The Chinese University of Hong Kong
Faculty of Medicine
Department of Microbiology
Joint Graduate Student Seminar 2016

Assembly of Human Gut Microbiota During Early Life

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◦ Human gut bacteria microbiota

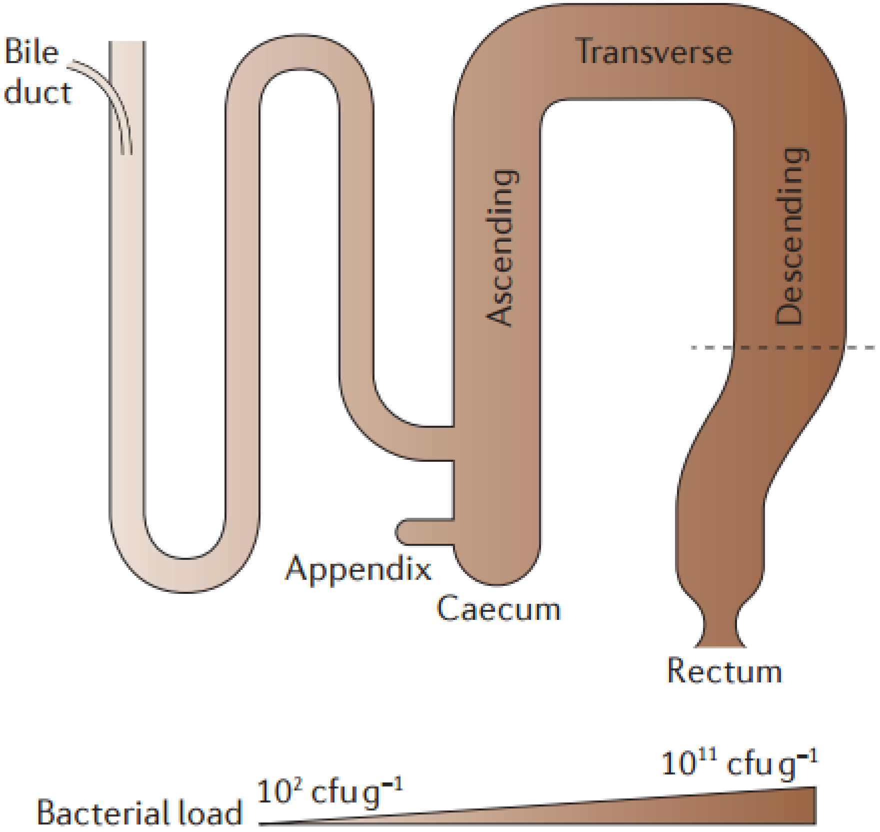


Figure 1. Donaldson et al., 2016

Examples of functions:

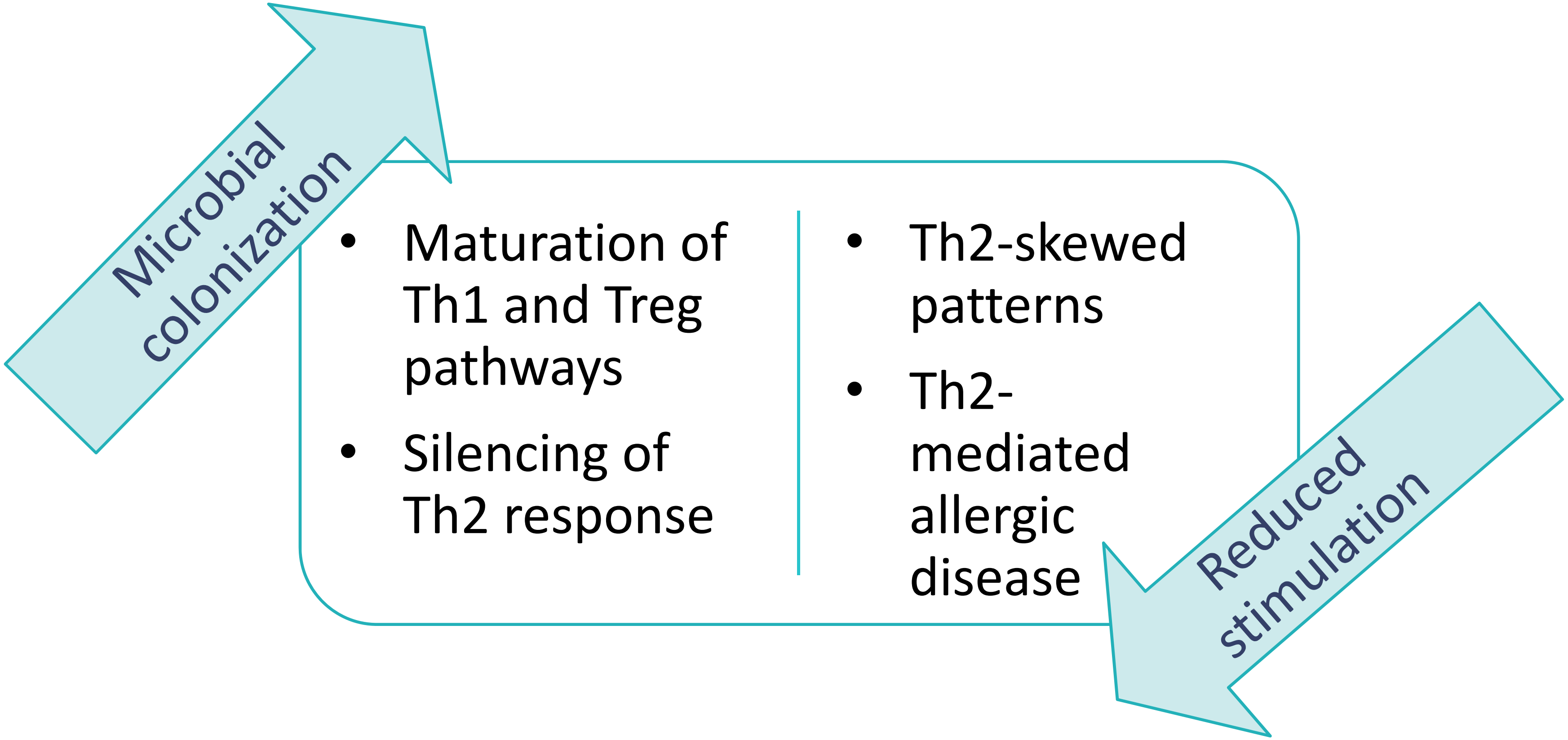
- SCFA production
- Vitamin synthesis
- Inhibition of pathogens

Dysbiosis associates with

- Inflammatory bowel disease
- Obesity
- Cancer

(Flint et al., 2012; Donaldson et al., 2016)

- Gut microbiota in early life influences…
- Immune system development



Microbial colonization

- Maturation of Th1 and Treg pathways
- Silencing of Th2 response

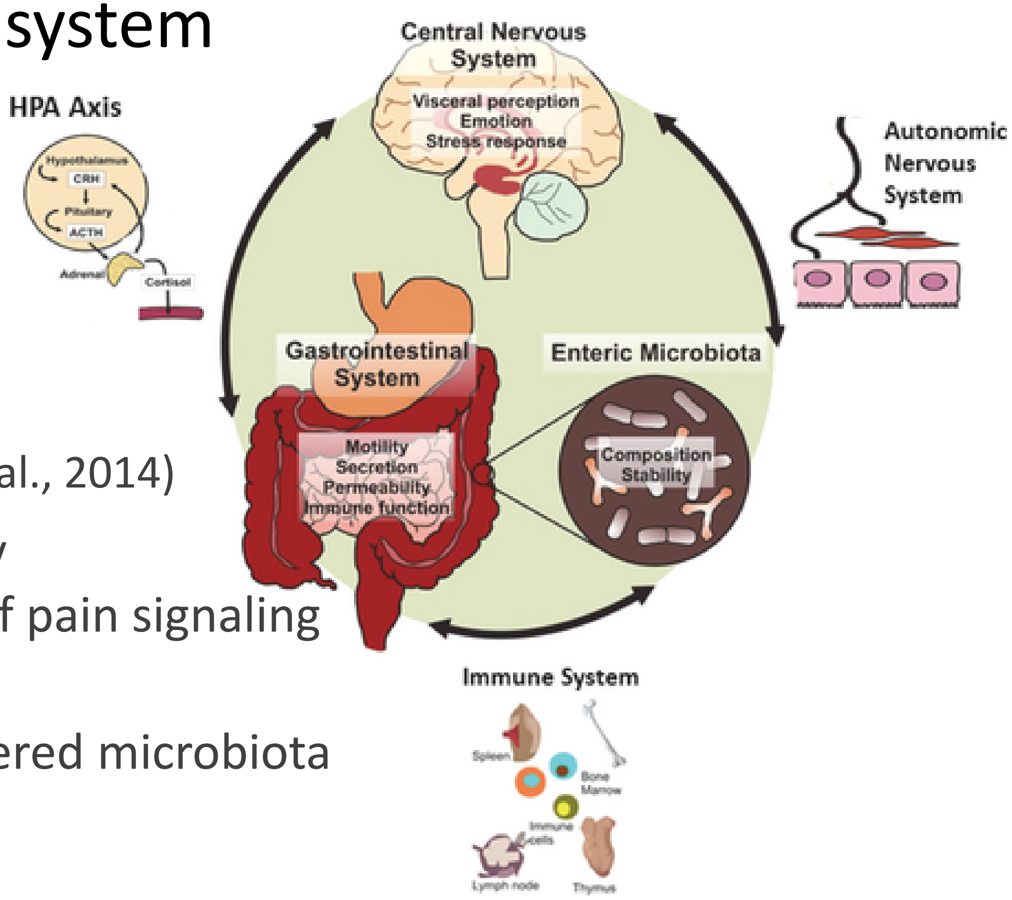
- Th2-skewed patterns
- Th2-mediated allergic disease

Reduced stimulation

- Low microbiota diversity in early infancy is related to asthma development (Abrahamsson et al., 2014)

(Jenmalm, 2011; West et al., 2015)

- Gut microbiota in early life influences…
- Behavior and Nervous system



- Microbiome-gut-brain axis
  (Cryan and O'Mahony, 2011)

- Studies on germ free mice:
  - Lack of microbiota
    → social deficits (Desbonnet et al., 2014)
  - Balanced microbial community
    → appropriate development of pain signaling (Amaral et al., 2008)
- Childhood Diseases linked to altered microbiota
  (O' Mahony et al., 2015)
  - Autism
  - Depression and anxiety

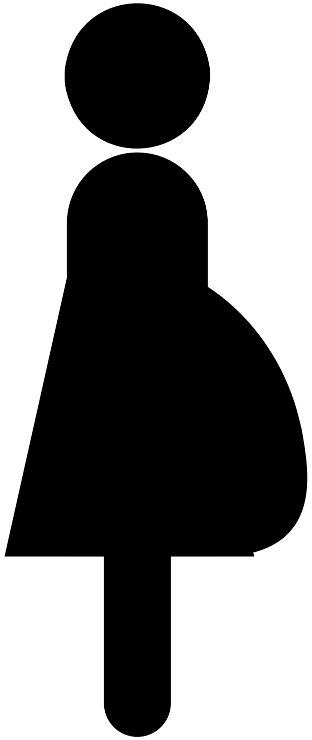
Figure 1. Clarke et al., 2014

Timeline of Assembly

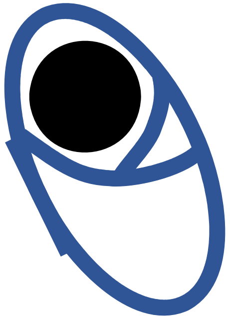
In utero

Birth

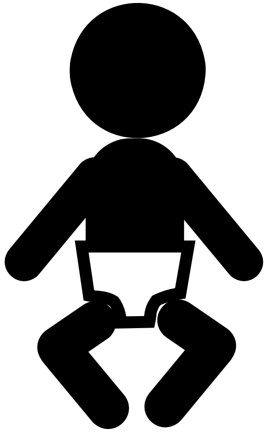
Postpartum



- Mode of delivery



- Intestine environment change
- Infant diet
- Secretory immunoglobulin A



- First question: When does colonization starts?

Widely accepted idea:

- Foetuses are sterile in utero
- Presence of any bacteria: Potential threat

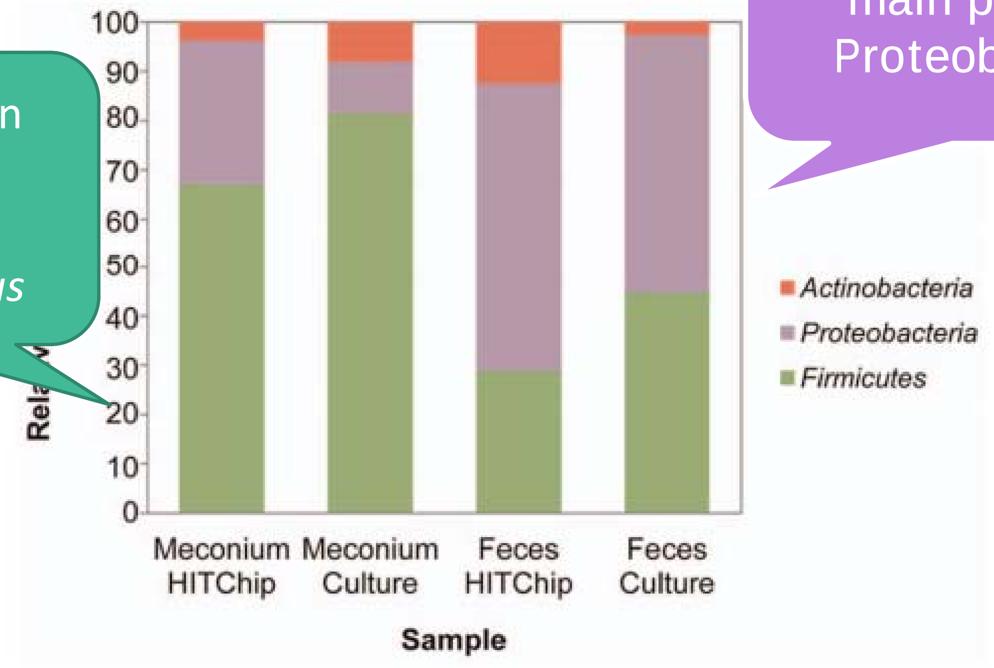
Therefore,

colonization should start on birth?

Not the case!

Meconium

- Not sterile (Jiménez et al., 2008)
- Specific microbiota
  - Complex microbial community
  - Differs from early fecal samples (Moles et al., 20



Fecal samples' main phylum: Proteobacteria

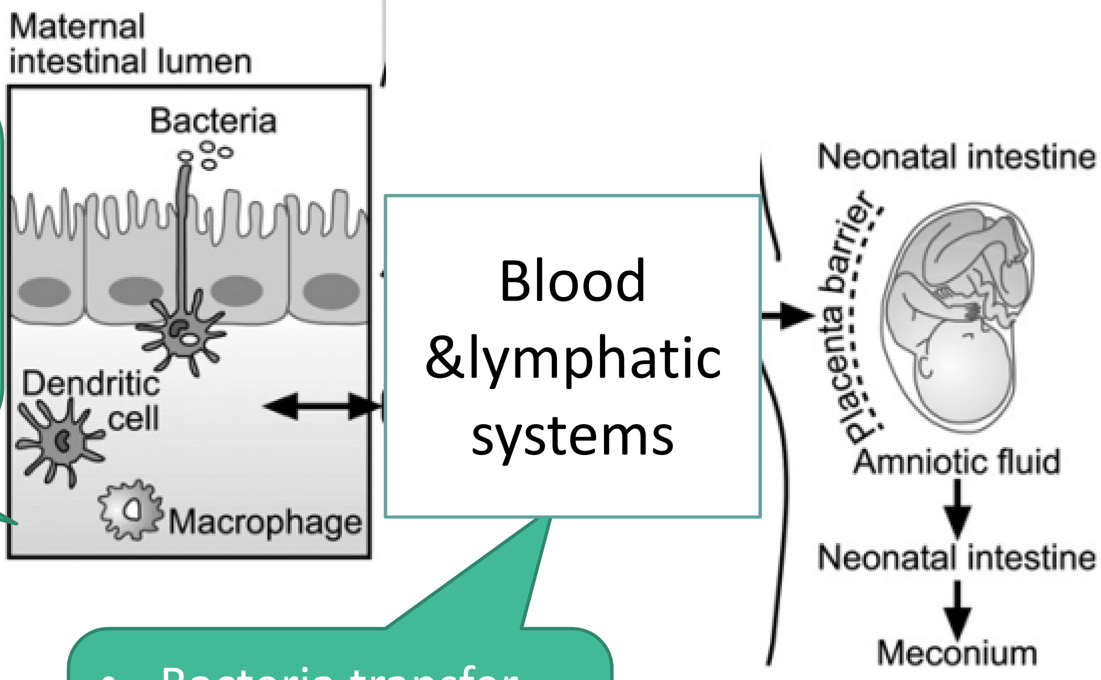
Meconium's main phylum: Firmicutes e.g. *Streptococcus*

Figure 6. Moles et al., 2013

How do bacteria get there?

- ◦ Mechanisms: not well understood
- ◦ Gut epithelial translocation suggested

- Microfold cells and dendritic cells
- Actively transfer bacteria across intestinal epithelial barrier



- Bacteria transfer through blood circulation

Figure 1. Thum et al., 2012

(Rescigno et al., 2001; Perez et al., 2007; Thum et al., 2012

- Studies by Jime´nez et al., 2005 and 2008

Pregnant mice

- Fed with genetically labeled *Enterococcus faecium*

Offspring

- Obtained by sterile Caesarean section

Labelled bacteria

- Found in
  - Amniotic fluid
  - Meconium of offspring

Birth

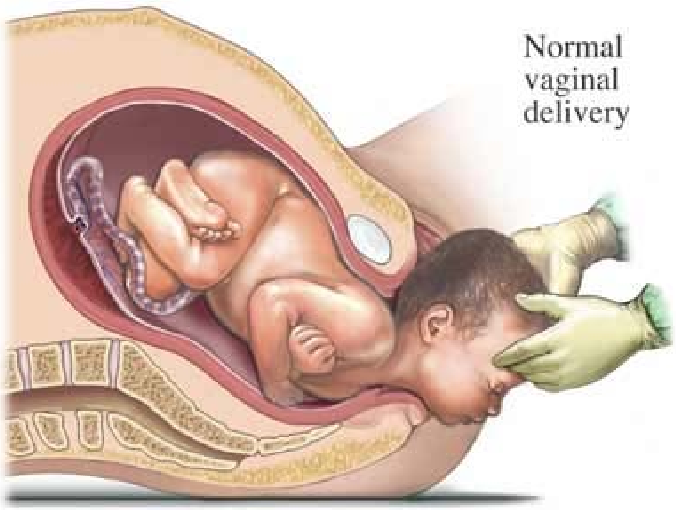
- Delivery progress:

Newborn’s first postnatal microbial exposure

Vaginally delivery

Newborn

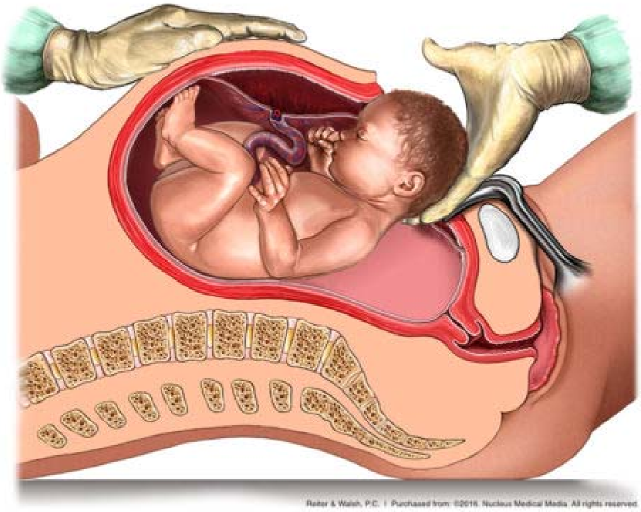
- passages through mother's vagina
- coated with maternal vaginal bacteria



http://www.womens-health-advice.com/assets/images/photos/childbirth-vaginal.jpg

Caesarean section

- Directly removed from uterus
- Not passages through vagina



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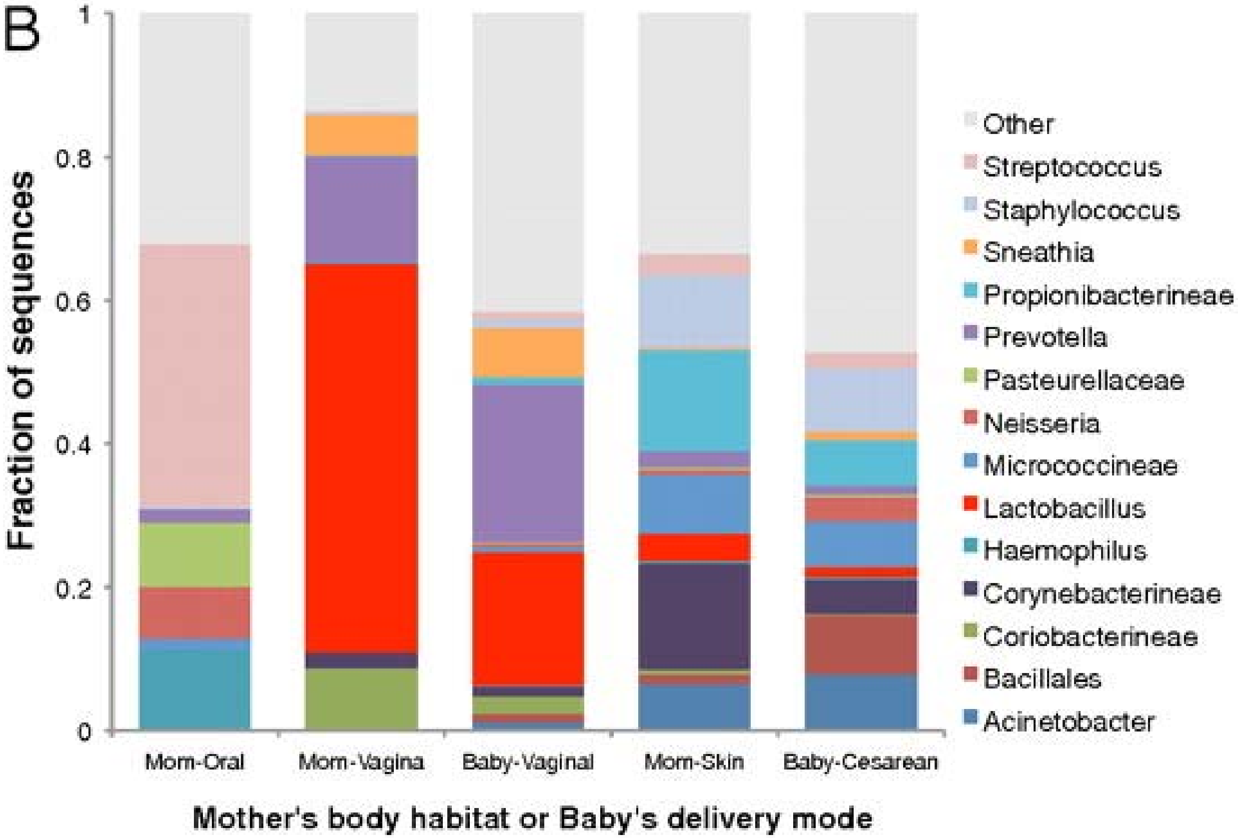
https://www.abclawcenters.com/wp-content/uploads/2016/08/C-Section-560x410.jpg

- Study by Dominguez-Bello et al., 2010
  - Composition of bacteria community of newborns
    - Skin, oral mucosa, and nasopharyngeal aspirate (<5min)
    - Meconium <24 h

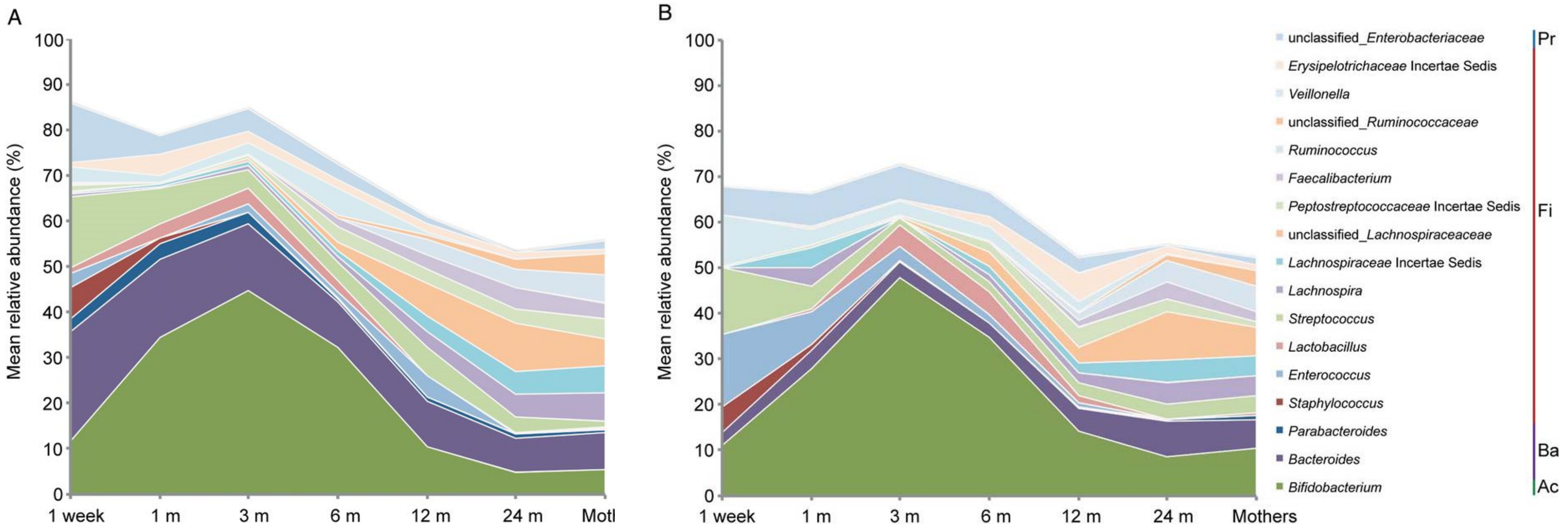
- Vaginally delivery:
  - Resembled mother's vaginal microbiota
- *Lactobacillus*, *Prevotella*, or *Sneathia* spp.

- C-section:
  - Similar to mother's skin surface
- *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp.



(Figure 1B. Dominguez-Bello et al., 2010)

- Mode of delivery influences infant gut microbiota
- Study by Jakobsson et al., 2013
  - Composition of gut microbiota of infants



Vaginally delivery

Caesarean section

Higher abundance of *Bacteroides* (1-12m)

Higher abundance of *Enterococcus* (at 1 mo)

(Figure 2. Jakobsson et al., 2013)

- Mode of delivery influences infant gut microbiota
- Study by Jakobsson et al., 2013
  - C-section born infants
    - lower diversity of
      - Total microbiota
      - Bacteroidetes phylum
    - Reduced Th1 response

Postnatal

Intestine environment change,

Pre-weaning infant diet, Passive Secretory immunoglobulin A,

Weaning and introduction of solid food

- Intestine environment change
  - Oxygen content in newborn intestine
    - High just after birth
    - Decreases over time after birth

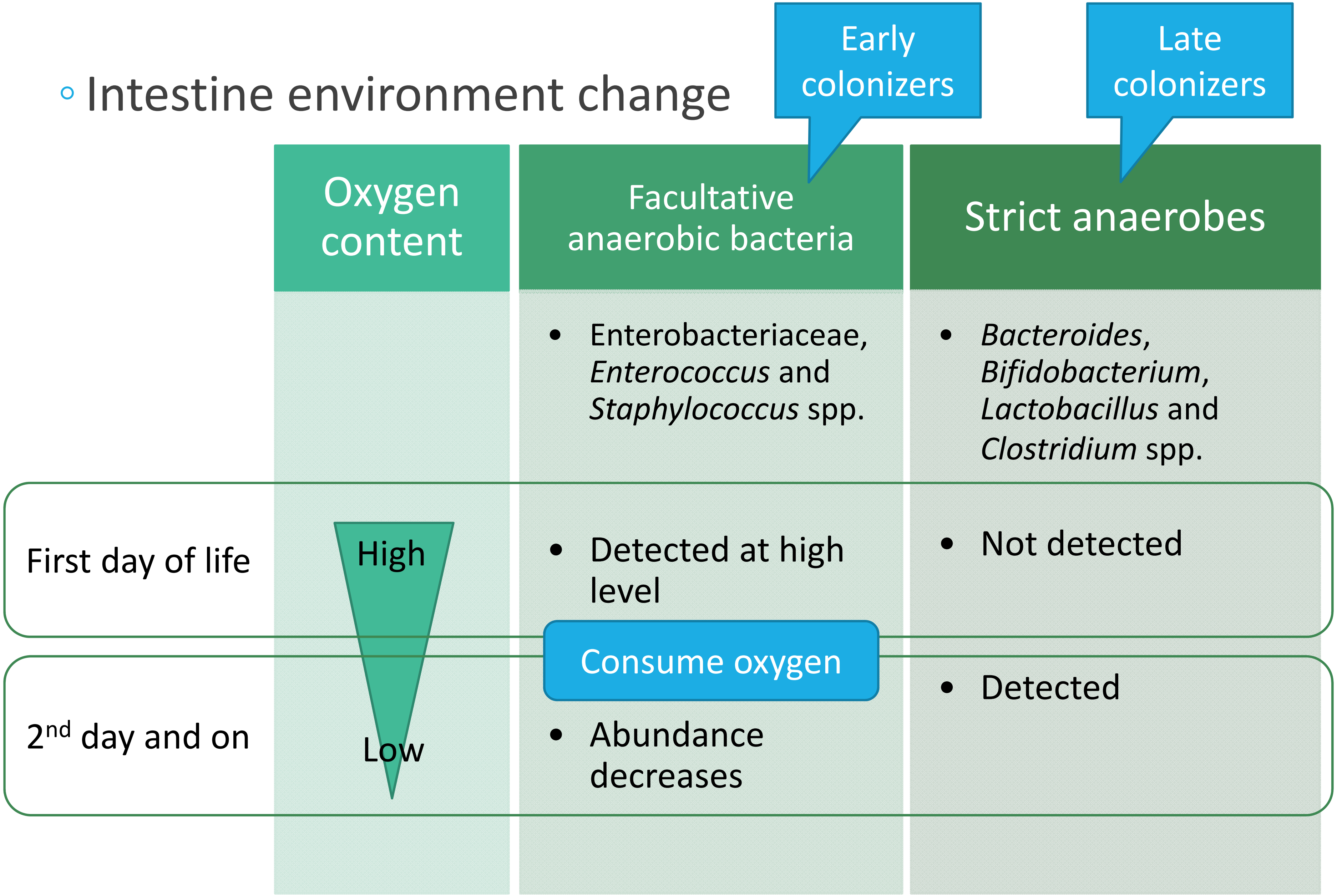
Redox potential in faeces

- Meconium: +175mV
- 1 to 2 d faeces: -113mV
- After weaning: -348mV

(same to adult)

(Tannock, 1994)

Intestine environment change



	Oxygen content	Facultative anaerobic bacteria	Strict anaerobes
		Enterobacteriaceae, *Enterococcus* and *Staphylococcus* spp.	*Bacteroides*, *Bifidobacterium*, *Lactobacillus* and *Clostridium* spp.
First day of life	High	Detected at high level	Not detected
2nd day and on	Low	Abundance decreases	Detected

Early colonizers

Late colonizers

Consume oxygen

(Orrhage and Nord, 1999)

- Pre-weaning infant diet

Breast milk microbiota

  - Core genera include *Staphylococcus* and *Streptococcus* spp.
  - Several hundred species present
  - Composition affected by delivery mode, mothers’ weight and lactation time

Breast milk Human milk oligosaccharides(HMOs)

  - with prebiotic activity
  - stimulate growth of specific groups
  - *Streptococcus* and *Bifidobacterium* spp.

(Hunt et al., 2011; Cabrera-Rubio et al., 2012; Jost et al., 2015)

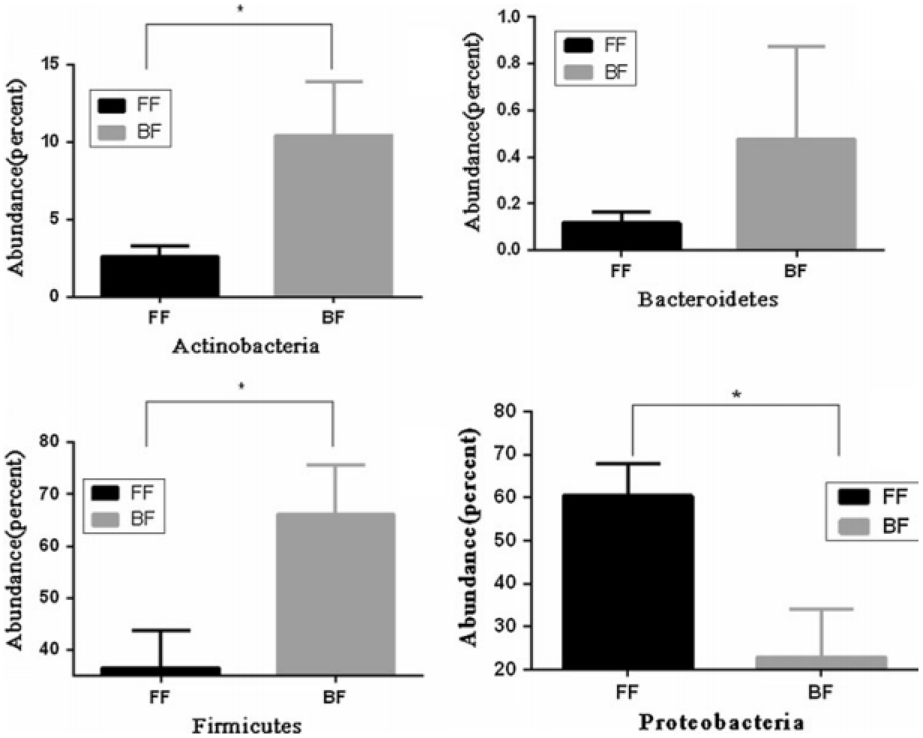
- Pre-weaning infant diet

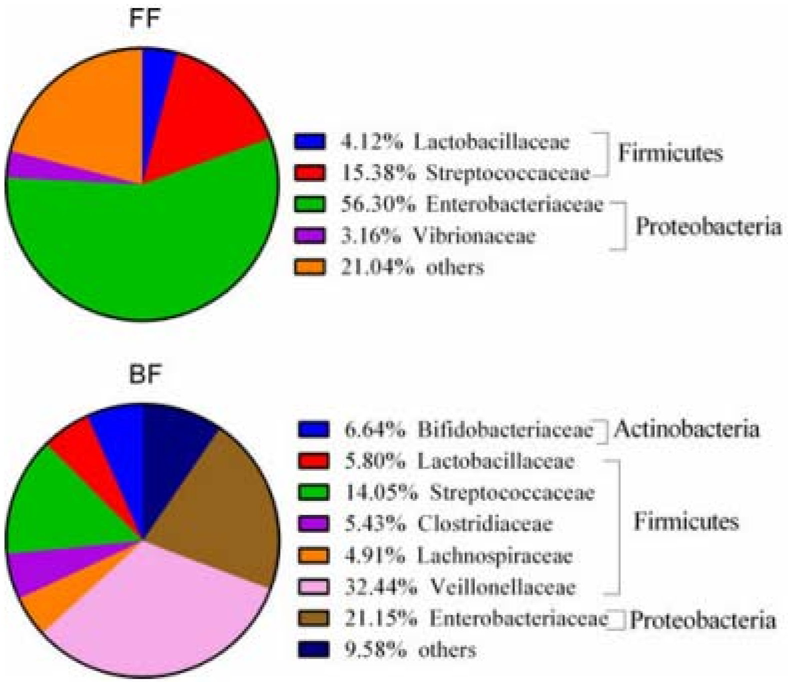
Formula milk

  - Compare with breastmilk
    - Lack of microbiota, HMOs, SIgA
    - Different nutrient content
  - Different gut microbiota from breast-fed
    - ↑Abundance of *Bacteroides* spp., Enterobacteriaceae
    - ↓ Abundance of *Bifidobacterium* spp.

(Harmsen et al., 2000; Fallani et al., 2010)

- Study by Fan et al., 2013
  - Comparison of microbiota of 12 Chinese infants
    - Breast-fed(BF) and Formula-fed(FF)





(Figure 1 & 2. Fan et al.,2013)

- Passive received secretory immunoglobulin A (SIgA)
  - Secretory immunoglobulin A (SIgA)
    - Dominant antibody type in mucosal secretion
    - Secreted by plasma cells in gut lamina propria
    - Involves in homeostasis of gut microbiota

SIgA sources in early life

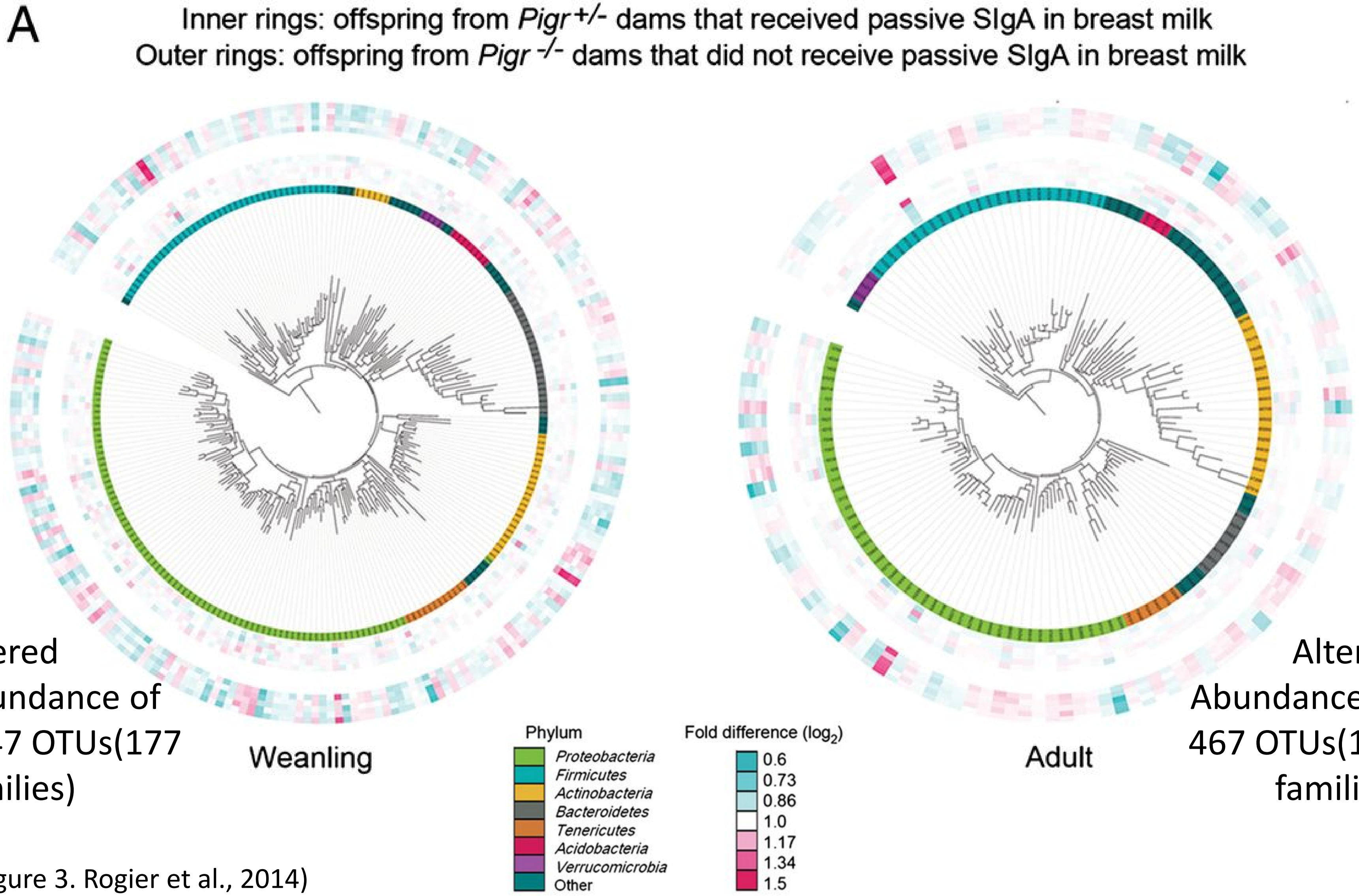
- Maternal IgA through milk
- Endogenous IgA production
  - Mice: starts after weaning
  - Human: starts several months after birth

(Pabst et al, 2016)

- Passive SIgA helps shaping infant gut microbiota
- Study by Rogier et al., 2014
  - IgA-deficient mice
    - Polymeric Ig receptor gene knockout *Pigr*$^{-/-}$
    - *Pigr*$^{-/-}$ female's milk: SIgA absent

  - Offspring fed by
    - *Pigr*$^{-/-}$ female: No passive SIgA
    - *Pigr*$^{+/-}$ female: Receive passive SIgA

- Composition of faecal microbiota
  - Detected by PhyloChip microarray hybridization
  - Differences persisted in to adulthood



Altered Abundance of 1047 OTUs(177 families)

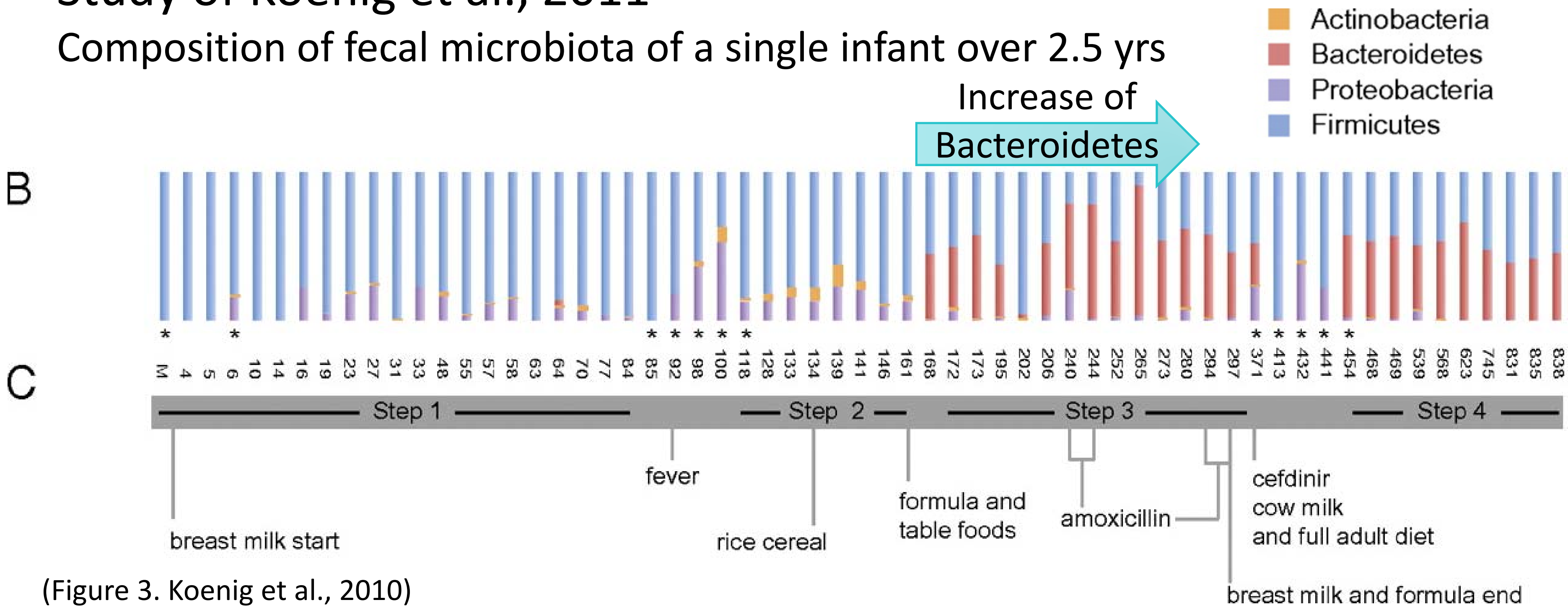
Altered Abundance of 467 OTUs(104 families)

(Figure 3. Rogier et al., 2014)

- Weaning & introduction of complementary feeding
  - Increased species diversity
  - Increasing butyrate producing Bacteria
  - Adult-like major Phyla: Bacteriodetes & Firmicutes

Study of Koenig et al., 2011

Composition of fecal microbiota of a single infant over 2.5 yrs



(Figure 3. Koenig et al., 2010)

(Eckburg et al., 2005; Voreades et al., 2014)

- Study by Bergström et al., 2014
  - Composition of microbiota of Danish infants at 9, 18 & 36 months
  - Significant changes from 9 to 18 months
    - Induced by weaning and introduction of complementary feeding
  - Dominating species:

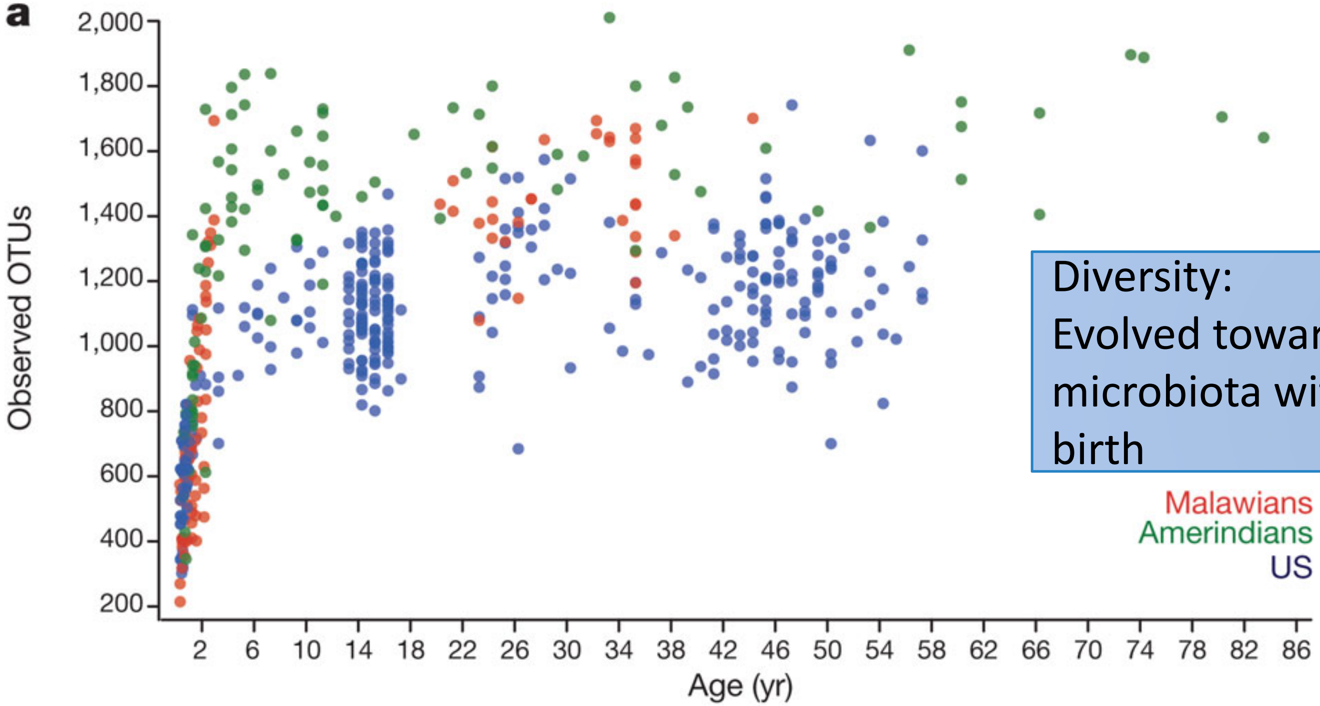
Lacobacillius, *Bifidobacterium* spp. and Enterobacteriaceae

↓

Clostridium spp. and *Bacteroides* spp.

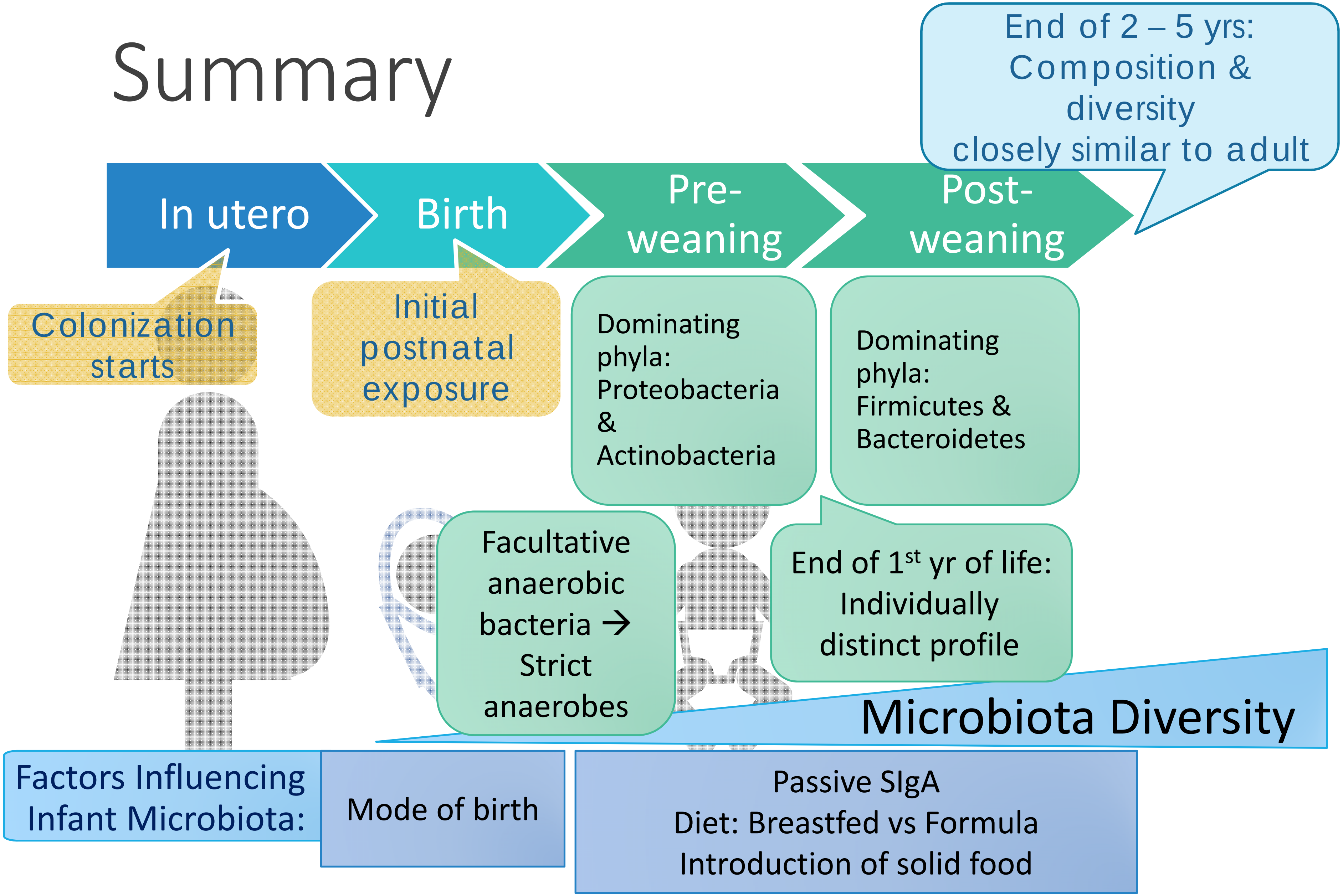
- Resembling adult-like microbiota
  - End of 2 to 5 years of life

    (Palmer et al., 2007; Koenig et al., 2011; Yatsunenko et al., 2012)
  - Induced by introduction of solid food (Bergström et al., 2014)
  - Study by Yatsunenko et al., 2012
  - Composition of gut microbiota of children and adults from Venezuela, Malawi and USA



Diversity:
Evolved towards adult-like microbiota within 3 years after birth

Summary



End of 2 – 5 yrs: Composition & diversity closely similar to adult

In utero

Birth

Pre-weaning

Post-weaning

Colonization starts

Initial postnatal exposure

Dominating phyla: Proteobacteria & Actinobacteria

Dominating phyla: Firmicutes & Bacteroidetes

Facultative anaerobic bacteria → Strict anaerobes

End of 1st yr of life: Individually distinct profile

Microbiota Diversity

Factors Influencing Infant Microbiota:

Mode of birth

Passive SIgA
Diet: Breastfed vs Formula
Introduction of solid food

(Palmer et al., 2007; Rodríguez et al., 2015)

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