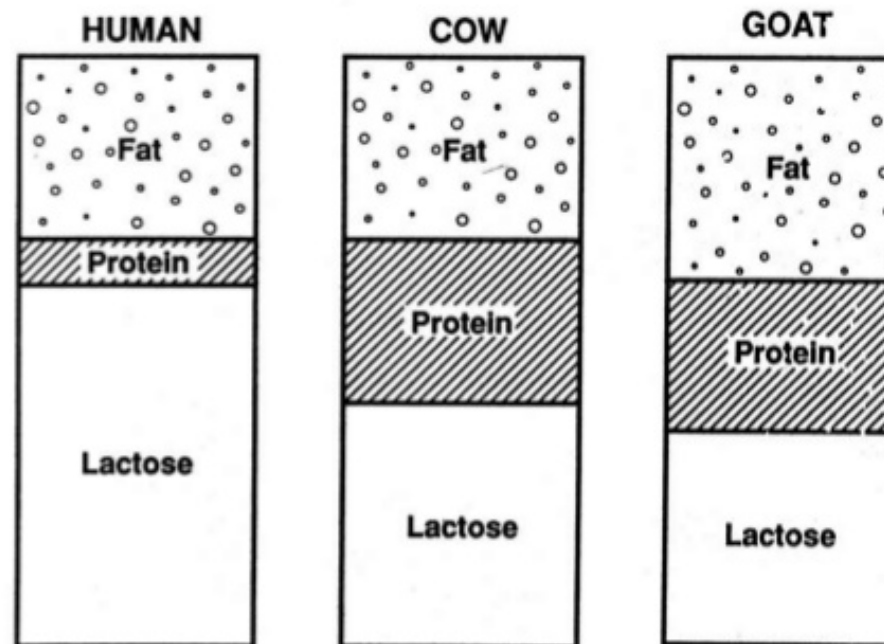
**Breast milk composition:  
Beyond nutrition**



## BREASTMILK: *PERSONALIZED* INFANT NUTRITION

### Nutrients in Human & Animal Milk

What are the differences between these milks?



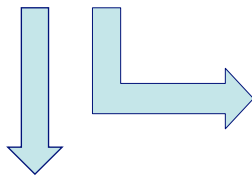
# Breastfeeding: What is in human milk?

## BREASTMILK: *GOLD STANDARD* OF INFANT NUTRITION

Bioactive compounds  
Breast milk

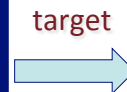


Abstent in food for infants  
(INFANT FOODS)



Growth modulation *in vivo*, gastrointestinal  
development and functionality

- protein, peptides and aminoacids
- nucleotides
- hormones
- Growth factors
- Immunomodulatory and anti-inflammatory agents
- Oligosaccharides
- Microbes



- Intestinal epitelia**, absortion of nutrients, mucosal permeability, celular proliferation, **intestinal microbiota**, induction of superficial molecules and regulation of cytokine production
- enteric nervous system**
- mucosal immune system**

**Human breast milk** is a constant source of **microbes** which have an impact on infant's microbial colonization

- Culture dependent & independent-methods
- New technologies → increase the knowledge



*Staphylococcus*

*Streptococcus*

*Bifidobacterium*

*Lactobacillus*

*Enterococcus*

*Lactococcus*

*Leuconostoc*

**Others:**

Gram + : *Actinomyces* spp. / *Corynebacterium* spp./*Kocuria* spp./

Gram - : *Escherichia coli* / *Klebsiella* / *Ralstonia* spp



**$10^4$ - $10^5$  cfu/mL**

**Structural**

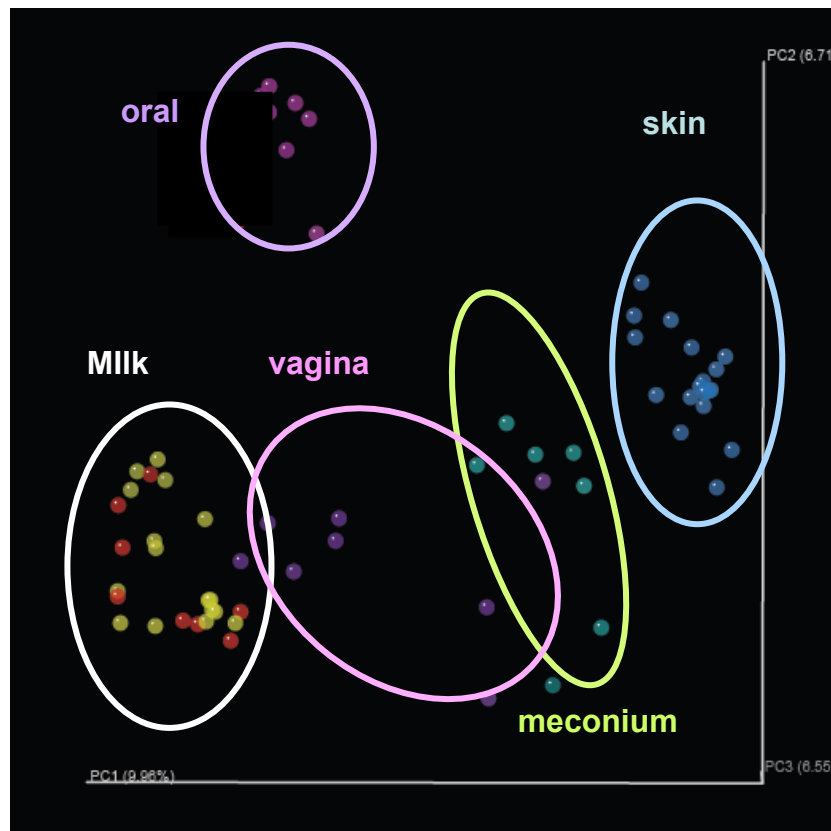
**Metabolic**

**Protector**

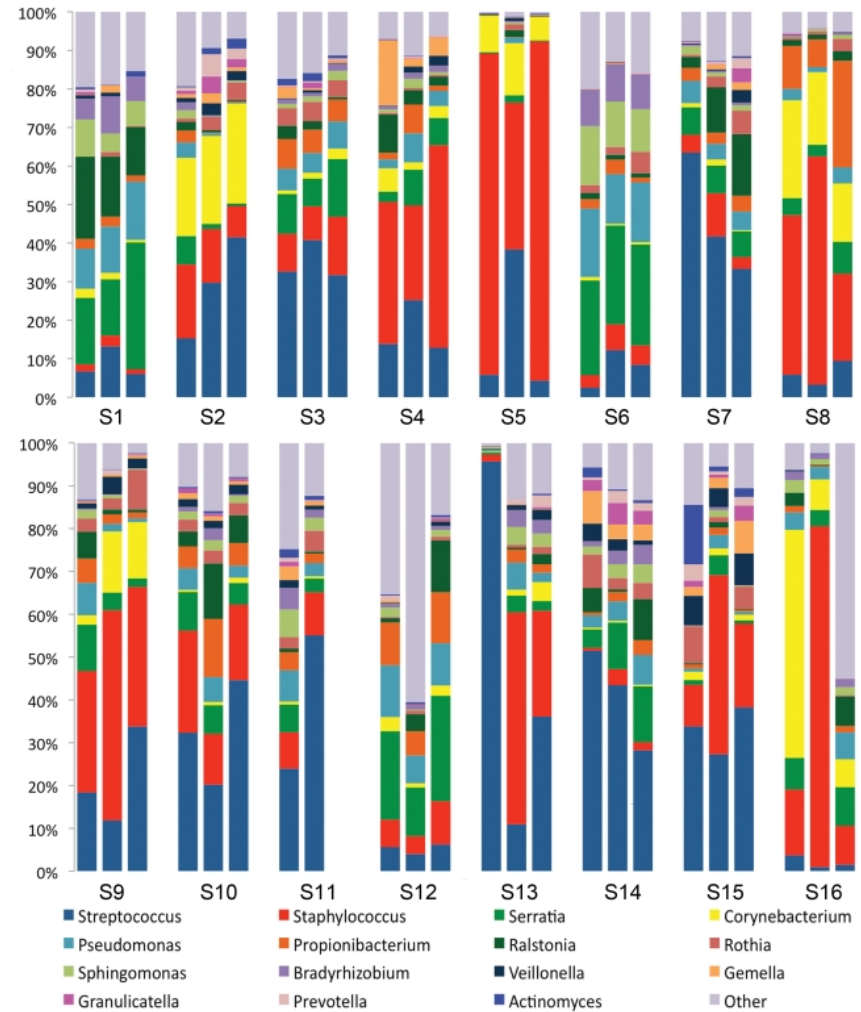
# Breastfeeding

## BREASTMILK: *source of microbes*

Great Variation from mother to mother



Cabrera-Rubio et al. 2011



Hunt et al., 2011

NIH-PA Author Manuscript



## Public Access Author Manuscript

*J Primatol.* Author manuscript; available in PMC 2013 July 01.

Presented in final edited form as:

*J Primatol.* 2011 February ; 40(1): 52–58. doi:10.1111/j.1600-0684.2010.00450.x.

### Species diversity and relative abundance of lactic acid bacteria in the milk of rhesus monkeys (*Macaca mulatta*)

L. Jin<sup>1</sup>, K. Hinde<sup>2,3</sup>, and L. Tao<sup>1</sup>

## Journal of Dairy Research



Article

Supplementary materials

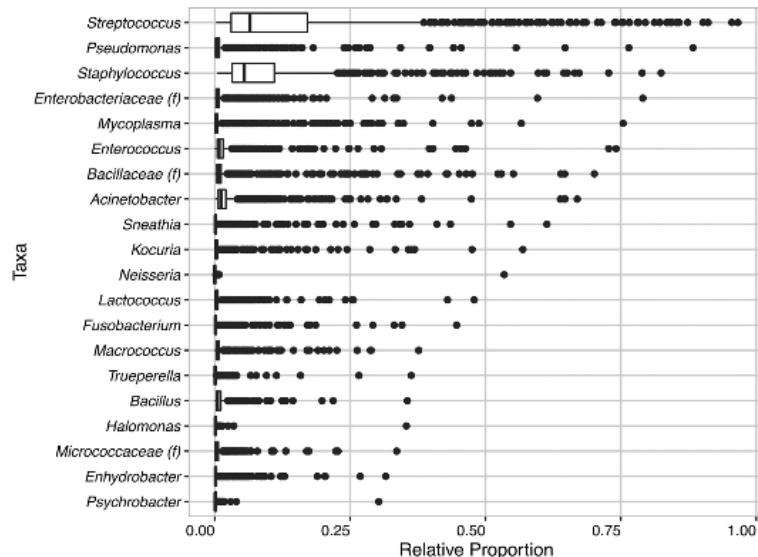
Metrics

Volume 83, Issue 3 August 2016, pp. 383-386

### Isolation and characterisation of lactic acid bacteria from donkey milk

María de los Dolores Soto del Río (a1), Christian Andrighetto (a2), Alessandra Dalmasso (a1), Angiolella Lombardi (a2) ...

DOI: <https://doi.org/10.1017/S0022029916000376> Published online: 07 September 2016



## Food Microbiology

Volume 46, April 2015, Pages 121–131



### Analysis of raw goat milk microbiota: Impact of stage of lactation and lysozyme on microbial diversity

Elizabeth A. McInnis<sup>a</sup>, Karen M. Kalanetra<sup>b</sup>, David A. Mills<sup>b</sup>, Elizabeth A. Maga<sup>a</sup> ...

Show more



RESEARCH ARTICLE



### The Core and Seasonal Microbiota of Raw Bovine Milk in Tanker Trucks and the Impact of Transfer to a Milk Processing Facility

Mary E. Kable<sup>a</sup>, Yanin Srisengfa<sup>a</sup>, Miles Laird<sup>a</sup>, Jose Zaragoza<sup>a</sup>, Jeremy McLeod<sup>b</sup>, Jessie Heidenreich<sup>b</sup>, Maria L. Marco<sup>a</sup>  
 Department of Food Science and Technology, University of California Davis, Davis, California, USA<sup>a</sup>; Hillmar Cheese Company, Hillmar, California, USA<sup>b</sup>





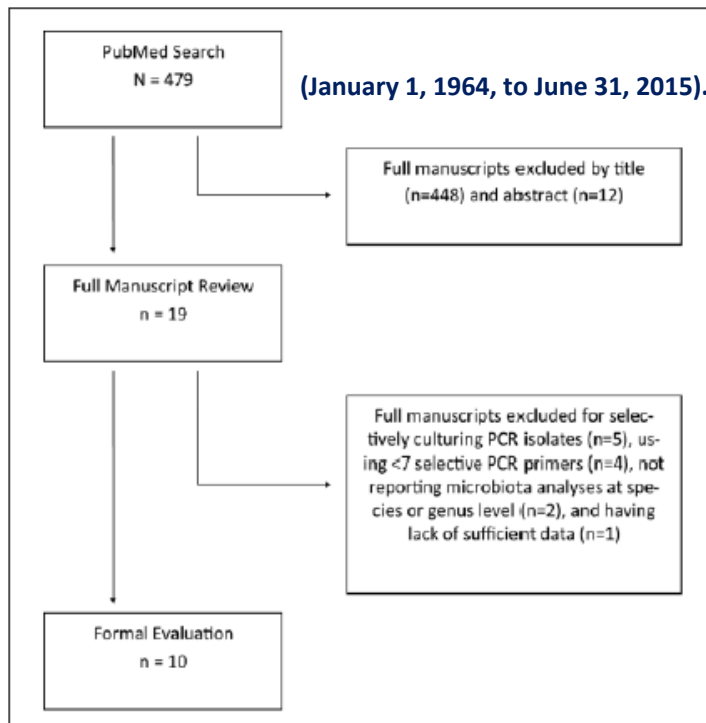
Review

## Systematic Review of the Human Milk Microbiota

John L. Fitzstevens<sup>1</sup>; Kelsey C. Smith, MA<sup>2,3</sup>; James I. Hagadorn, MD<sup>2,3,4</sup>;  
Melissa J. Caimano, PhD<sup>5,6</sup>; Adam P. Matson, MD<sup>3,4,7</sup>;  
and Elizabeth A. Brownell, PhD<sup>2,3,4</sup>



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***Streptococcus* and *Staphylococcus* are the predominant genera in the human milk microbiota.**

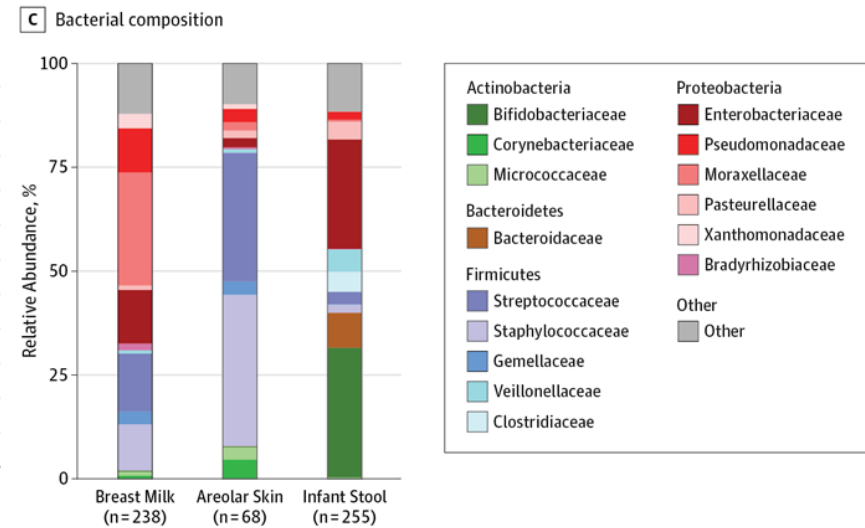
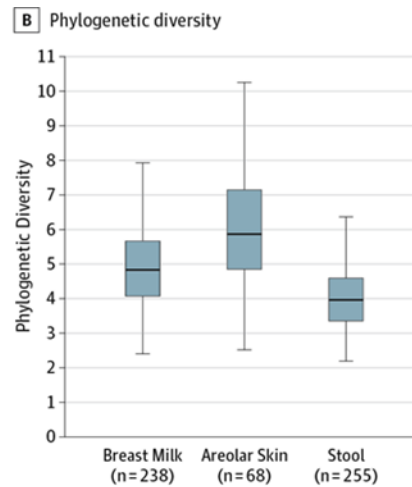
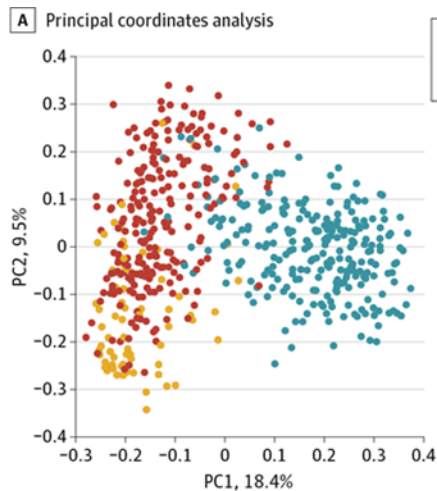
These 2 genera may be universally predominant in the human milk microbiota, independent of geographic location or milk collection technique and may have been underestimated in previous work using conventional PCR methods.

JAMA Pediatrics | Original Investigation

# Association Between Breast Milk Bacterial Communities and Establishment and Development of the Infant Gut Microbiome

May 8, 2017

Pia S. Pannaraj, MD, MPH; Fan Li, PhD; Chiara Cerini, MD; Jeffrey M. Bender, MD; Shangxin Yang, PhD; Adrienne Rollie, MS; Helty Adisetiyo, PhD; Sara Zabih, MS; Pamela J. Lincez, PhD; Kyle Bittinger, PhD; Aubrey Bailey, MS; Frederic D. Bushman, PhD; John W. Sleasman, MD; Grace M. Aldrovandi, MD



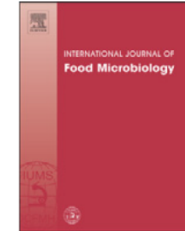
Breastfed infants received 27.7% of their gut bacteria from breast milk and 10.4% from areolar skin during the first month of life



Contents lists available at [SciVerse ScienceDirect](https://www.sciencedirect.com)

## International Journal of Food Microbiology

journal homepage: [www.elsevier.com/locate/ijfoodmicro](http://www.elsevier.com/locate/ijfoodmicro)



Short communication

### Fungal diversity in cow, goat and ewe milk

Emilie Delavenne, Jerome Mounier, Katia Asmani, Jean-Luc Jany, Georges Barbier, Gwenaelle Le Blay\*

Université Européenne de Bretagne, France

Université de Brest, EA3882 Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, IFR148 ScInBioS, ESMISAB, Technopôle de Brest Iroise, 29280 Plouzané, France



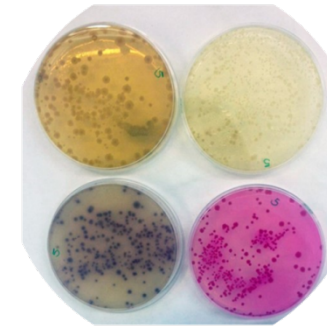
Fungal species in dairy animals' milk: *Candida*, *Cryptococcus*, *Debaryomyces*, *Malassezia*, *Pichia*, *Rhodotorula*, *Aspergillus*, etc.

# SCIENTIFIC REPORTS

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

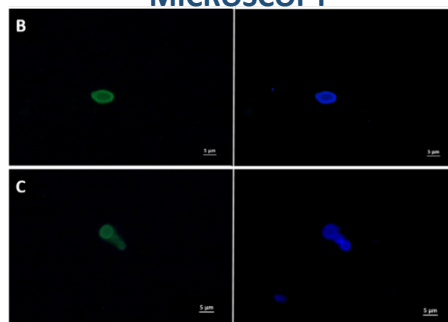
Received: 10 May 2017  
 Accepted: 21 September 2017  
 Published online: 12 October 2017

Alba Boix-Amorós<sup>1,2</sup>, Cecilia Martínez-Costa<sup>3</sup>, Amparo Querol<sup>1</sup>, María Carmen Collado<sup>1</sup> & Alex Mira<sup>2</sup>



Alba Boix-Amorós

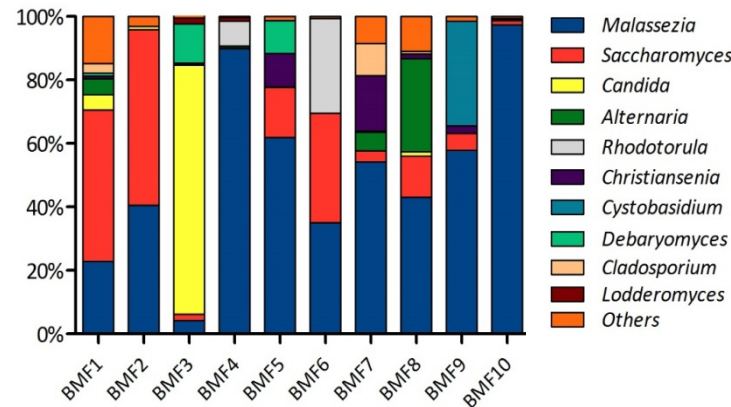
### FISH HYBRIDIZATION AND MICROSCOPY



FISH probe specific for 18S rRNA gene

Calcofluor White Stain

### 28S rRNA gene

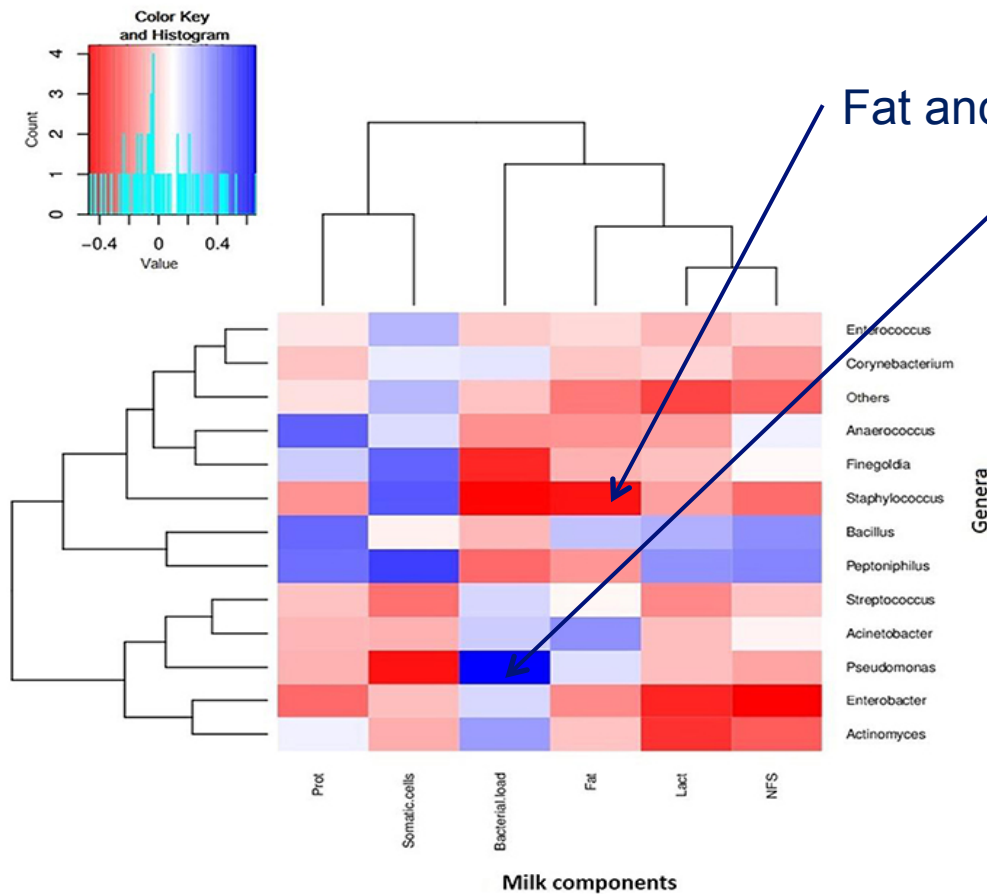


Species	%	Prevalence
<b>Malassezia globosa</b>	34,63	6/10
<b>Candida sp. HA1671</b>	19,54	3/10
<b>Saccharomyces cerevisiae</b>	14,56	7/10
<b>Malassezia restricta</b>	9,07	10/10
<b>Alternaria arborescens</b>	4,82	4/10
<b>uncultured Candida</b>	3,48	2/10
<b>Cladosporium bruhnei</b>	3,28	1/10
<b>Alternaria sp. NT-2015a</b>	2,67	5/10
<b>Candida sake</b>	1,93	2/10
<b>Alternaria tenuissima</b>	1,57	5/10
<b>Debaryomyces hansenii</b>	1,24	3/10
<b>Cystobasidium sp. CBS7295</b>	1,20	1/10
<b>uncultured Debaryomyces</b>	0,63	3/10

# Factors influencing Milk Microbiome

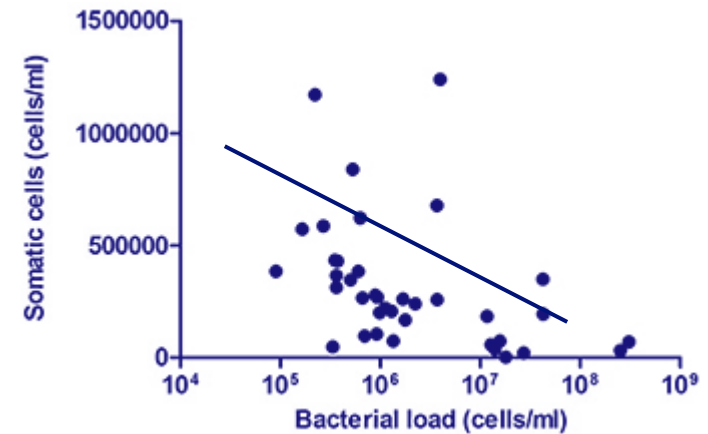
## Milk microbiome is associated to macronutrients profile in breast milk ?

Relationships between bacterial composition and nutritional or cellular content of HM



Fat and *Staphylococcus*

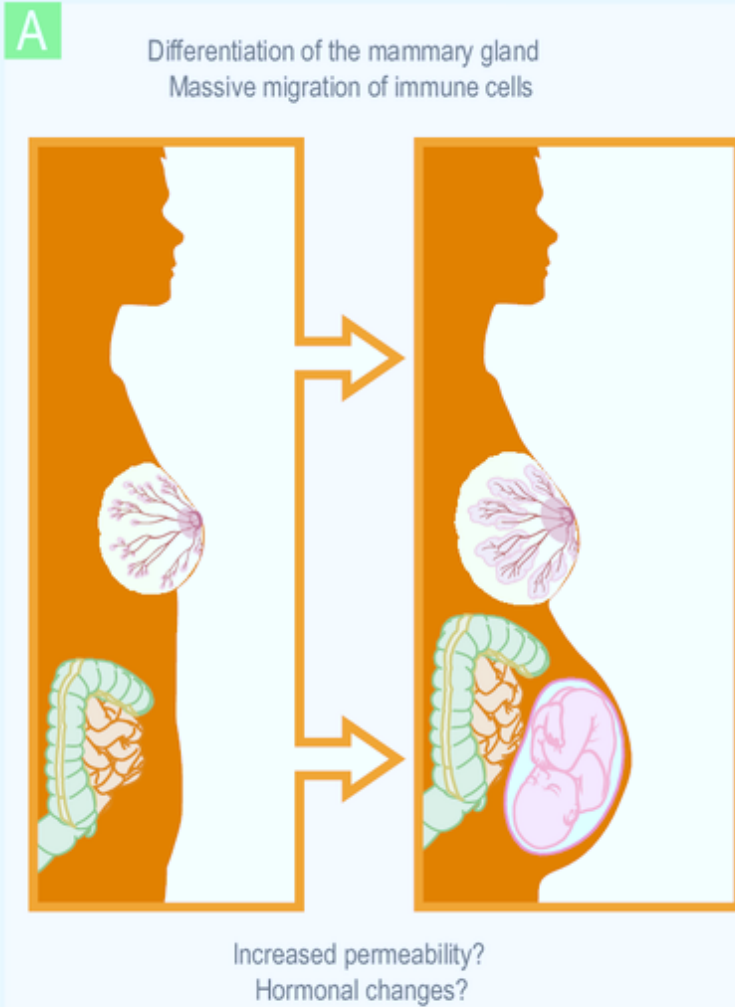
Bacterial load and *Pseudomonas*



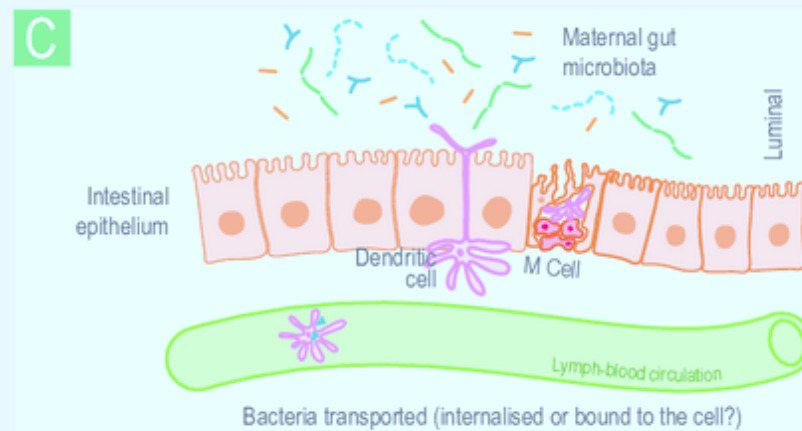
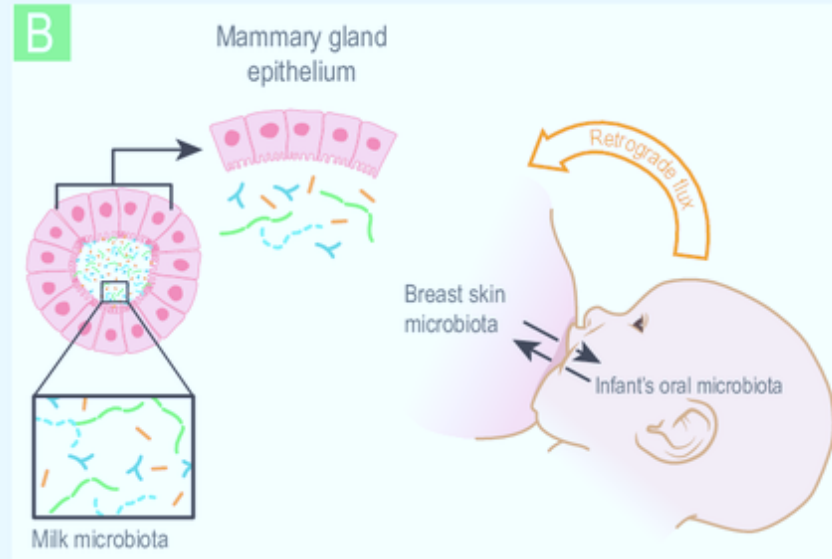
**Breast milk microbiota:  
where does it come from?**

# Origin and Source of breast milk microbes

## Physiological changes during pregnancy



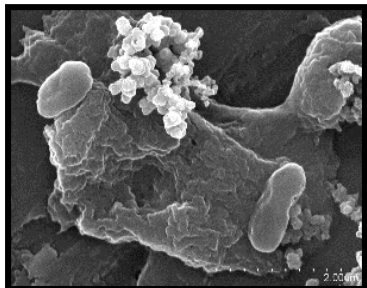
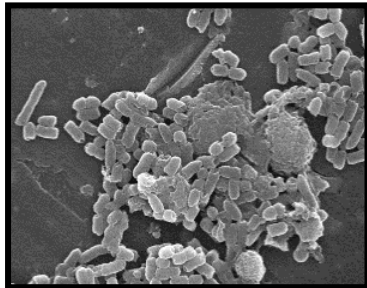
## Potential routes



**Factors influencing  
Breast milk composition**



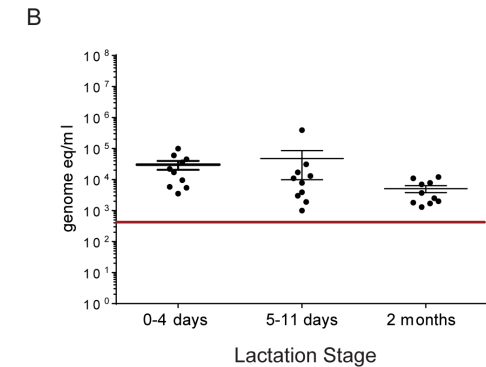
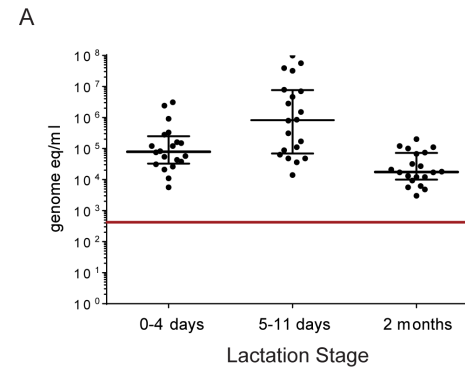
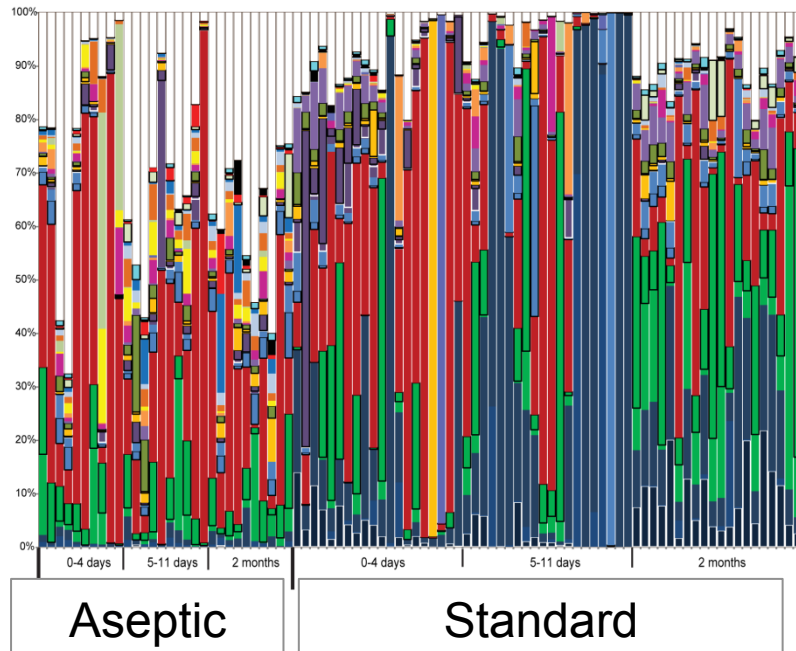
FACTORS AFFECTING HUMAN BREAST MILK COMPOSITION



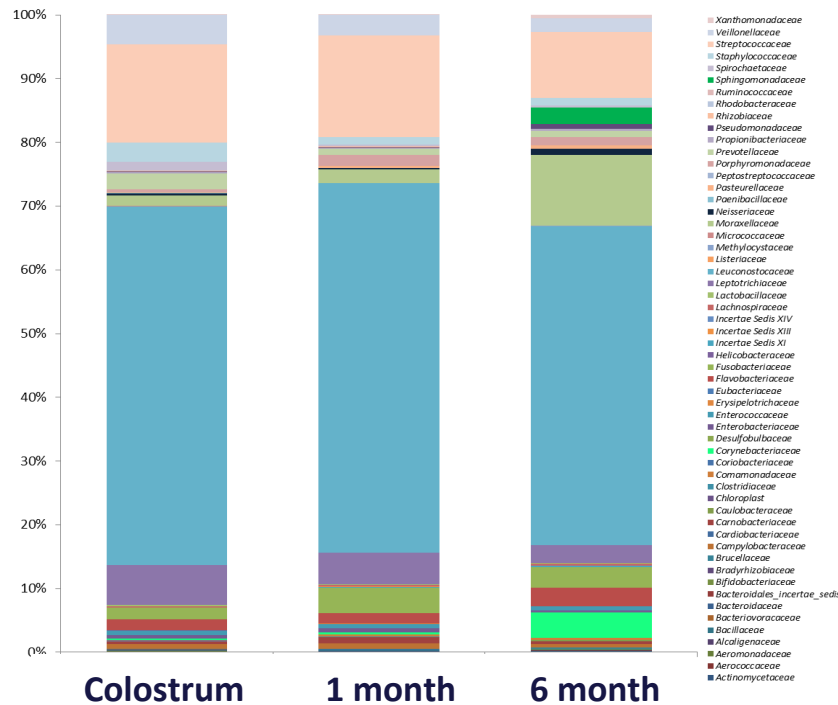
# Factors influencing Milk Microbiome

## Milk microbiome is influenced by sampling protocol?

*Staphylococcus* and *Streptococcus* spp. were equally abundant in both sample-type, ↑*Acinetobacter* sp. in standard protocol, but not in strict aseptic protocol



Milk microbiome is influenced by lactation stage

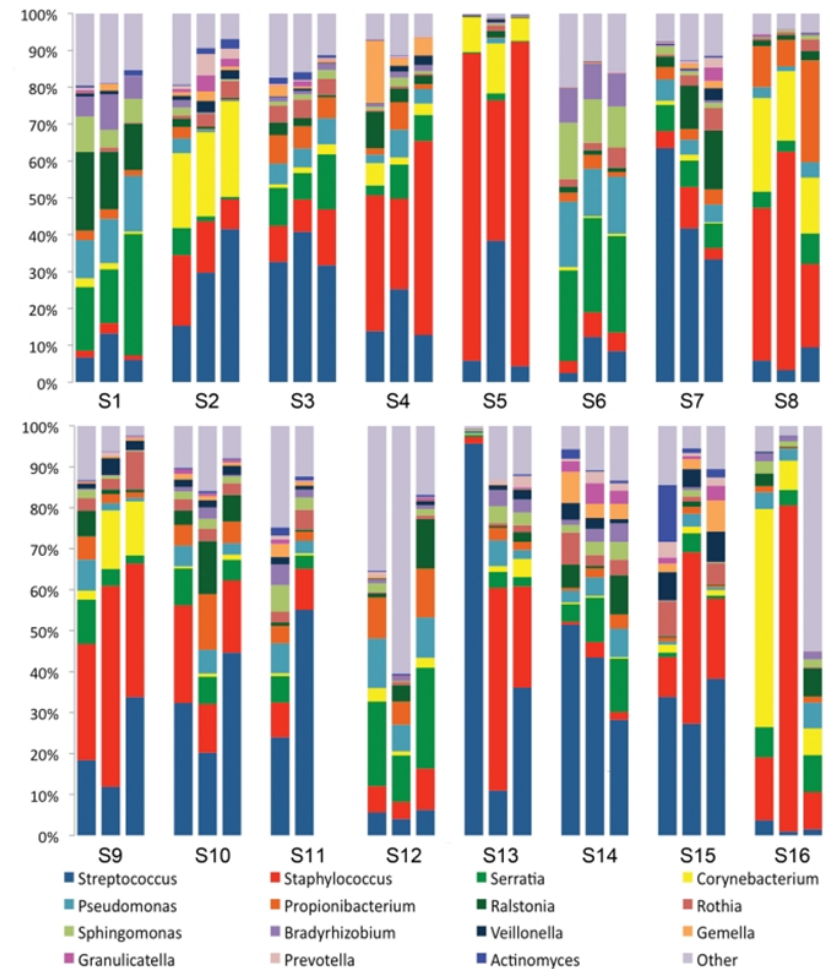


**Colostrum:** *Weisella* y *Leuconostoc* (*Lactobacillales*)

*Staphylococcus*, *Streptococcus* and *Lactococcus*.

**Mature milk:** Lactic Acid bacteria but increase of oral

bacteria as *Veillonella*, *Leptotrichia*, *Prevotella*.



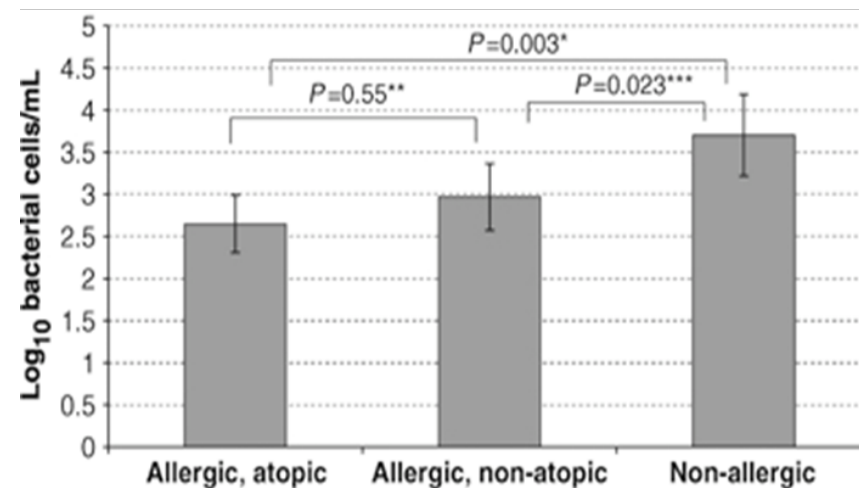
Hunt et al., 2011

## Maternal Allergy influence milk and infant microbiota composition

- ↓ *Bifidobacterium* group numbers in milk samples of allergic mothers than non-allergic mothers

- 61 mother-infant pairs
- Breast milk samples 1 month
- All infants colonized with bifidobacteria, exception;
  - 1 born by CS
  - 1 having received antibiotics

Breast milk *Bifidobacterium* levels



Gronlund et al., 2007

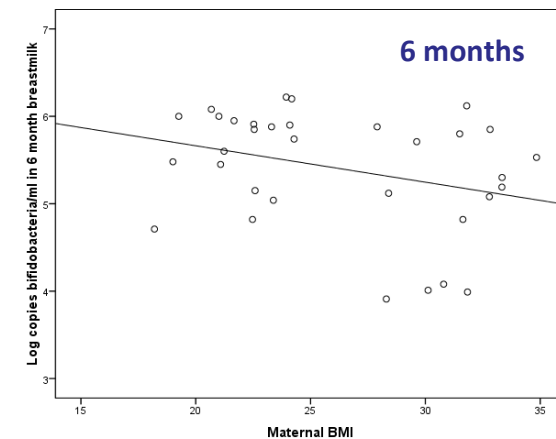
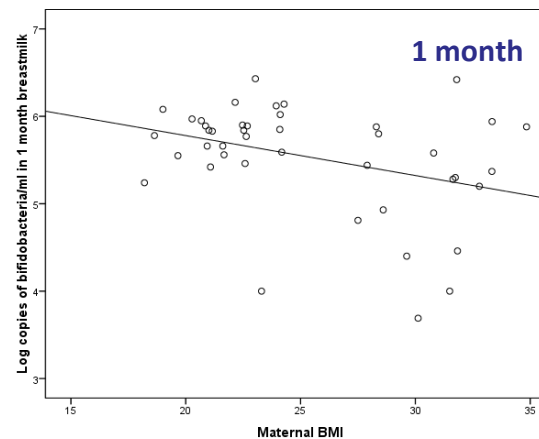
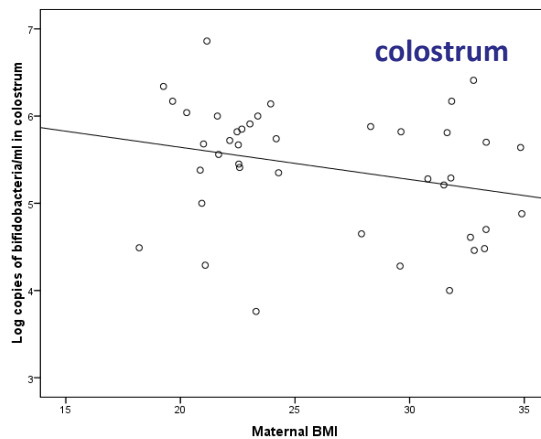
- Maternal allergy and gestational exposures can alter the concentration of Type-1/Type-2/T-regulatory markers in breast milk

Bursch et al., 2013



## Maternal obesity influence milk and infant microbiota composition

- ↓ *Bifidobacterium* group & ↑ *Staphylococcus* group numbers in milk samples of **obese mothers** than normal-weight mothers



- **TGF- $\beta$ 2** and **sCD14** levels in the breast milk of overweight mothers tended to be lower than the levels in that of normal-weight mothers

*Collado et al., 2012*

- Infants born to obese mothers were exposed to 2-fold ↑ **HM insulin** and **leptin** ( $P < 0.01$ ) and showed a significant reduction in the early pioneering bacteria Gammaproteobacteria

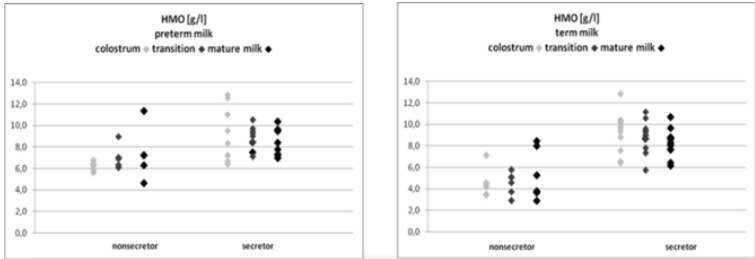
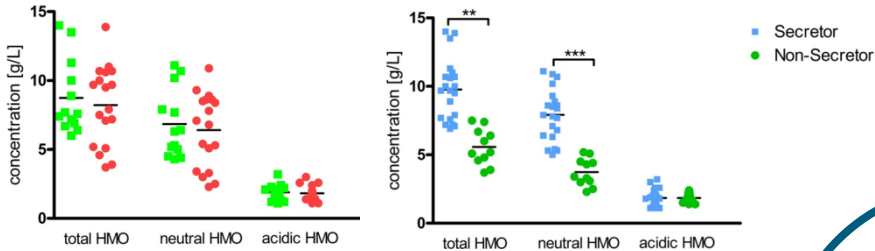
*Lemas et al., 2016 AJCN*



# Breast Milk Bioactive compounds

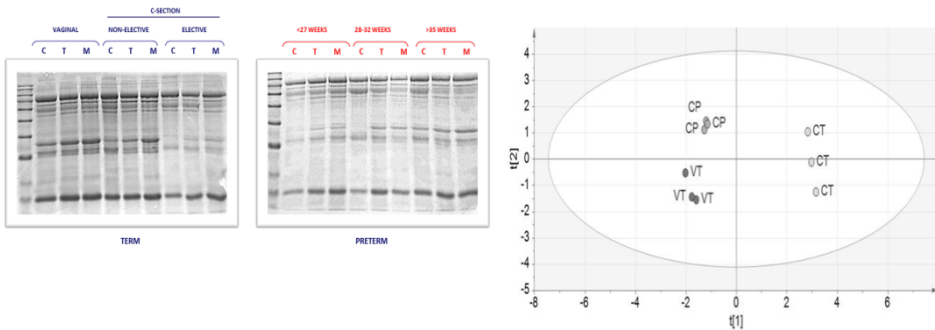
## Human Milk Oligosaccharides

Hypothesis: HMO specifically interact with milk microbes



## Protein & Peptides

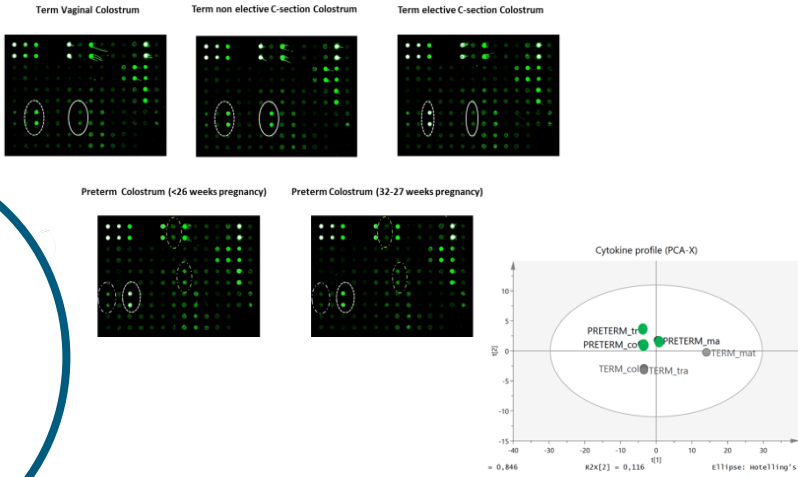
Multi-level approach : protein-peptide strategies followed by state-of-the-art Mass Spectrometry-based methodologies



## Growth Factors, Cytokine & other

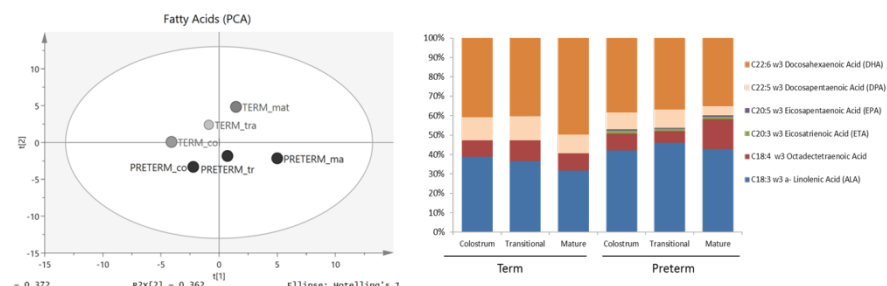
Complex interactions of cytokines and microbiota in breast milk

Collado et al., 2012



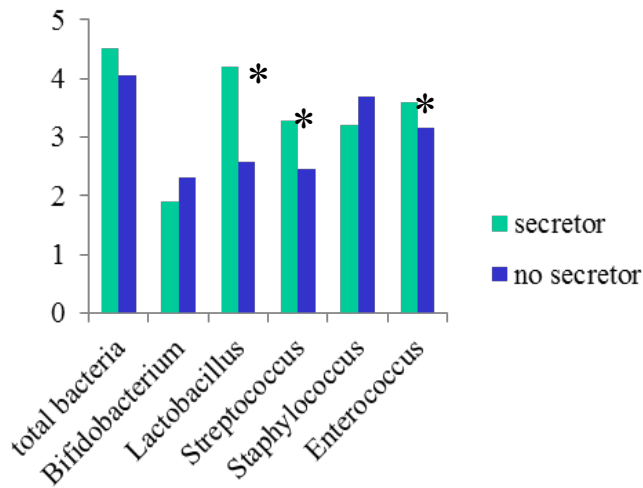
## Fatty ACids

Omega fatty acids measured gas chromatography (GC) Fatty acids have been shown to be modified by time and diet

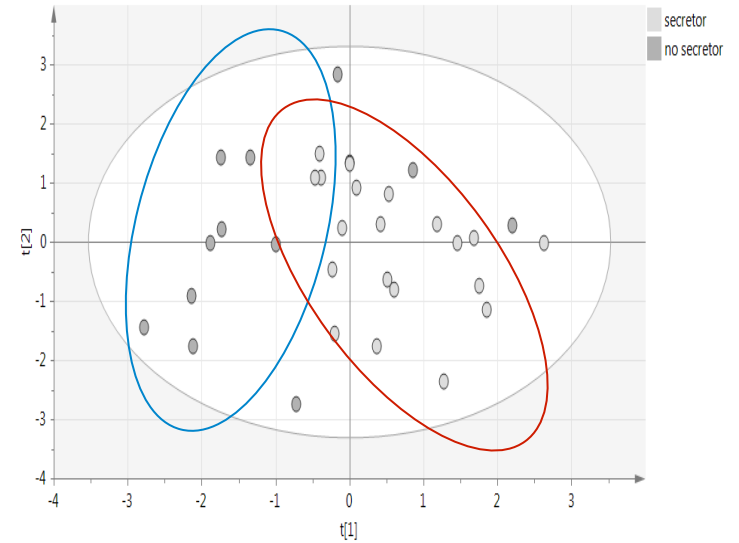
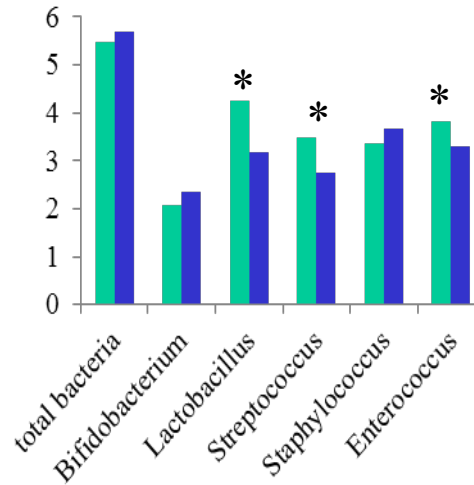


Milk microbiome is influenced by maternal genotype?

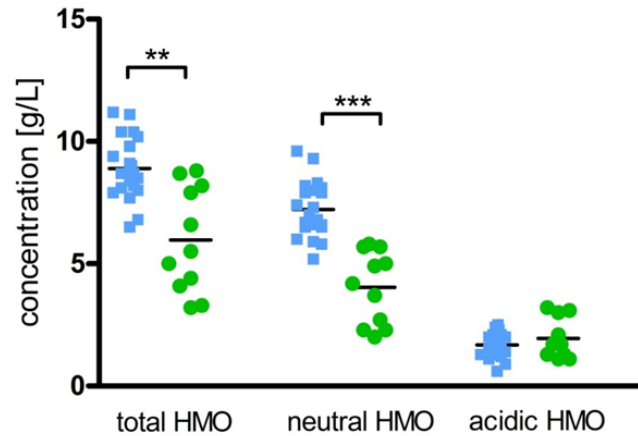
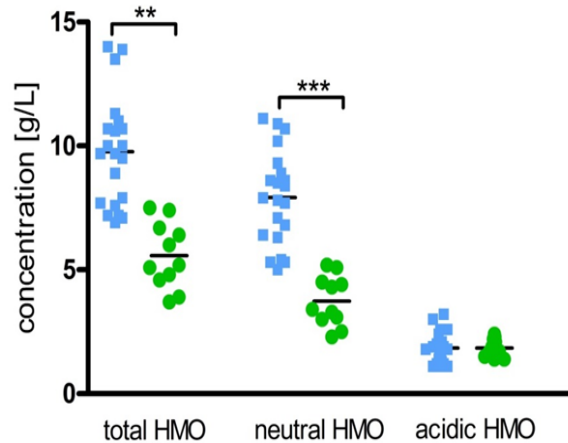
Colostrum



Mature milk



Cabrera-Rubio et al. 2019 JPGN



Kunz et al. 2016 JPGN

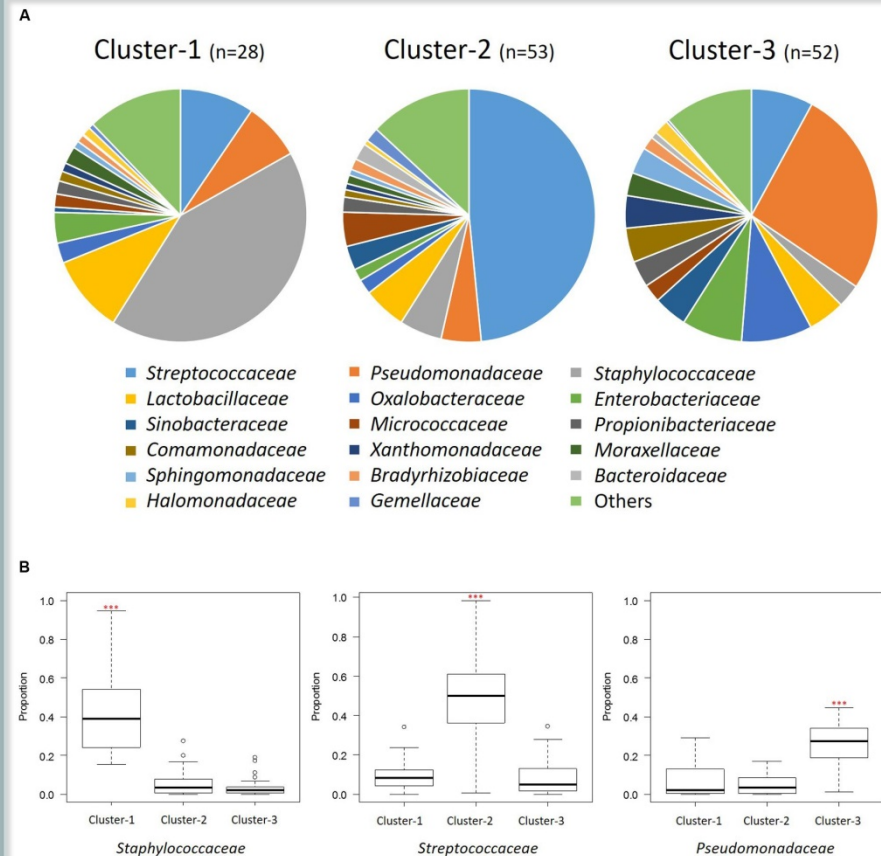
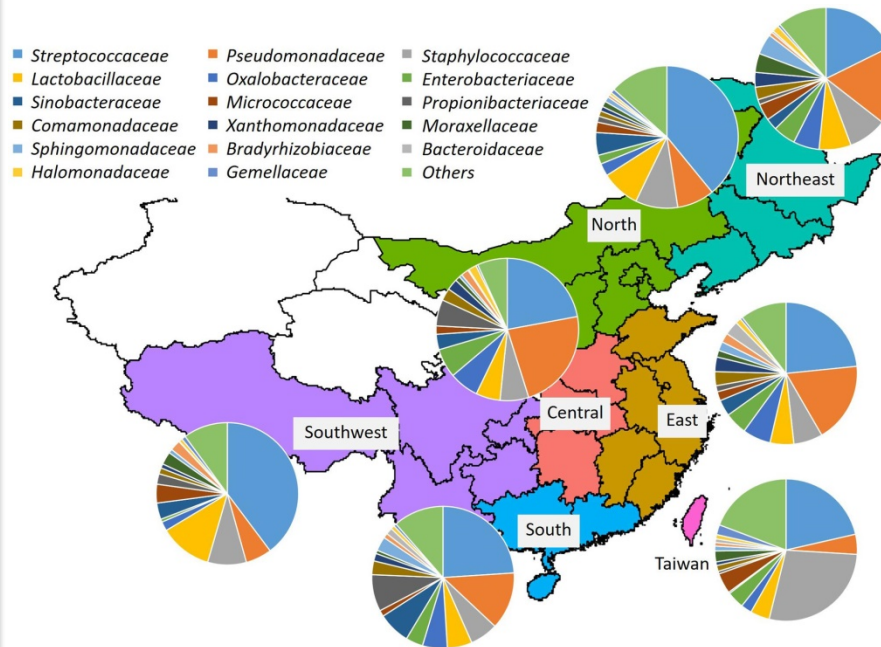




# Factors influencing Milk Microbiome

## Milk microbiome is influenced by geographical location or diet?

133 healthy mothers in Taiwan and in six regions of mainland China (Central, East, North, Northeast, South, and Southwest China)





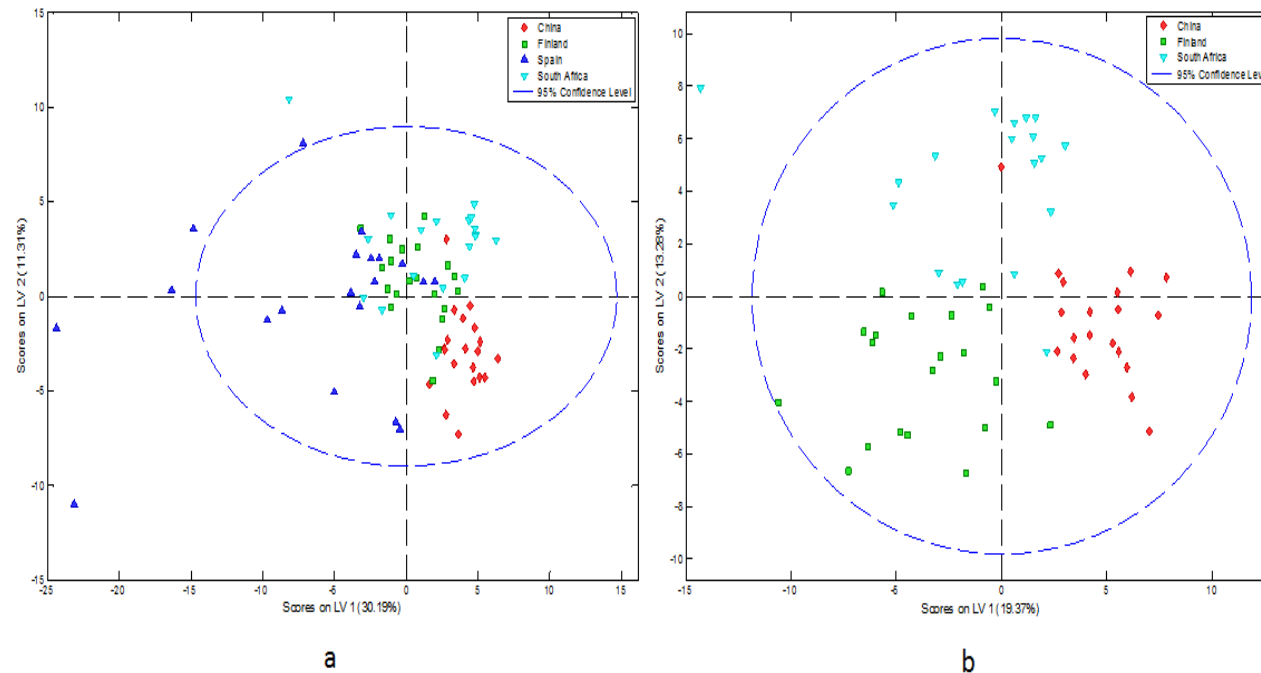
## HUMAN BREAST MILK METABOLOMIC PROFILE DIFFERS ACROSS COUNTRIES



Article

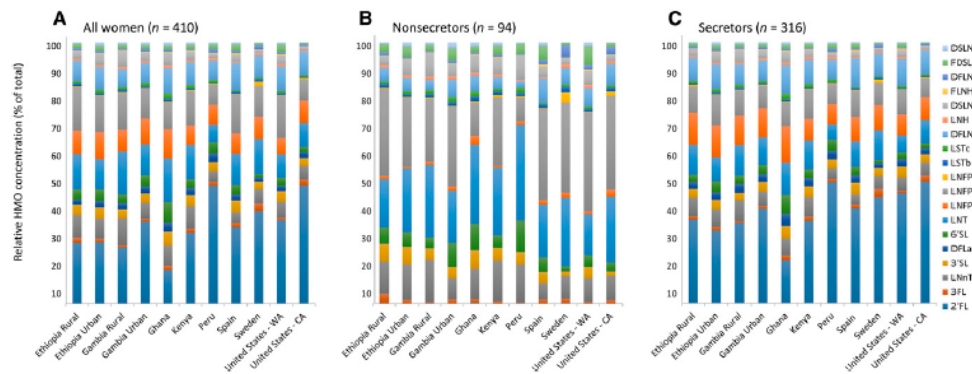
### Human Breast Milk NMR Metabolomic Profile across Specific Geographical Locations and Its Association with the Milk Microbiota

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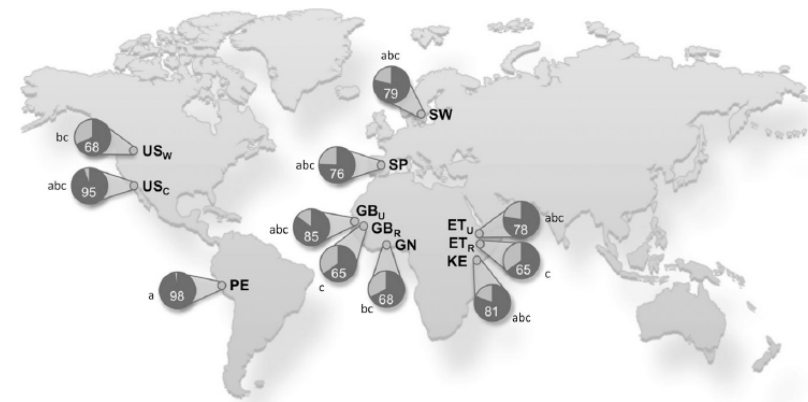


## What's normal? Oligosaccharide concentrations and profiles in milk produced by healthy women vary geographically<sup>1,2</sup>

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**FIGURE 2** Mean  $\pm$  SEM relative abundance of HMO concentrations of all women combined (A), nonsecretors (B), and secretors (C) in each cohort. Note that there was only one nonsecretor subject each in Peru and United States - CA. CA, California; DFLac, difucosyllactose; DFLNH, difucosyllacto-*N*-hexaose; DFLNT, difucosyllacto-*N*-tetraose; DSLNH, disialyllacto-*N*-hexaose; DSLNT, disialyllacto-*N*-tetraose; FDSLNH, fucodisialyllacto-*N*-hexaose; FLNH, fucosyllacto-*N*-hexaose; HMO, human milk oligosaccharide; LNFP, lacto-*N*-fucopentaose; LNH, lacto-*N*-hexaose; LNnT, lacto-*N*-neotetraose; LNT, lacto-*N*-tetraose; LSTb, sialyl-lacto-*N*-tetraose b; LSTc, sialyl-lacto-*N*-tetraose c; WA, Washington; 2'FL, 2'-fucosyllactose; 3'FL, 3'-fucosyllactose; 3'SL, 3'-sialyllactose; 6'SL, 6'-sialyllactose.



**FIGURE 3** Percentages of women in each cohort categorized as secretors. Cohorts that do not share a common lowercase letter differ ( $P < 0.05$ ) in terms of their percentages of women who were secretors with the use of a chi-square test with Benjamini and Hochberg false-discovery-rate corrections. ET<sub>u</sub>, rural Ethiopia; ET<sub>r</sub>, urban Ethiopia; GB<sub>u</sub>, rural Gambia; GB<sub>r</sub>, urban Gambia; GN, Ghana; KE, Kenya; PE, Peru; SP, Spain; SW, Sweden; US<sub>c</sub>, United States-California (Hispanic); US<sub>w</sub>, United States-Washington.



Article

## Colostrum and Mature Human Milk of Women from London, Moscow, and Verona: Determinants of Immune Composition

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## What's Normal? Immune Profiling of Human Milk from Healthy Women Living in Different Geographical and Socioeconomic Settings

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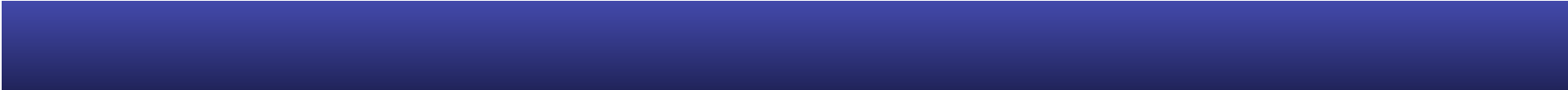
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**To increase our understanding of the role of maternal bacteria in infant microbiota as it will help to develop new dietary strategies based on microbial modulation, aimed at the beneficial microbiological, immunological and metabolic programming of child health.**

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