COST Action FA1401
European network on the factors affecting the gastro-intestinal microbial balance and the impact on the health status of pigs (PiGutNet)

Action MC Chair: Prof. Paolo Trevisi

Action MC Vice-Chair: Prof. Jürgen Zentek
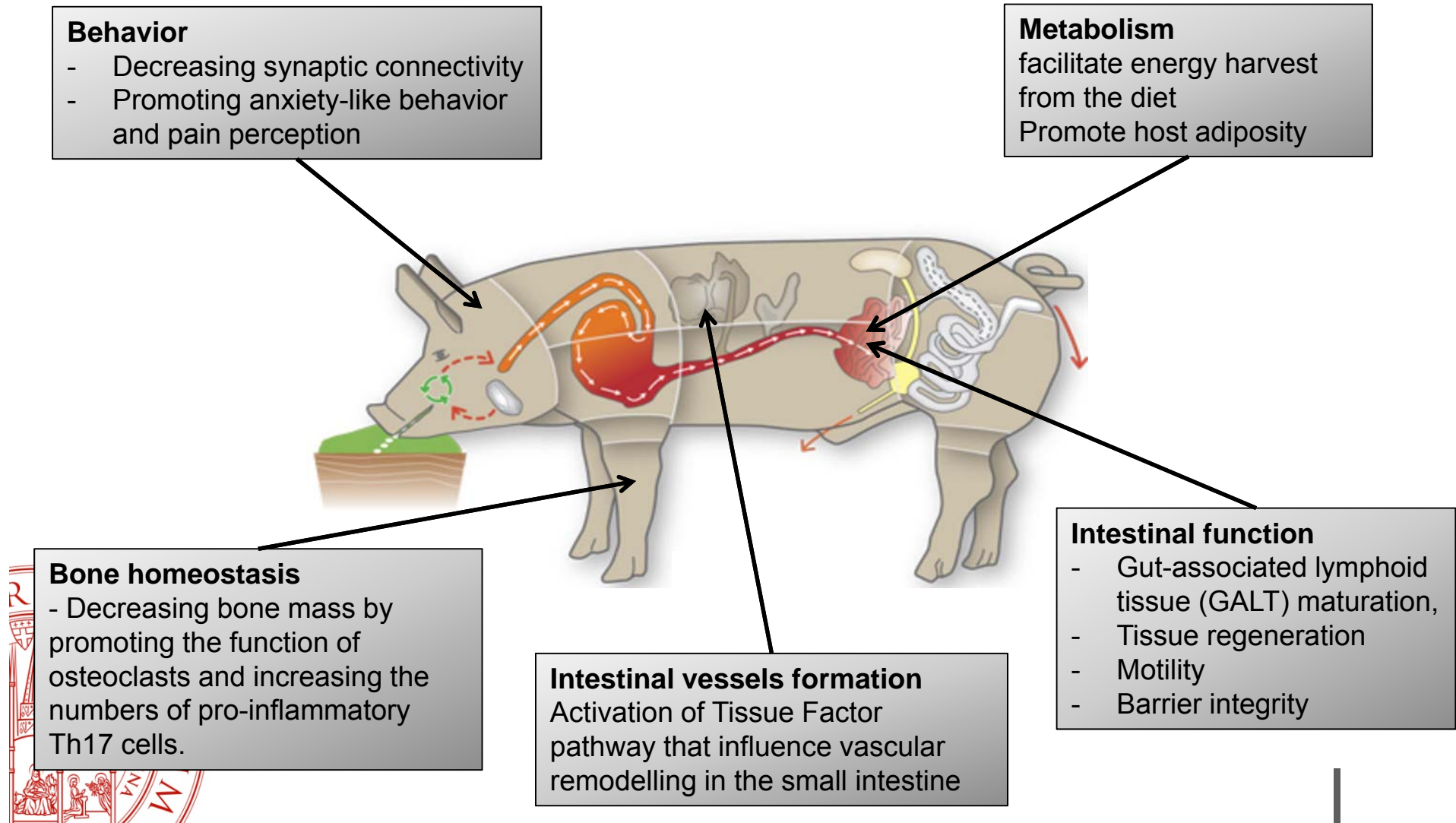

cost
EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

COST is supported by
the EU Framework Programme
Horizon 2020

Role of microbiota on host physiology

Behavior
- Decreasing synaptic connectivity
- Promoting anxiety-like behavior and pain perception

Metabolism
facilitate energy harvest from the diet
Promote host adiposity

Bone homeostasis
- Decreasing bone mass by promoting the function of osteoclasts and increasing the numbers of pro-inflammatory Th17 cells.

Intestinal vessels formation
Activation of Tissue Factor pathway that influence vascular remodelling in the small intestine

Intestinal function
- Gut-associated lymphoid tissue (GALT) maturation,
- Tissue regeneration
- Motility
- Barrier integrity

How to analyze the microbiota modifications?

Metataxonomic and Metagenomic Approaches vs. Culture-Based Techniques
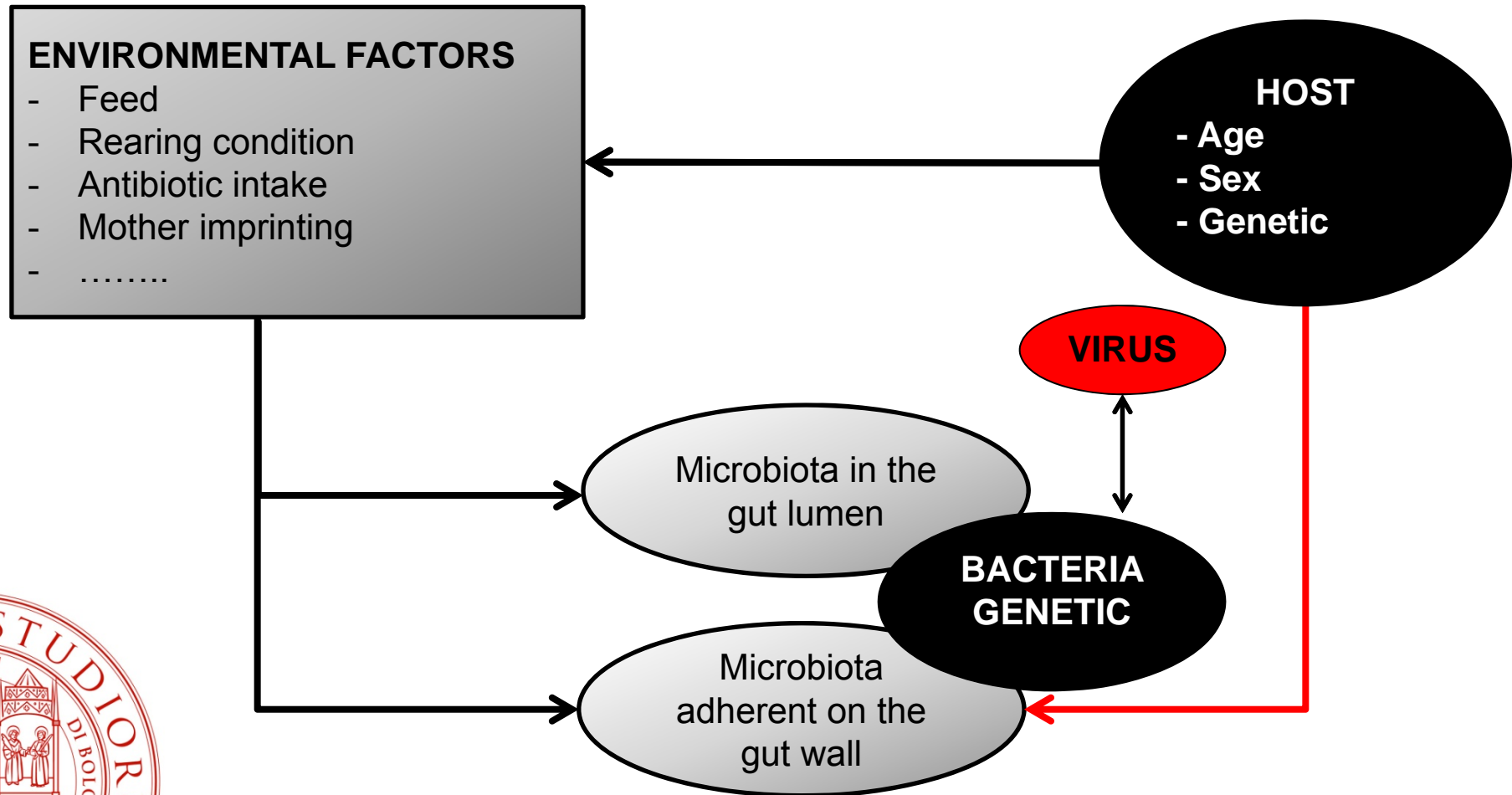
(Hilton *et al*. 2016. *Frontiers in Microbiology*. Volume 7: Article 484

Technique	Speed (days)	Cost	Accuracy	Resistance mutations	Multispecies	High-throughput	Emergent pathogens	Reference update
Culture	2–14	$	Genus or Species	No, additional assays	No	No	No	No
PCR	2	$	Genus or Species	No, additional assays	No, additional assays	No	No	No
Microarray	2	$	Genus or Species	Yes	Yes	Yes	No	Annually but costly
16S	1.5–2	$$	Genus or Species	No	Yes	Yes	Yes	Every run
Metagenomic	2–3	$$	Species or Strain	Yes	Yes	Yes	Yes	Every run

HMP

NIH HUMAN MICROBIOME PROJECT
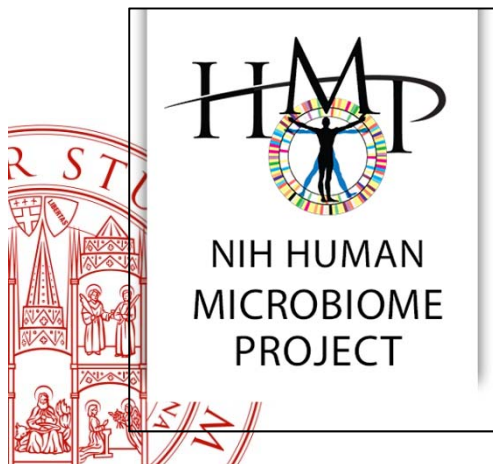
Xiao *et al*. **Sept. 2016**. A reference gene catalogue of the pig gut microbiome. *Nature Microbiology* 1:16161

Factor affecting the gut microbial colonization/balance
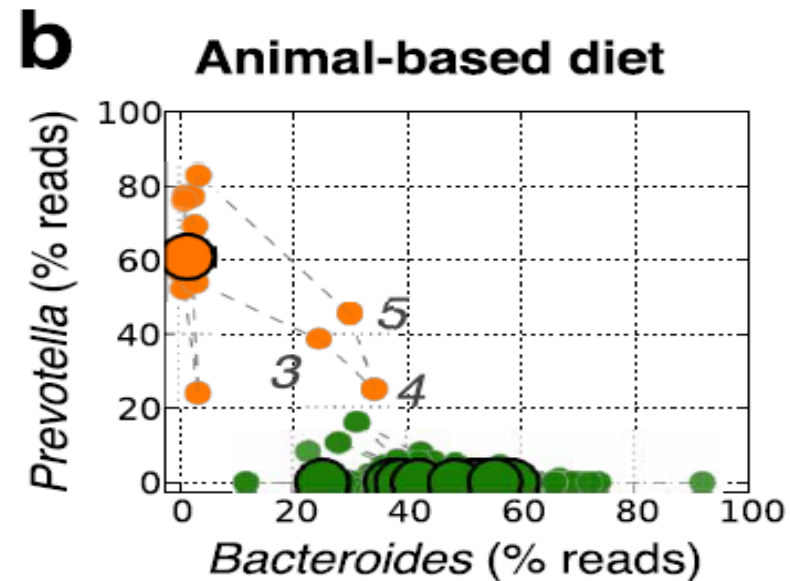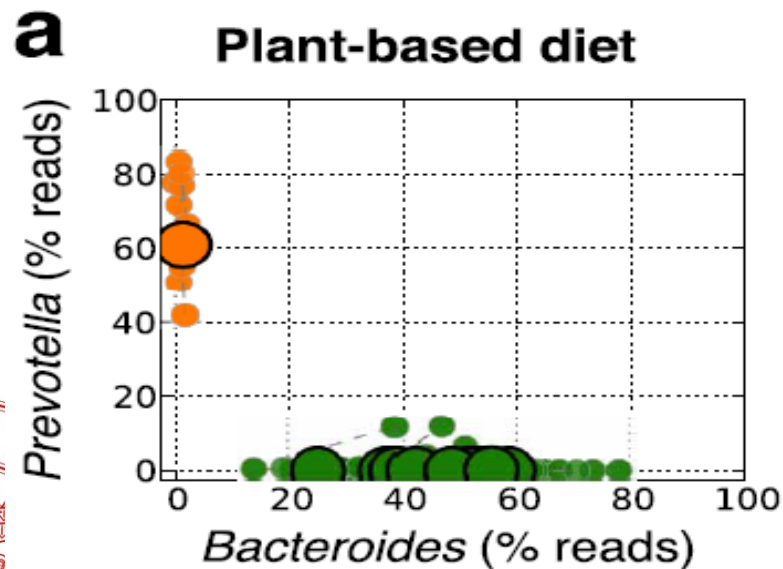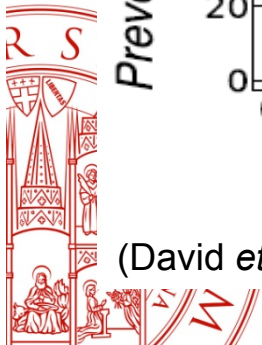

ENVIRONMENTAL FACTORS
- Feed
- Rearing condition
- Antibiotic intake
- Mother imprinting
- ……..

HOST
- Age
- Sex
- Genetic

VIRUS

Microbiota in the gut lumen

BACTERIA GENETIC

Microbiota adherent on the gut wall

Environment *vs* Microbiota

Diet can affects the microbial balance
➤ Protein and Fiber content/source;
➤ Additive;
➤ Antibiotic;
➤ Particle size;
➤ Liquid/solid;
➤ etc.

a Plant-based diet

b Animal-based diet

(David *et al*. 2014. Nature. Vol. 505:559-563)

Cronic stress

Systemic inflammation
↑ IFN-*γ*; TNF-*α;* etc.

Anorexia
↓energy intake, ↓enteral stimulation

Gut epithelium
↑ epithelial permeability
↓antimicrobial peptide secretion

▼

↑bacterial translocation
↑passage of feed antigens

Gut epithelium
↓ activity of brush border enzyme
↓activity of enterocyte transport of nutrient

▼

↓ digestive and absorptive capacity

GALT
↑ intestinal inflammation
↓ antimicrobial peptide secretion

Gut epithelium
↓ antimicrobial peptide secretion

Gut microbiota
Changes in gut microbiota composition
and function

▼

Energy harvest
Changes in fermentation capacity

Protein and energy wasting

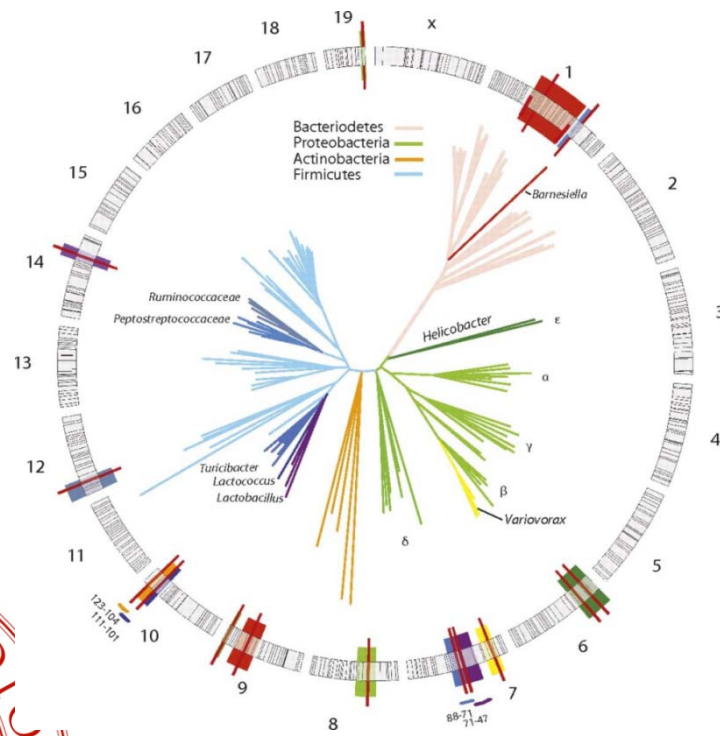Adapted from (Genton *et al.,* 2015. Clin. Nutr. 34:341)

Influence of breed, age and sex on the pig gut microbiota composition

(Xiao *et al*. Sept. 2016. Nature Microbiology. Article n. 16161)
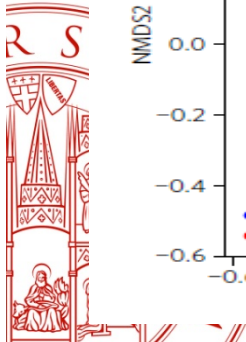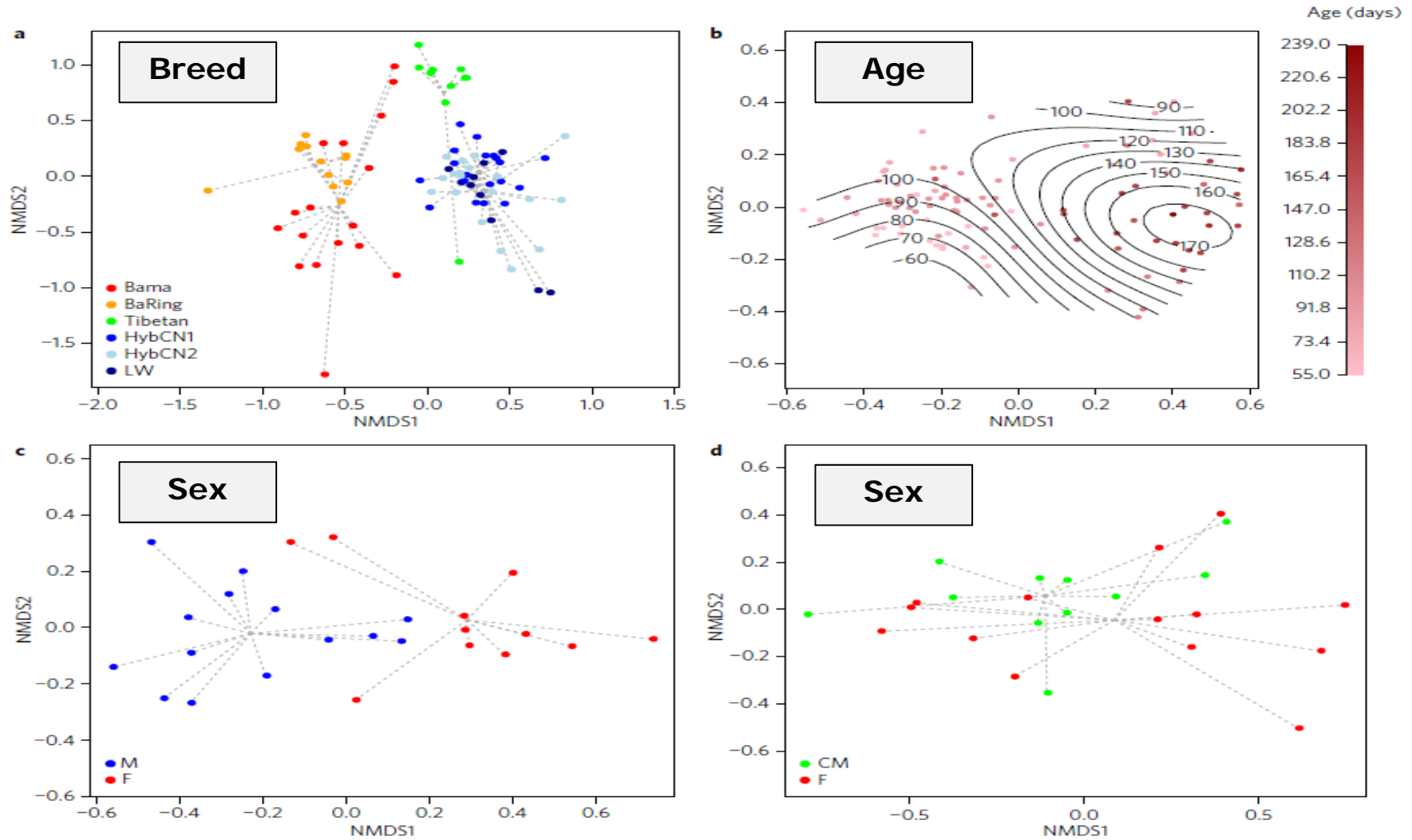
Host genotype *vs* Microbiota

Mapping of murine Quantitative Trait Loci (QTL) to the murine gut microbiota.

These QTL affect microbiota community:

❖ **some loci control single species**

❖ **other control taxa**

(Benson A K *et al*. 2010 PNAS. 107:18933-18938)

Host genotype *vs* Microbiota

In pig:

➢ Linkage observed between the gene Mucin4 (chromosome 13) and the gene for the production of the specific *E. coli* F4ab/ac receptors

➢ Polymorphism on FUT1 (chromosome 6) explains susceptibility to *E. coli* F18

Allele
RESISTENTE
(MUC4-/-)

Allele
SUSCETTIBILE
MUC4 +/+

Table 2 Allele frequencies of the *MUC4* g.8227C>G polymorphism in different Italian pig breeds

Breed	No. of Pigs	Allele Frequency	
		C	G
Italian Large White[a]	541	0.507	0.493
Italian Landrace[b]	65	0.540	0.460
Italian Duroc[b]	48	0.917	0.083
Calabrese	15	0.830	0.170
Casertana	27	0.910	0.090
Cinta Senese	22	1.000	0.000
Nero Siciliano	30	0.720	0.280

[a]Two generation unrelated pigs of the selective genotyping study using backfat thickness estimated breeding value.
[b]Two generation unrelated pigs of the selective genotyping study using average daily gain estimated breeding value.
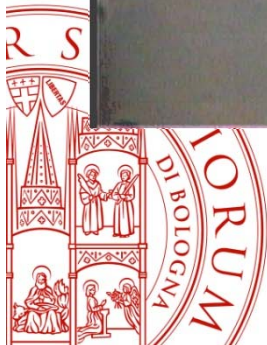
Host genotype *vs* microbiota

Host genotype affect the presence of specific receptors for ETEC F4ac on the intestinal villi of pig
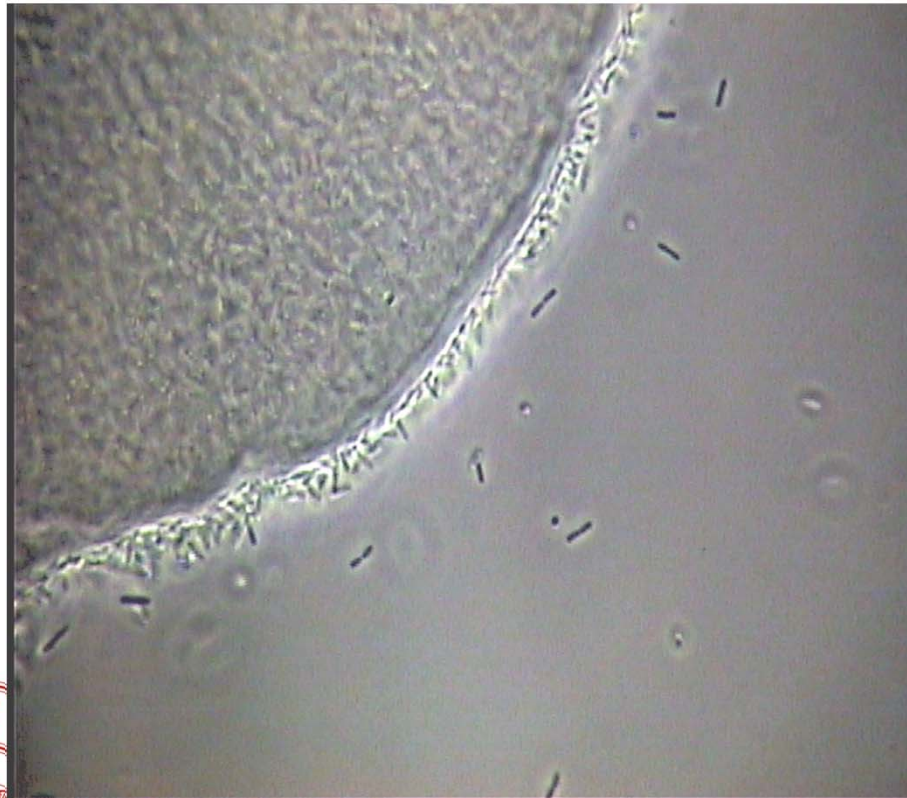
Susceptible subject   **Not susceptible subject**

Host genotype *vs* Microbiota
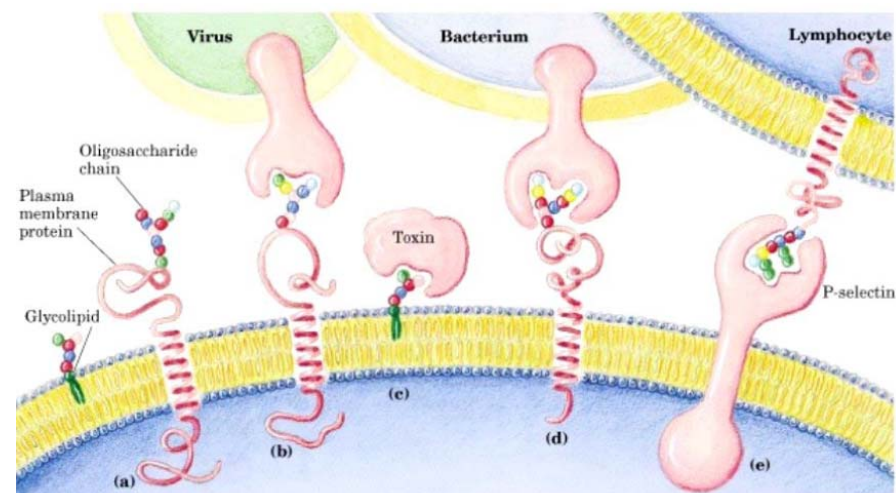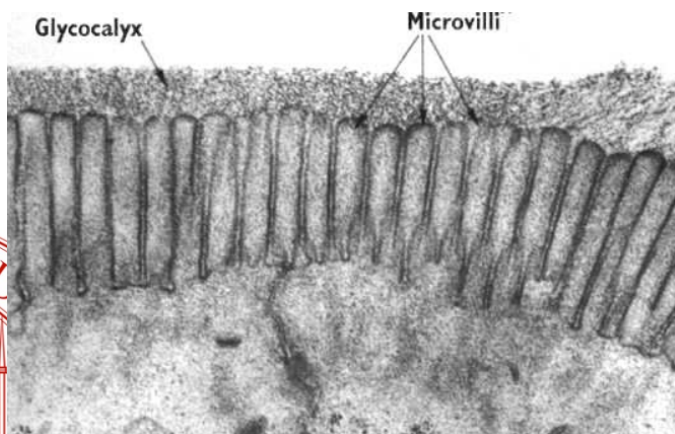
Genotype blood types and Glycomic

RESEARCH **Open Access**

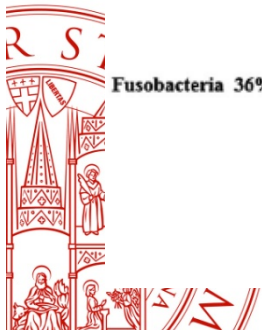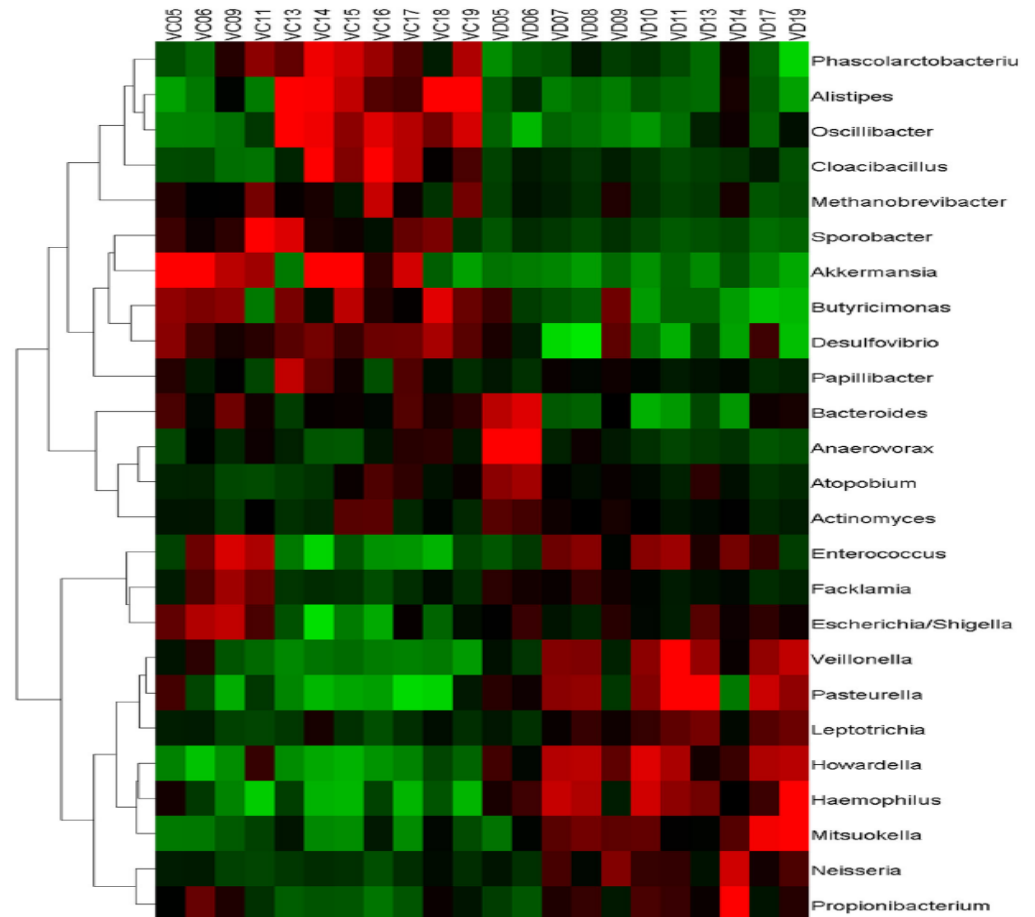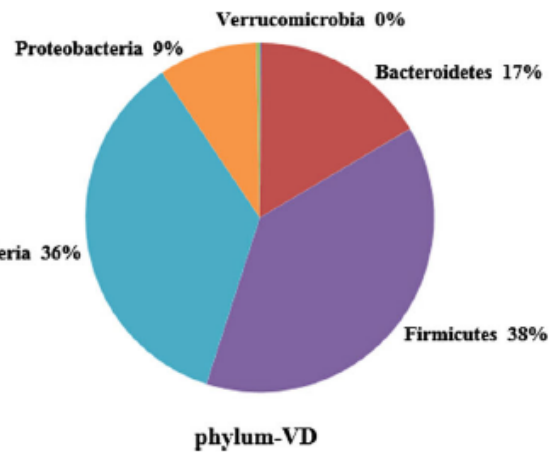# Association between the ABO blood group and the human intestinal microbiota composition
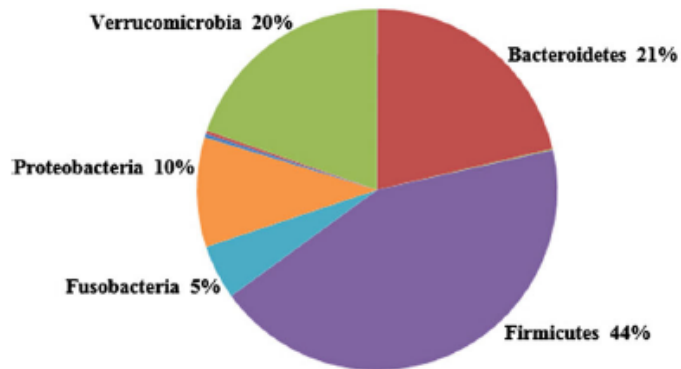
Harri Mäkivuokko[1*], Sampo J Lahtinen[2], Pirjo Wacklin[1], Elina Tuovinen[1], Heli Tenkanen[1], Janne Nikkilä[1], Marika Björklund[2], Kari Aranko[1], Arthur C Ouwehand[2] and Jaana Mättö[1]

Virus *vs* Microbiota

Porcine Epidemic Diarrhoea (PED) infection affects faecal microbiota in pigs

(Liu *et al*., 2015. Curr Microbiol. 71:643-649)

Why is COST the best mechanisms for PiGutNet?
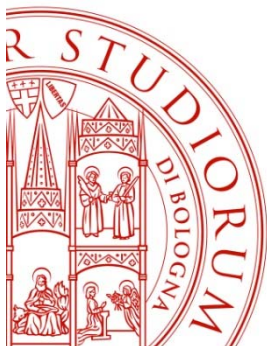
Local, Regional, EU projects

A multidisciplinary network to join the pieces

The work is not still finished

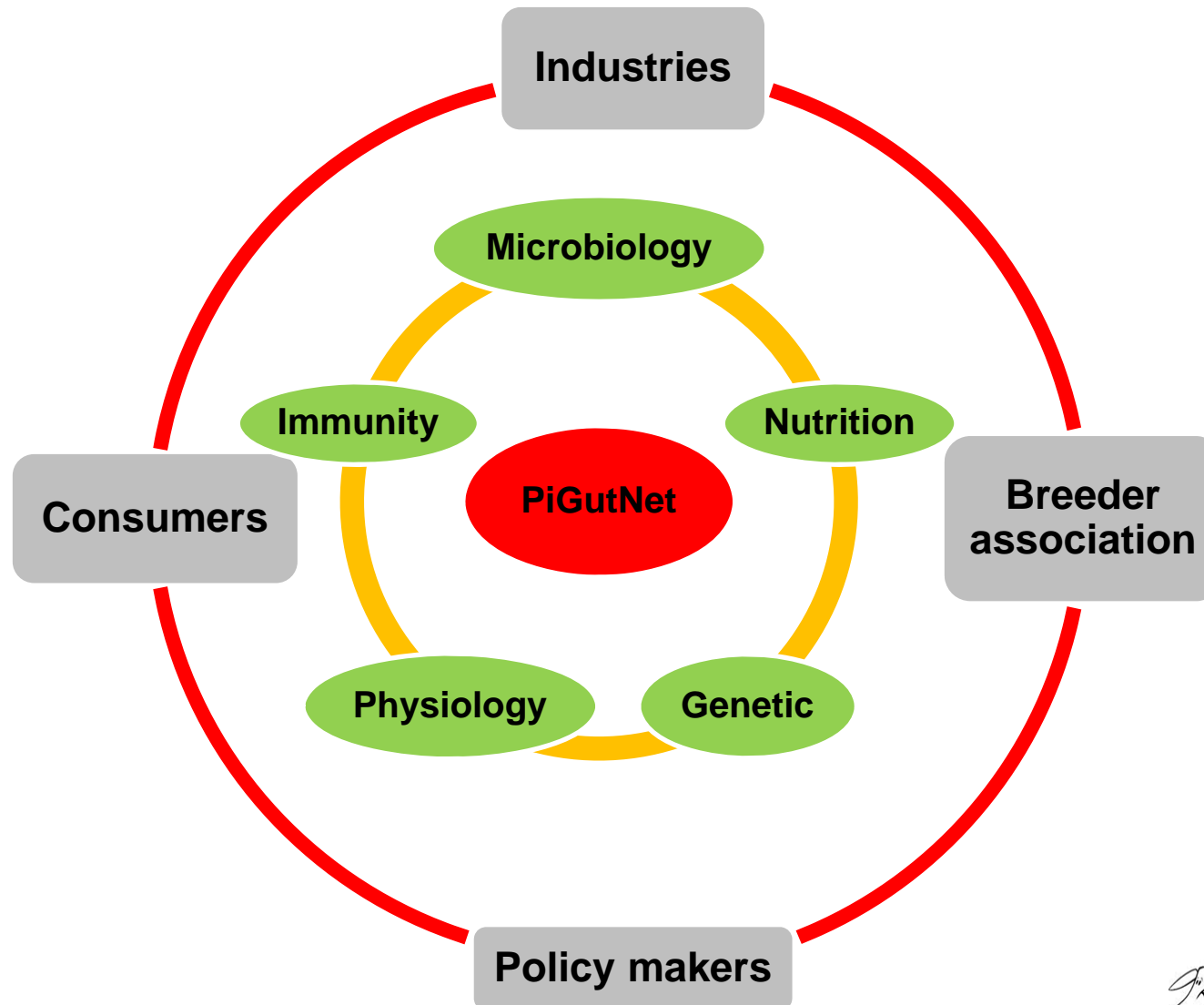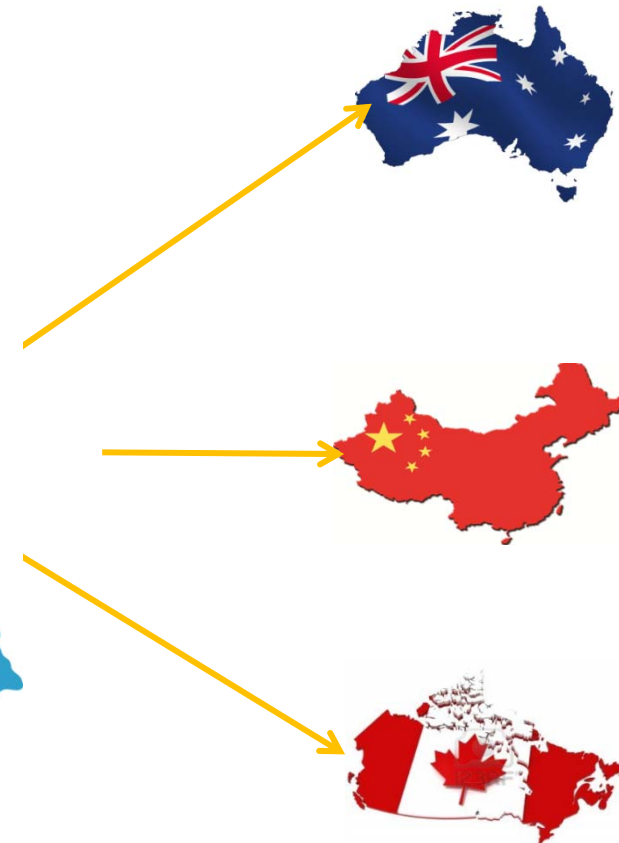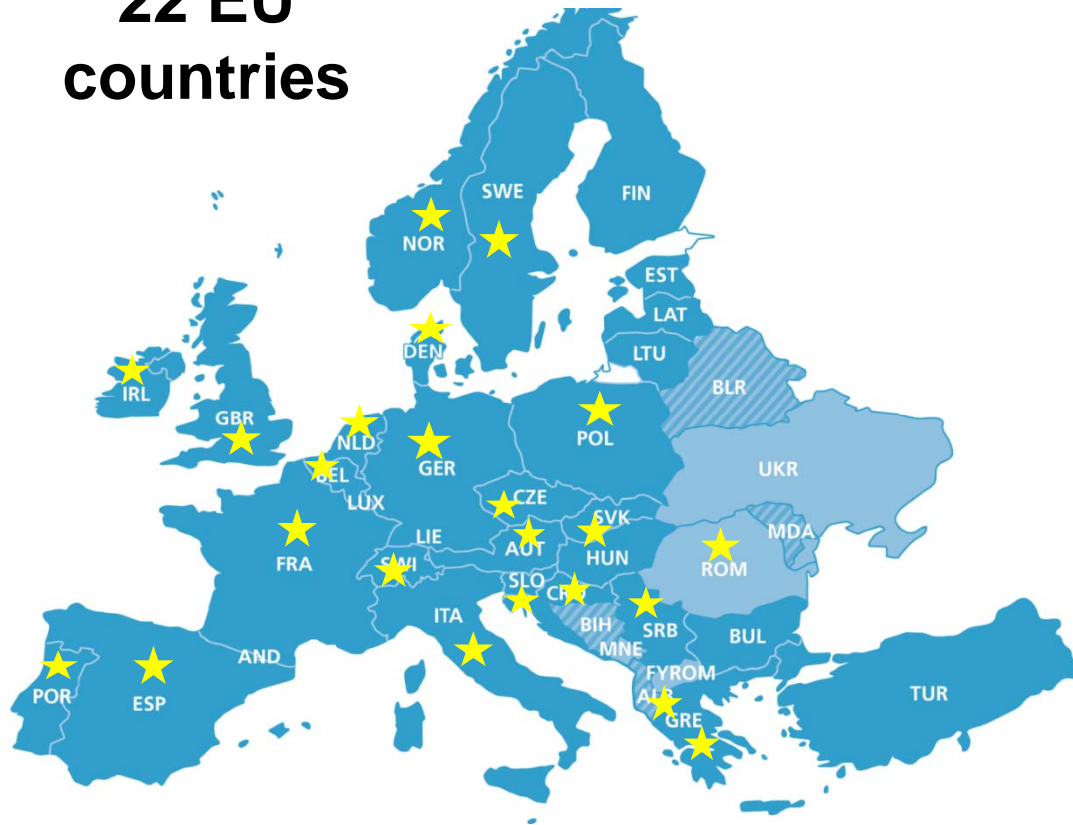**Disseminate the knowledge**

Target groups/end users
Multidisciplinary and Multiplayer Approach

The PiGutNet network

22 EU countries

- ✓ **46 Research Institutions**
- ✓ **10 companies**
- ✓ **5 breeders associations**
- ✓ **(EAAP)**

+

- ✓ **3 extra-EU countries**
- - **3 Research institutions**
    +
    Albania

Dimension of the Action

- **Status of Action, including participating countries**
  - ➢ Duration: 4 years - end 2018
  - ➢ Participants: 22 EU countries from 57 institutions, 10 companies, EAAP
  - ➢ Murdoch University, Chinese Agricultural University, University of Tirana, University of Saskatchewan

- **Action budget status**

➢ Budget assigned to PiGutNet until today: **394.660 Euro**
  - - GP1: 130. 000 Euro
  - - GP2: 42.660 Euro
  - - GP3: 111.000 Euro
  - - GP4: 111.000 Euro

Primary objective of PiGutNet - listed in the MoU

To increase the knowledge about the effect/interaction of environmental and genetic factors on the composition of the microbiota in the GIT of pigs and to improve the risk management associated with antibiotic resistance in pig production.

SCIENTIFIC FOCUS

Factors affecting the gastro-intestinal microbial balance

4 GAPS TO CONSIDER:

Gap 1
- **Molecular microbiology**

Gap 2
- **Environment and host genetic**

Gap 3
- **Feeding strategies**

Gap 4
- **Antibiotic resistance**

PiGutNet

SCIENTIFIC WORK PLAN

PiGutNet

WG1
Microbial
characterization

WG2
Genetic /
Environment

WG3
Feeding
strategies

WG4
Antibiotic and
dysbiosis

WG5

Dissemination

PiGutNet
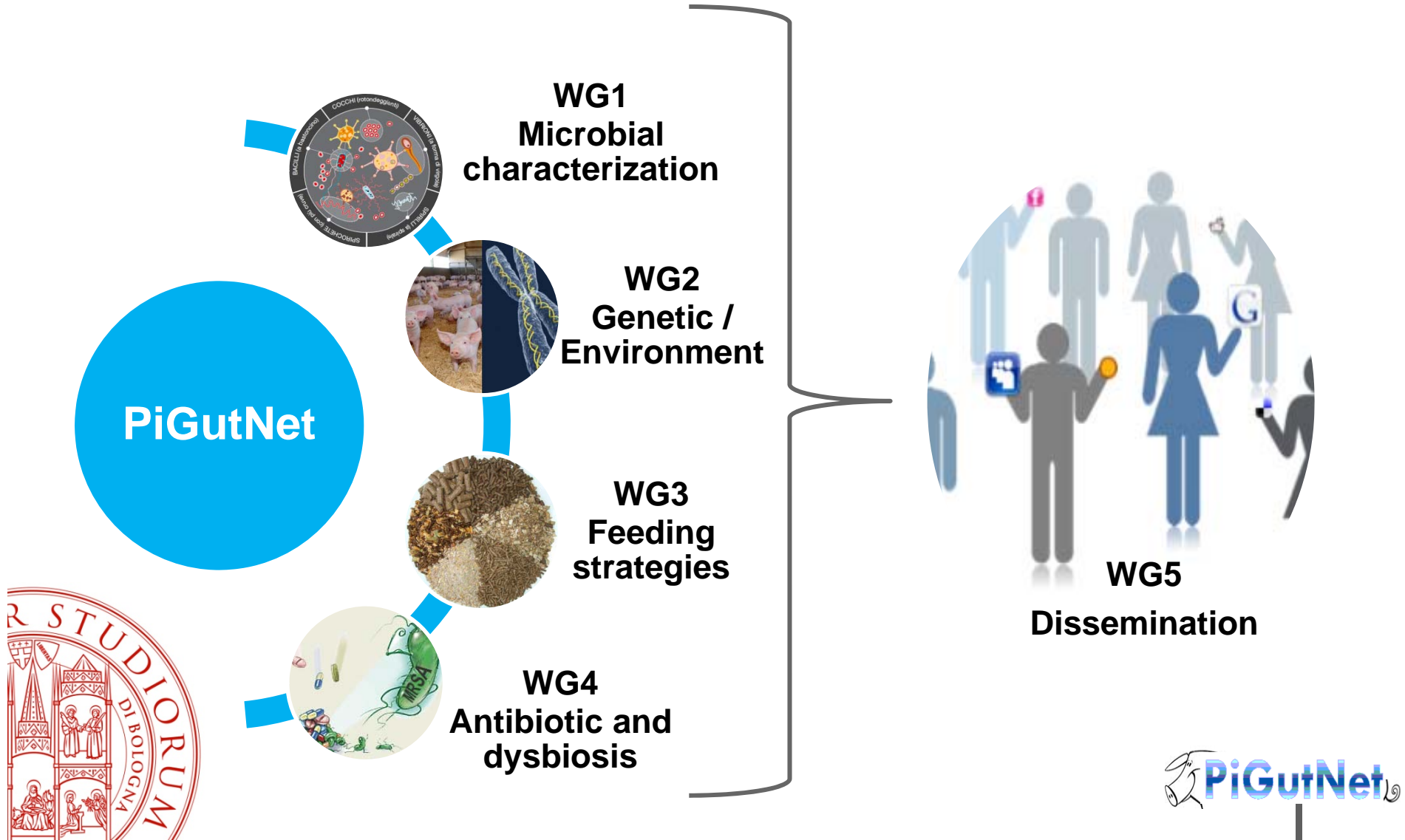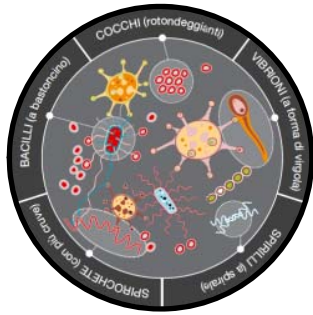
WORKING GROUP 1

Functional and genetic characterization of microbial communities in the gastrointestinal tract of pigs

Leader: Dr. Sara Botti (IT)
Co-Leader: Prof. Mick Bailey (UK)

WORKING GROUP 2

Genetic and environmental factors to understand dysbiosis including their interaction (epigenetics)

Leader: Prof. Charlotte Lauridsen (DK)
Co-Leader: Dr. Jordi Estelle (FR)

WORKING GROUP 3

Feeding strategy to maintain/restore the gut homeostasis

Leader: Prof. J. Francisco Perez (ES)
Co-Leader: Prof. Jan Erik Lindberg (SE)

WORKING GROUP 4

Antibiotics as a factor of dysbiosis and spread of antibiotic resistance genes

Leader: Prof. Papadopoulus Dimitris (EL)
Co-Leader: Dr. Geert Bruggeman (BE)

WORKING GROUP 5

Knowledge and management exchange

- Support exchanges across the working groups, and the dissemination towards external stakeholders

Leader: Prof. Olivera Djuragic(RS)
Co-Leader: Dr. Geert Bruggeman (BE)

Short Term Scientific Mission (STSM)

Leader: Dr. Marianna Roselli (IT)
Co-Leader: Prof. Hermann-Josef Rothkoetter (DE)

Some questions from the network

WHAT IS A HEALTHY PORCINE MICROBIOTA?

- ➢ **What is a healthy gut and a healthy microbiota?**

- ➢ **What is dysbiosis?**

- ➢ **What is the importance of the gut segment?**
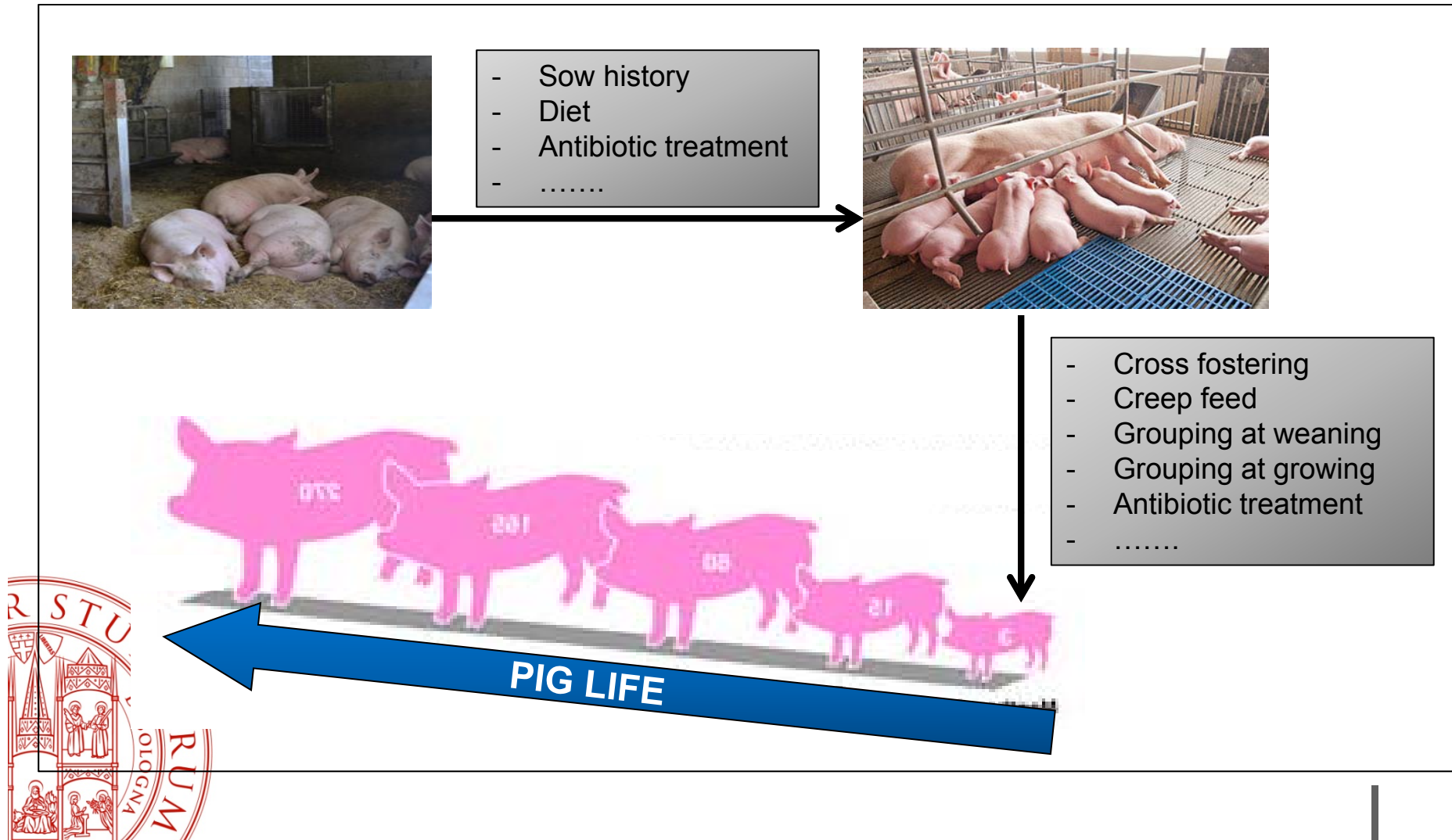

WHICH FACTORS AFFECT IT?

- ➢ **Which is the state of the art on the relative contribution of the host and the gut microbiome for the stability of the gut ecosystem?**

- ➢ **Which type of phenotypes, samples and metadata should be obtained in experiments concerning porcine gut microbiota analysis in relation to health?**

- ➢ **Which are the most adapted statistical approaches susceptible to contribute to an effective analysis of such datasets?**

- ➢ **Which recommendations in order to get ready for meta-analysis studies?**

The continuity concept

- Sow history
- Diet
- Antibiotic treatment
- ....

- Cross fostering
- Creep feed
- Grouping at weaning
- Grouping at growing
- Antibiotic treatment
- ....

PIG LIFE

Tools developed by PiGutNet

Virtual sample repository

Around Europe, there are thousands of samples collected during in vivo and in vitro trials and stored in the fridges. These samples could be deeply exploited to produce new data useful for several research topics. PiGutNet, set-up a virtual repository to favour the samples exchanges, in response to the new legislation on the 3R for the reduction of the animals for scientific purposes.

PiGutNet European Nucleotide Database 16sRNA

The WG2, highlighted the need to create a database to collect the data related with the sequencing of bacteria DNA of pigs. A dedicated area on the European Nucleotide Archive (ENA) managed from the European Bioinformatics Institute, was created. The EAA allow to have a protected area dedicated to PiGutNet. The objective is to stimulate partners to share the data

Final Considerations

➤ An unique profile to define the "balanced" or "optimal" microbiota in the gut of pigs is not applicable → Need to adopt a dynamic approach.

➤ Few data are available on the long term effect of an early perturbation of the microbiota on health status of pig during their life.

➤ Environmental factors play a crucial role in the settlement of the microbiota → Anyway the genetic background of the animals should be more considered to understand individual variability of the gut ecology.

➤ The taxonomic analyses could be not enough to understand the complex interaction between host/environment/bacteria → Need to consider the microbiota's functions (metagenomics).

➤ The interplay between the bacteria and the other organisms that constitute the gut microbiota must be deeply considered (virus, parasites, fungi, etc.)

➤ Analytical tools/platforms and bioinformatic approach are critical points, needs for a large consensus on basic tools.

An integrated approach is needed to improve the robustness of the animals
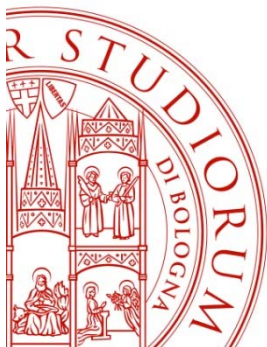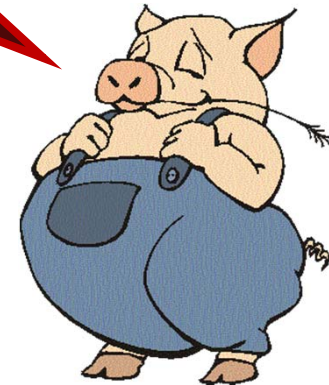
THANK YOU FOR YOUR ATTENTION!

Contacts:

Prof. Paolo Trevisi – **paolo.trevisi@unibo.it**

Project website - **www.pigutnet.eu**

Follow PiGutNet on Twitter: **@PiGutNet**

Take home message

"A microbial community highly efficient in recovering energy from food may constitute a risk factor for obesity in an organism with easy access to food, while it may be healthy in an individual with limited access to food". (Blaser *et al*., 2006)