Probiotics for early microbiota development

Lise Sanchez, Alexis Mosca, Philippe Langella, Sylvie Binda, Rebeca Martin Rosique

Probiotics for EArly miCrobiota dEvelopment (PEACE Project)

12TH PROBIOTICS, PREBIOTICS & NEWFOOD – Rome congress 2023

September 17, 2023

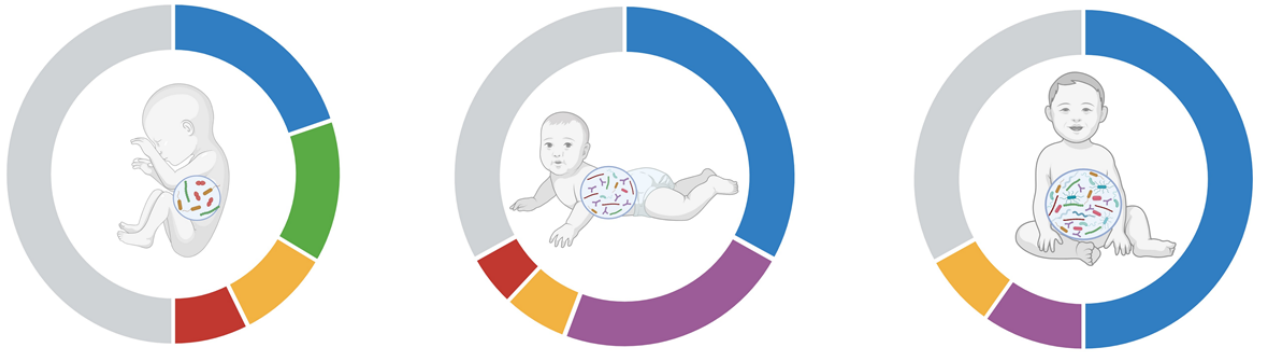
Lise Sanchez, Alexis Mosca, Philippe Langella, Sylvie Binda and Rebeca Martin Rosique

Background – Primocolonization

Mother-to-infant vertical transmission

Opportunity window

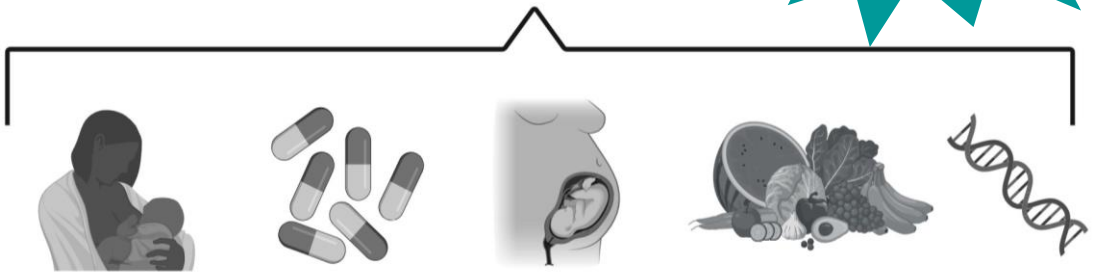
Birth ~1 month ~6 months



Infant gut microbial diversity and richness

Influencing factors

Probiotics



Breast feeding

Antibiotics

Delivery mode

Diet

Genetics

Maternal gut

Maternal vagina

Breast milk

Maternal oral cavity

Maternal skin

Non-maternal sources (Environment, family members, etc)

Schema from Wang and al (2019) modified with Biorender

Our hypothesis

- Vertical microbiota transmission plays a fundamental role in host homeostasis (Wang *et al.*, 2019). Western lifestyle practices can disrupt this process, negatively affecting host health by causing the loss of microbes across generations and thus leading to an imbalanced host-microbiota relationship (Ruiz-Nunez *et al.*, 2013, Bokulich *et al.*, 2016).

- It may be possible to counteract or mitigate this imbalance by supplementing the newborn (and/or the mothers) with beneficial bacteria (probiotics) (Martin *et al.,* 2016).

Our objectives

I. Isolation of candidate from human babies samples

II. *In vitro* characterization of isolated strains

Objective I – Newborn commensal bacteria isolation strategy

N = 6

Inclusion of babies based on various criteria

Full-term babies, breast-fed, no antibiotics or probiotics during pregnancy (mother and newborn), no chronic illness in the mother, etc

N = 32

Fecal samples collected

One sample per month during 6 months after birth (4 samples not received)

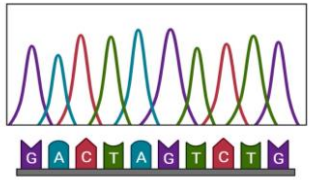
N = 984

Isolated bacteria

From odd month faecal samples only (15 samples)

N= 280

Identified bacteria



Bacteria exclusively isolated from a culture medium adapted to the family and genera of interest

N = 96

Pre-selected different species

Limited to the bacterial family and genera of interest

N = 11

11 bacteria of interest identified :

- ✓ LHS01
- ✓ LHS02
- ✓ LHS03
- ✓ LHS04
- ✓ LHS05
- ✓ LHS06
- ✓ LHS07
- ✓ LHS08
- ✓ LHS09
- ✓ LHS10
- ✓ LHS11

Selection of 1 strain per species per sample
(Avoid selecting the same strain twice)

➢ **11 strains of interest were found and selected for *in vitro* characterization (Objective II)**

Our objectives

I. Isolation of candidate from human babies samples

II. *In vitro* characterization of isolated strains

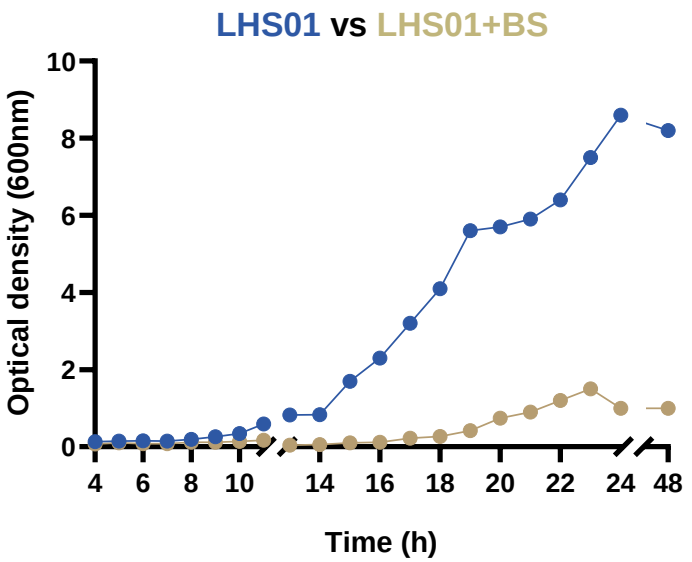
2.1 – Safety and survival properties

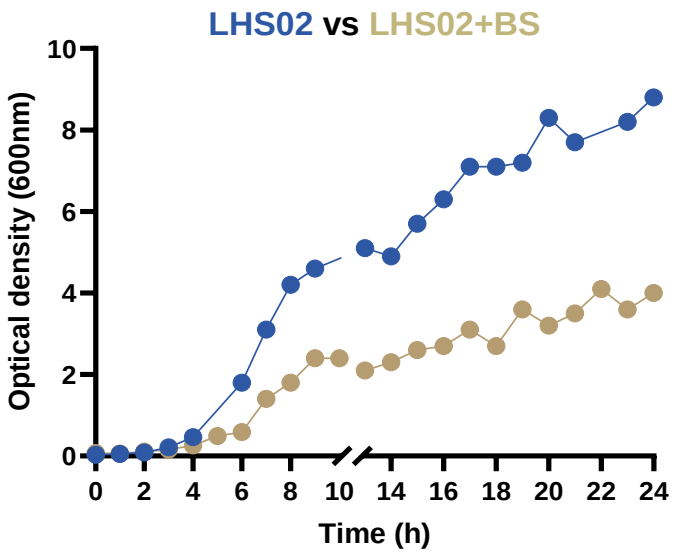
2.2 – Immunomodulatory and protective effect on the intestinal barrier

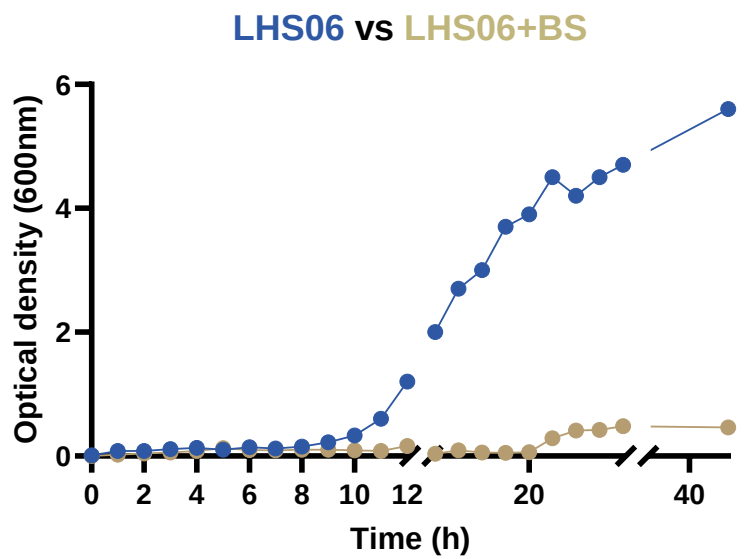
2.3 – Metabolic and anti-pathogenic effects

Objective II – 2.1 – Bile salts resistance

Growth curve experiments with 0,3% of bile salts (BS) over 48h



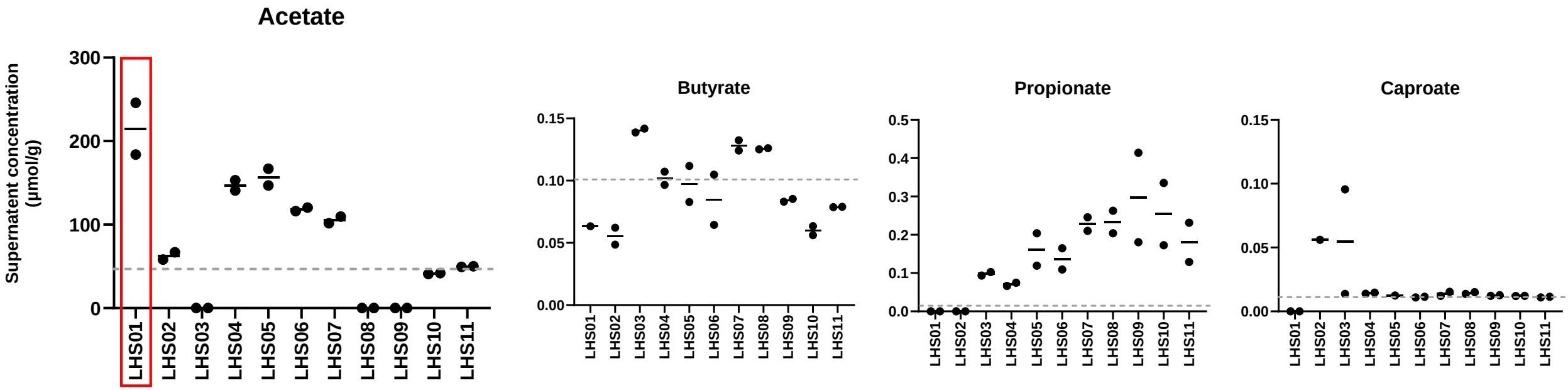




➢ Only 3 strains were not completely inhibited by bile salts

Objective II – 2.1 – Quantification of main Short Chain Fatty Acids (SCFA)

GC-MS analysis of bacterial supernatant collected after 12h growth



- Acetate is the only SCFA produced in significant amounts by our isolated strains
- Highest levels of acetate produced by LHS01

Objective II – 2.1 – Determination of Minimum Inhibitory Concentration (MIC)

MIC = lowest concentration of the antimicrobial that inhibits bacterial growth.

- **Susceptible** (-) : growth is inhibited at a concentration equal to or lower than the established cut-off value (mg/L)
- **Resistant** (+) : able to grow at a concentration higher than the established cut-off value (mg/L)

MIC determination for each strains according to EFSA guidelines

	Ampicillin	Vancomycin	Gentamycin	Kanamycin	Streptomycin	Erythromycin	Clindamycin	Tetracycline	Chloramphenicol
LHS01	-	-	-	-	-	-	-	-	-
LHS02	-	-	-	+	+	-	-	-	+
LHS03	-	-	-	-	-	-	-	-	-
LHS04	-	-	-	-	-	-	-	-	-
LHS05	-	-	-	-	-	-	-	-	-
LHS06	-	-	-	-	-	-	-	-	-
LHS07	-	-	-	-	-	-	-	-	-
LHS08	-	-	+	-	-	-	-	-	-
LHS09	-	-	+	-	+	-	-	-	-
LHS10	-	-	-	+	-	-	-	-	-
LHS11	-	-	+	+	+	+	-	-	+

➢ **We choose for the follow-up the 7 strains susceptible to all the antibiotics tested or with know "intrinsic resistance"**

Our objectives

I. Isolation of candidate from human babies samples

II. *In vitro* characterization of isolated strains

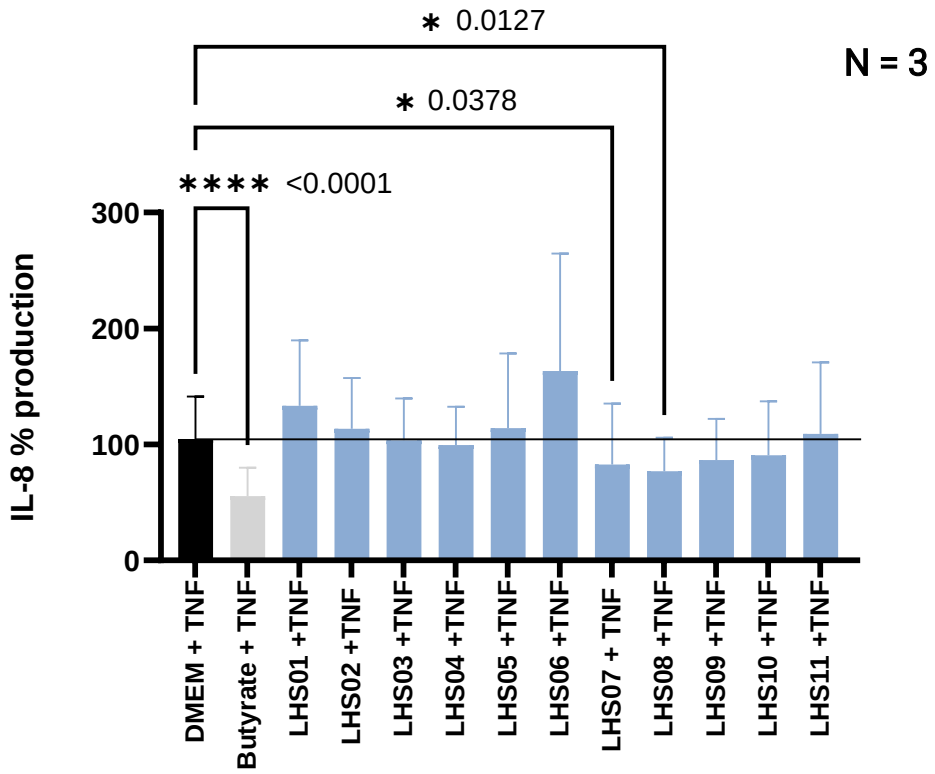
2.1 – Safety and survival properties

2.2 – Immunomodulatory and protective effect on the intestinal barrier

2.3 – Metabolic and anti-pathogenic effects

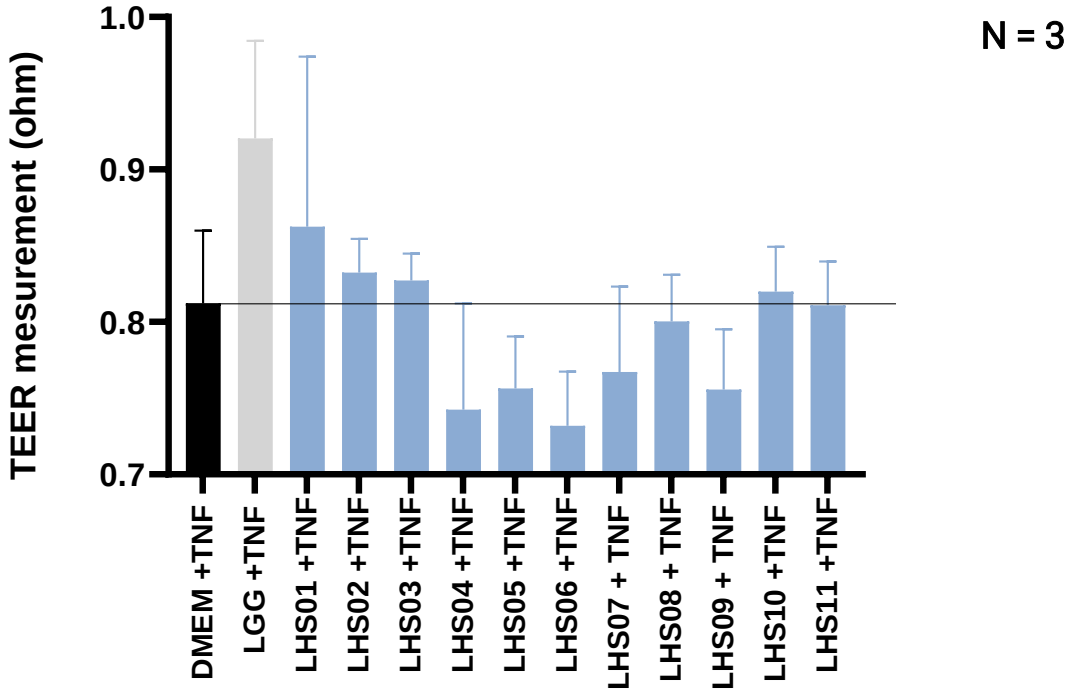
Objective II – 2.2 – Immunomodulatory and protective effects on the intestinal barrier

ELISA quantification of IL-8 after co-incubation with HT-29 cells line stimulated with TNF - α (6h)



➢ LHS07 and LHS08 seems the most promising strains

Transepithelial electric resistance (TEER) measurement after co-incubation with Caco-2 cells line stimulated with TNF – α (24h)

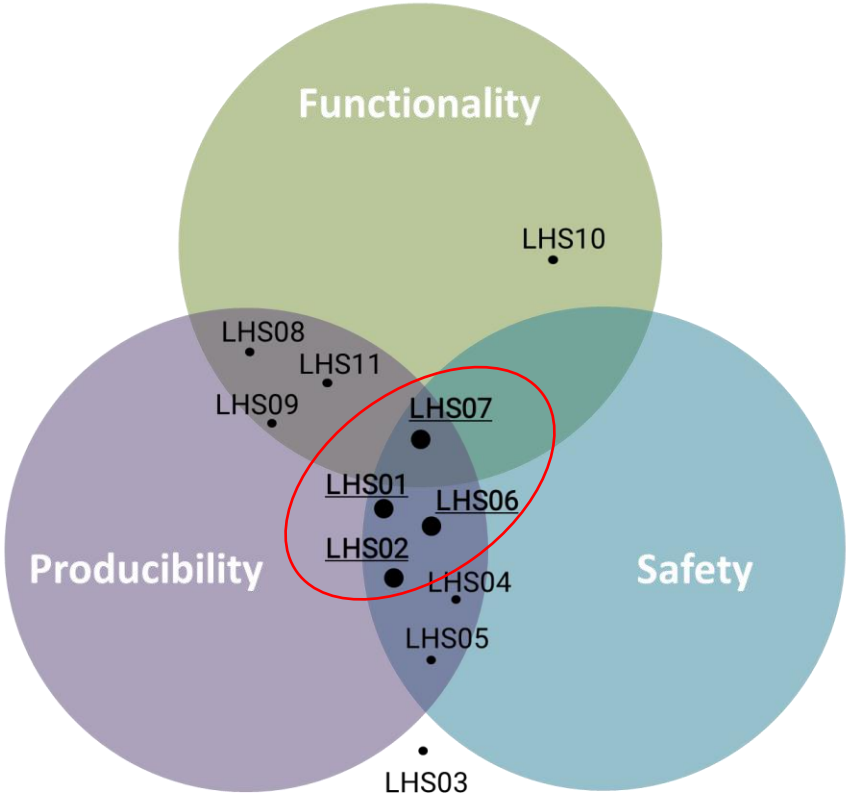


➢ No significant effect on TEER compared to DMEM + TNF-α

➢ LHS01 shows a tendency to increase the resistance

Conclusions – Next steps

Selection of four promising strains on the basis of functionality, producibility and safety



2.3 Human milk oligosaccharides (HMO) fermentation	2.3 Crossfeeding experiment	2.3 Whole genome sequencing

Selection of 1 or 2 strain(s)

Perspectives

Our potential probiotic(s)

Pre-clinical trial

Objective III
In vivo confirmation in mouse model of altered vertical transmission

Clinical trial

Phase I	Phase II	Phase III	Phase IV
			
Safety	Efficacy and safety	Confirmed results	Review and approval

A **new probiotic** to promote the **early establishment** of the **intestinal microbiota**

Acknowledgements

INRAE

Rebeca Martin Rosique

Phillipe Langella

Luis Bermudez-Humaran

Florian Chain

Anne Aucouturier

Sead Chadi

Véronique Robert

Frédéric Pepke

LALLEMAND

Sylvie Binda

ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

Alexis Mosca