

COST Action FA1401

European network on the factors affecting the gastrointestinal microbial balance and the impact on the health status of pigs (PiGutNet)



Action MC Chair:

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Action MC Vice-Chair:

Prof. Jürgen Zentek

COST
EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

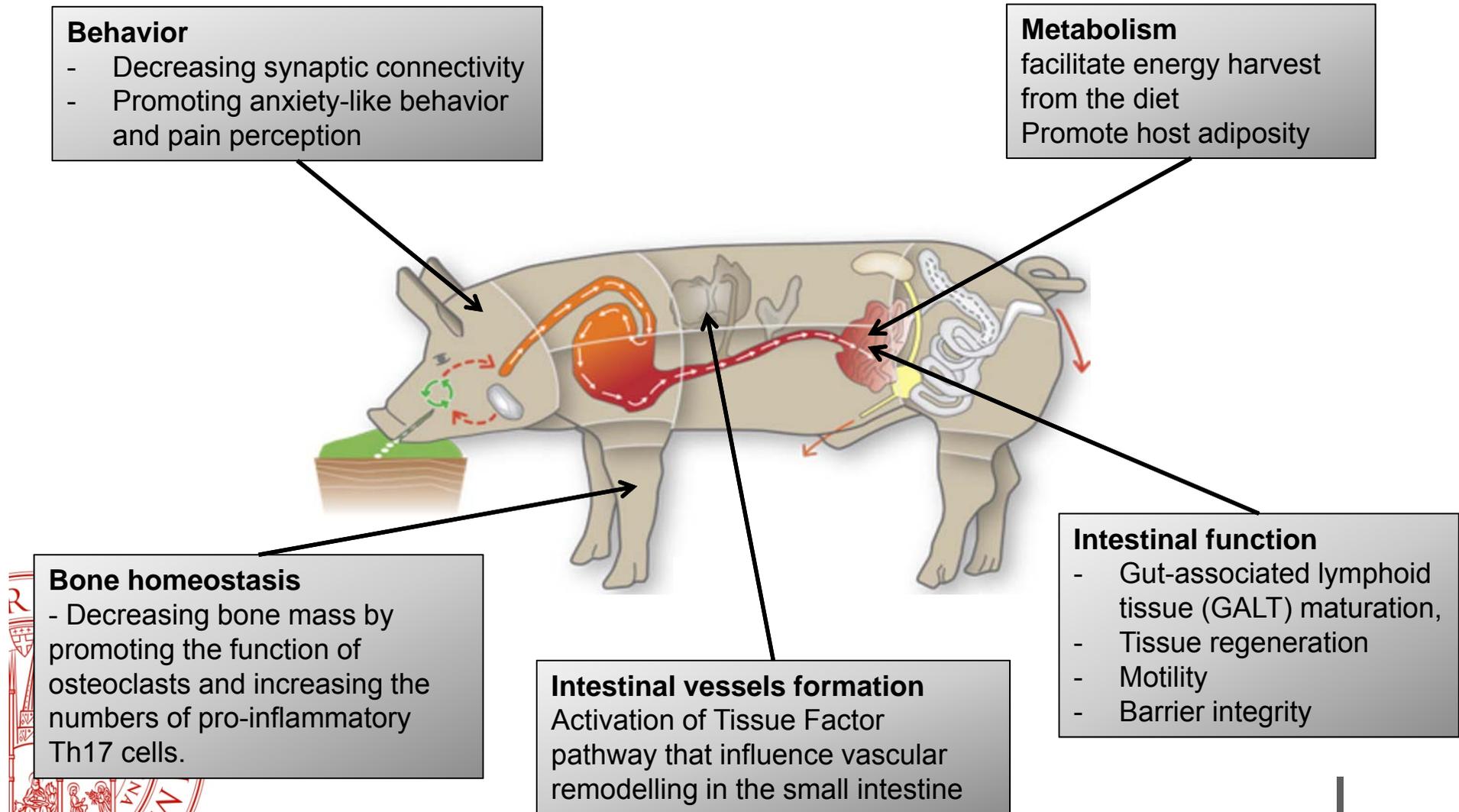


COST is supported by
the EU Framework Programme
Horizon 2020

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA

IL PRESENTE MATERIALE È RISERVATO AL PERSONALE DELL'UNIVERSITÀ DI BOLOGNA E NON PUÒ ESSERE UTILIZZATO AI TERMINI DI LEGGE DA ALTRE PERSONE O PER FINI NON ISTITUZIONALI

Role of microbiota on host physiology

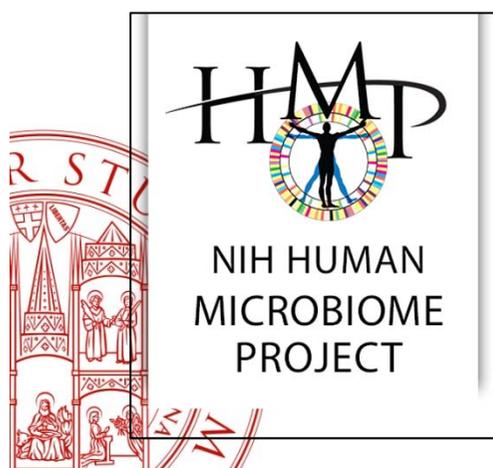


How to analyze the microbiota modifications?

Metataxonomic and Metagenomic Approaches vs. Culture-Based Techniques

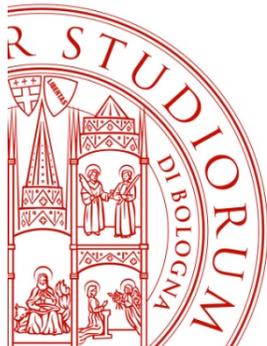
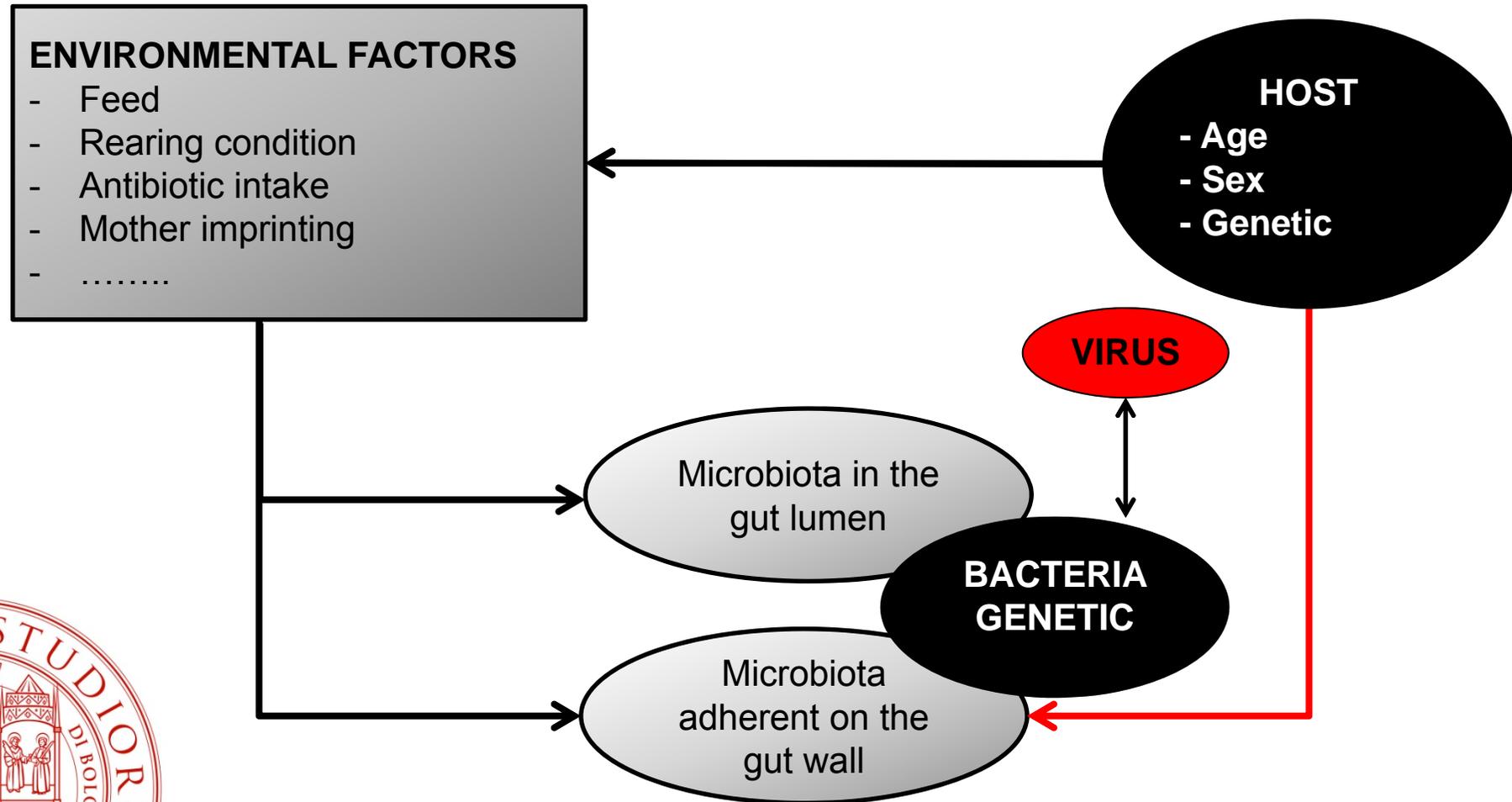
(Hilton *et al.* 2016. *Frontiers in Microbiology*. Volume 7: Article 484)

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



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

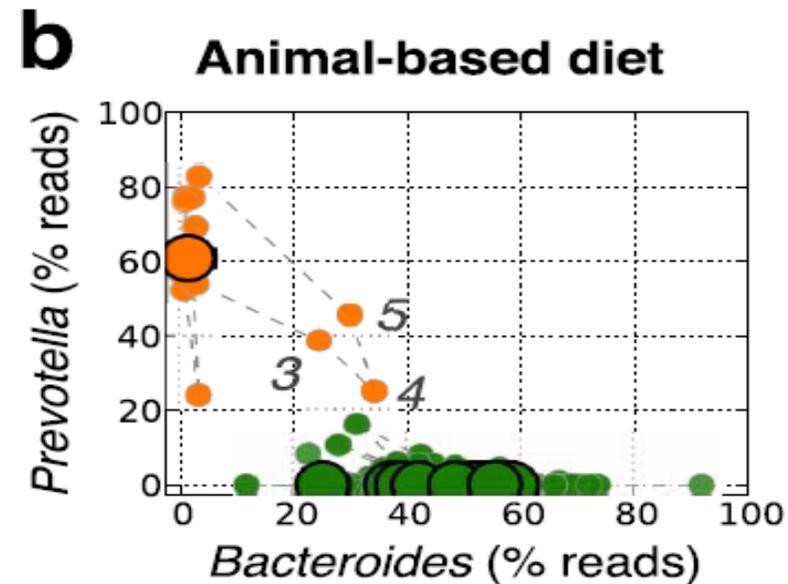
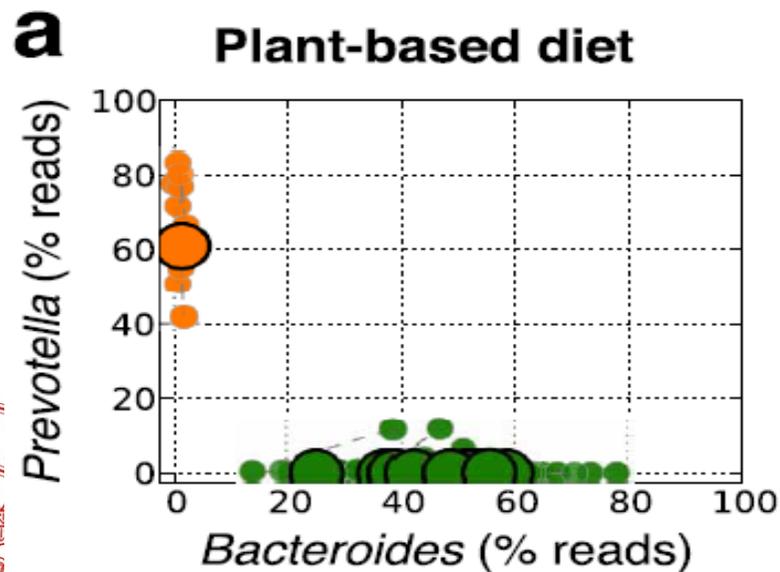
Factor affecting the gut microbial colonization/balance



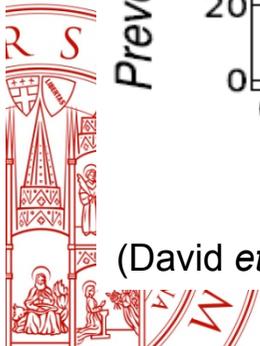
Environment vs Microbiota

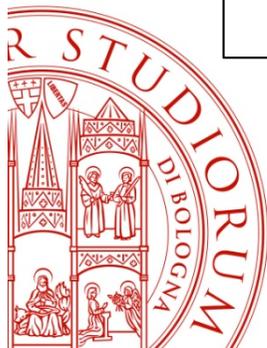
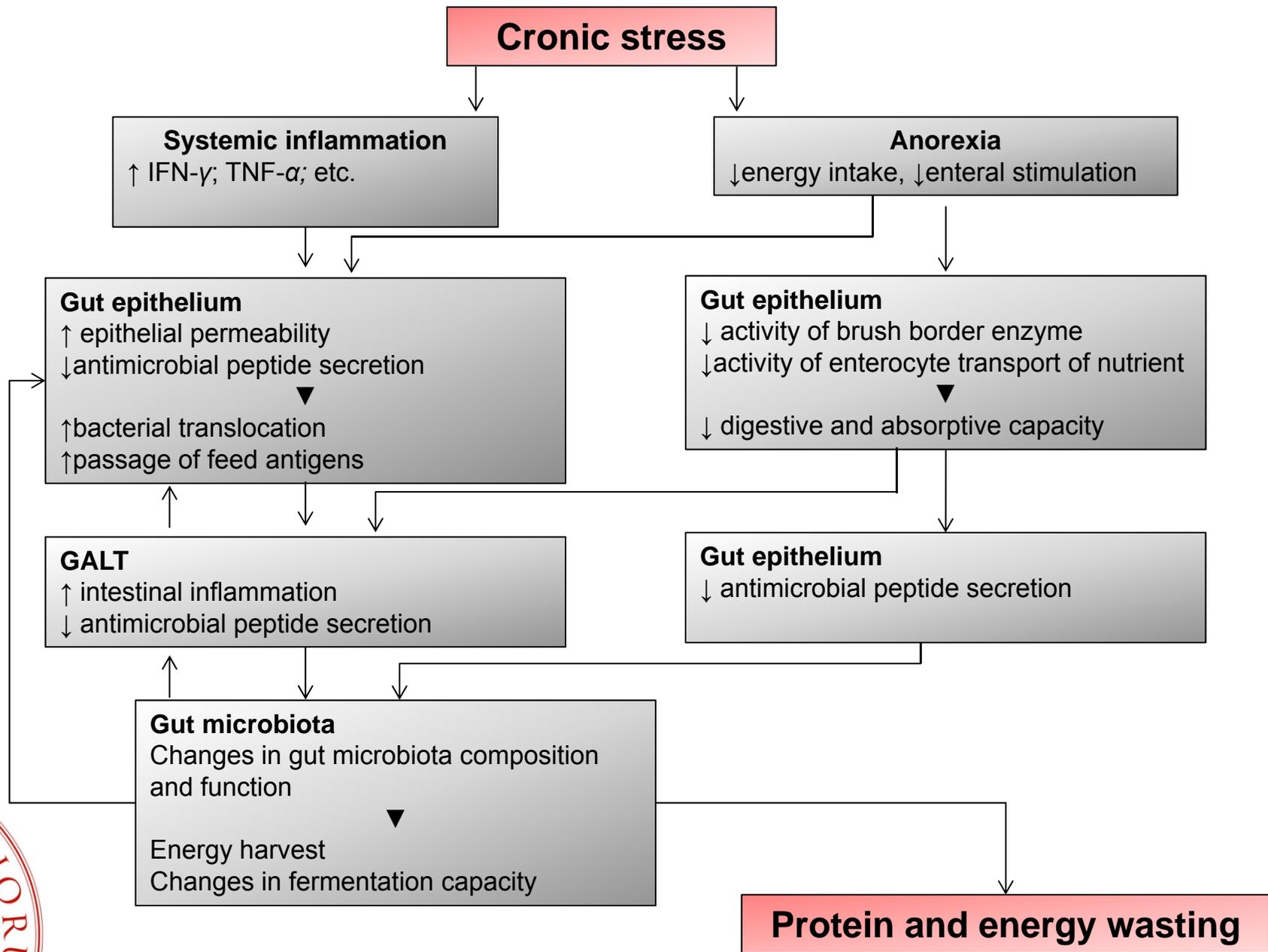
Diet can affect the microbial balance

- Protein and Fiber content/source;
- Additive;
- Antibiotic;
- Particle size;
- Liquid/solid;
- etc.



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

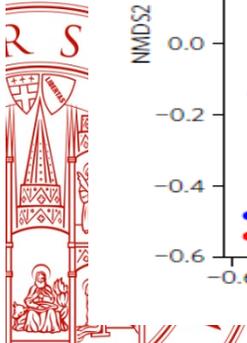
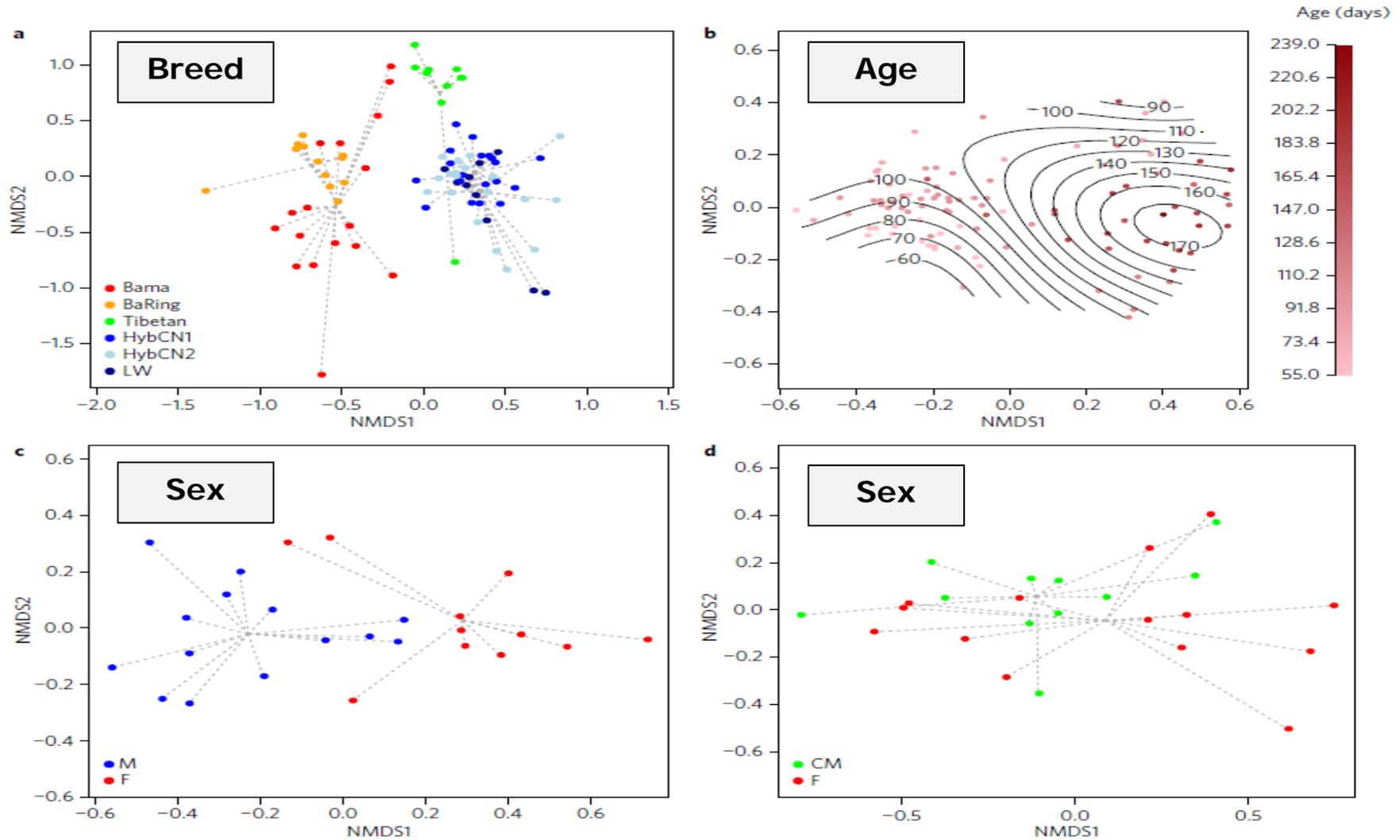




Adapted from (Genton *et al.*, 2015. Clin. Nutr. 34:341)

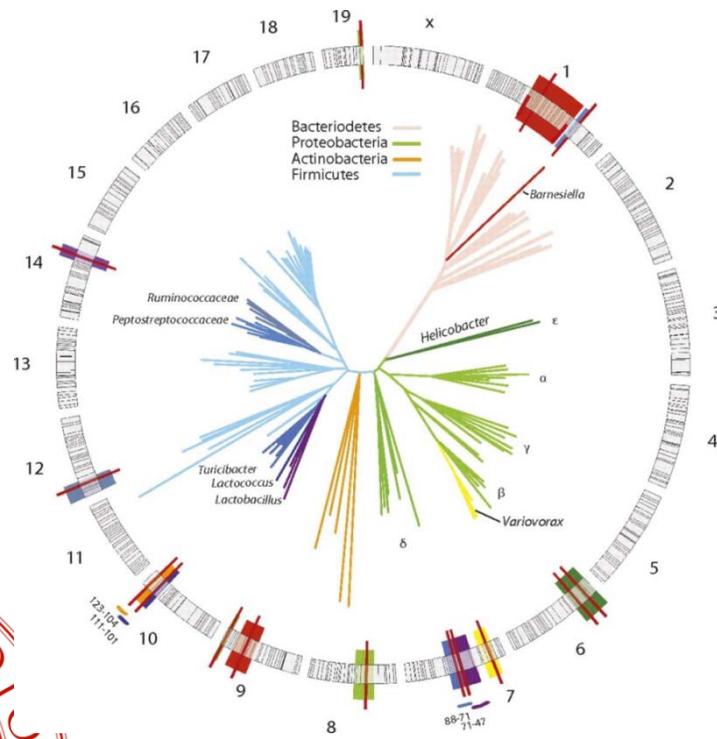
Influence of breed, age and sex on the pig gut microbiota composition

(Xiao *et al.* Sept. 2016. Nature Microbiology. Article n. 16161)



Host genotype vs Microbiota

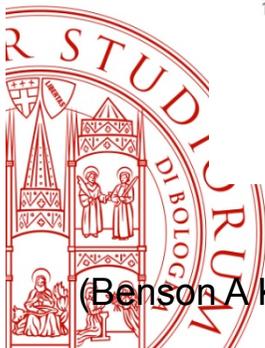
Mapping of murine Quantitative Trait Loci (QTL) to the murine gut microbiota.



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

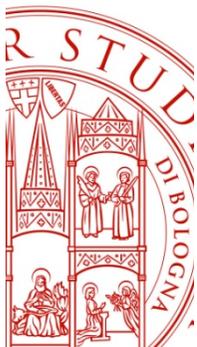


Table 2 Allele frequencies of the *MUC4* g.8227C>G polymorphism in different Italian pig breeds

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

^aTwo generation unrelated pigs of the selective genotyping study using backfat thickness estimated breeding value.

^bTwo generation unrelated pigs of the selective genotyping study using average daily gain estimated breeding value.

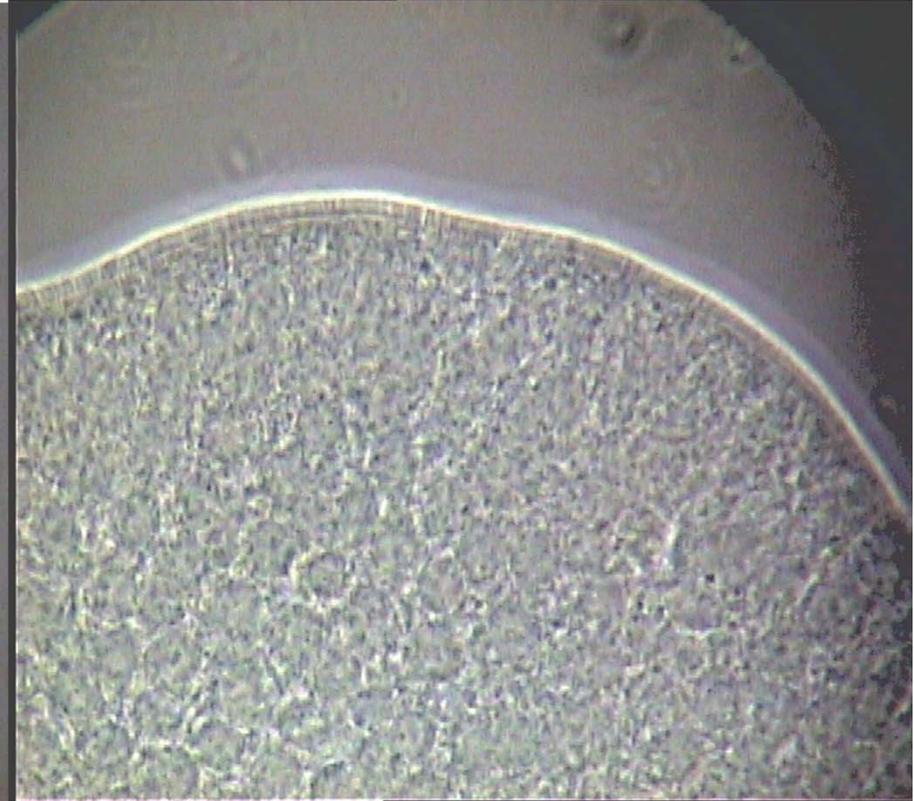


Host genotype vs microbiota

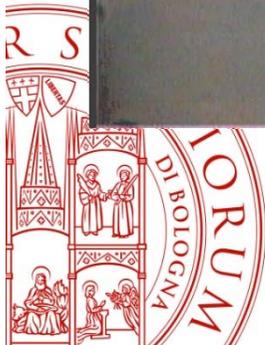
Host genotype affect the presence of specific receptors for ETEC F4ac on the intestinal villi of pig



Susceptible subject



Not susceptible subject



Host genotype vs Microbiota

Genotype blood types and Glycomic

Mäkivuokko et al. *BMC Microbiology* 2012, **12**:94
<http://www.biomedcentral.com/1471-2180/12/94>

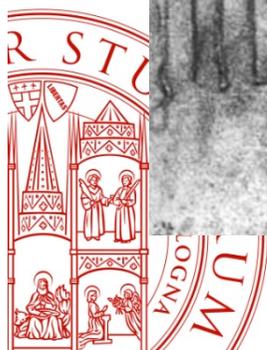
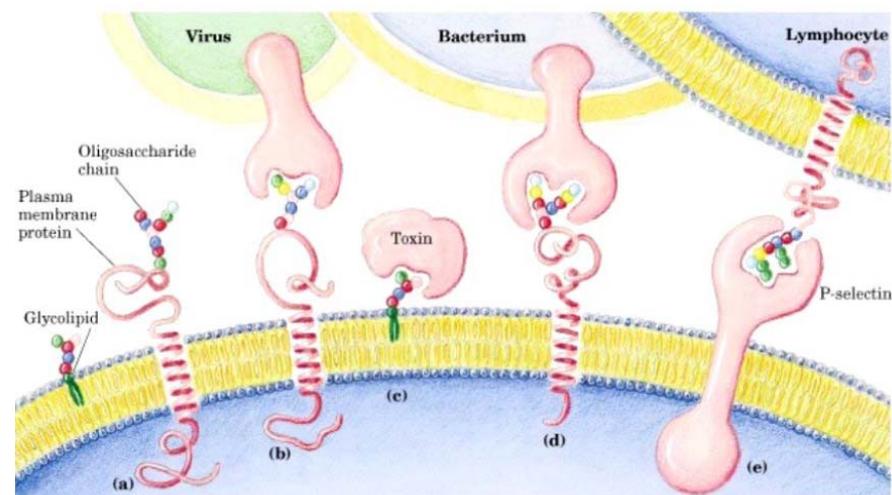
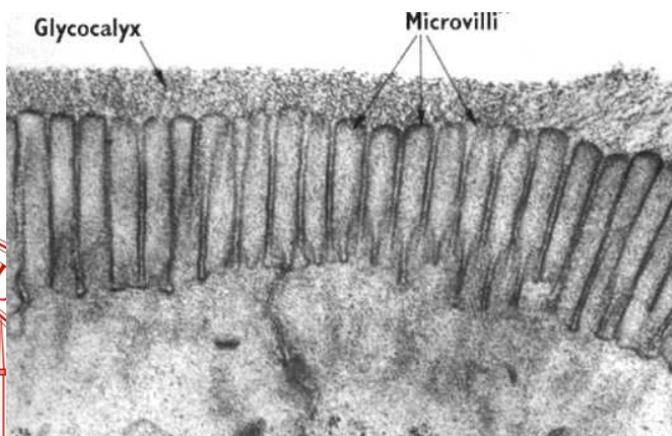


RESEARCH

Open Access

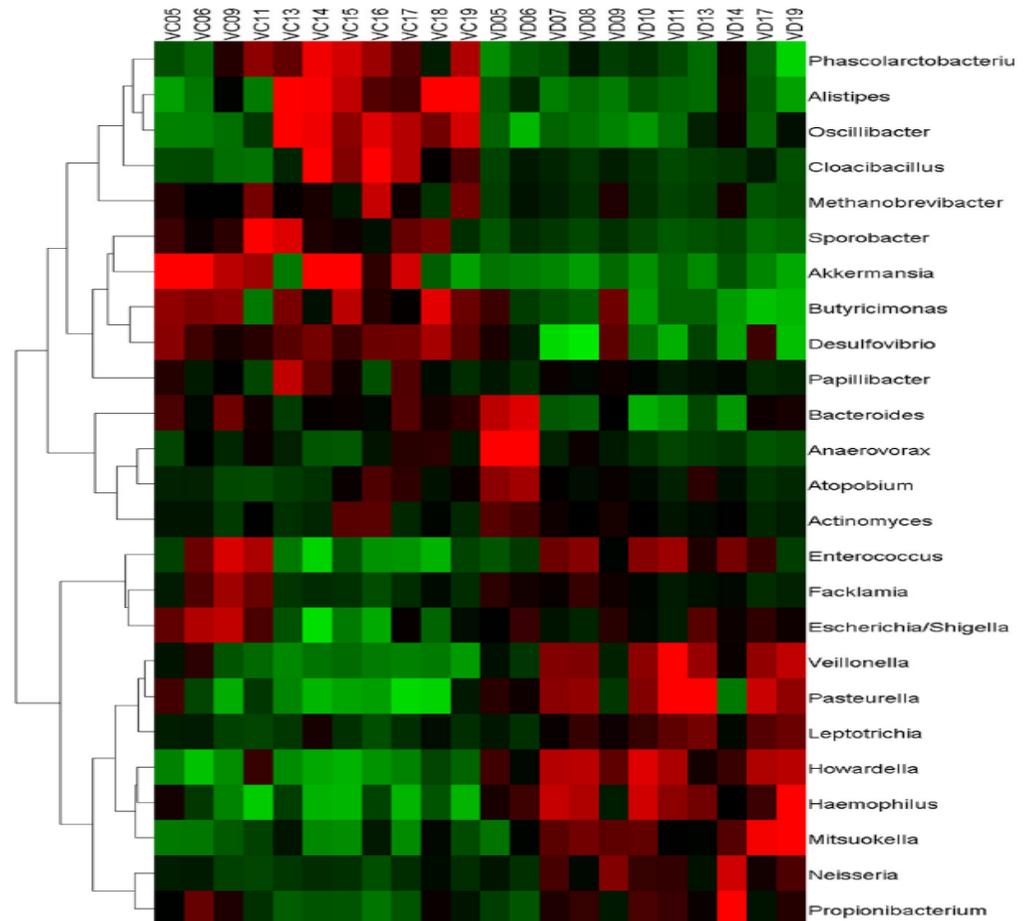
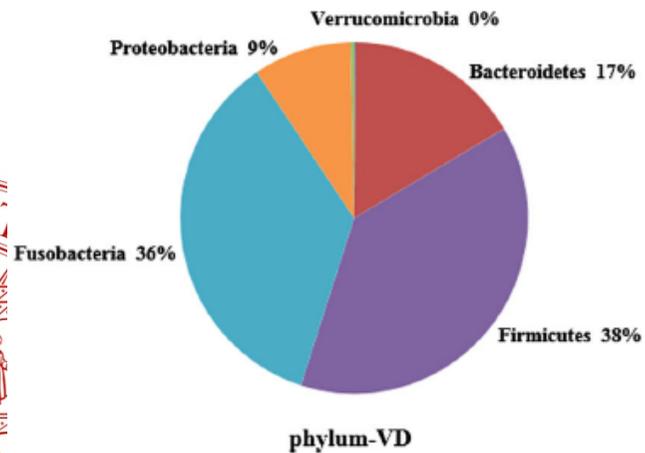
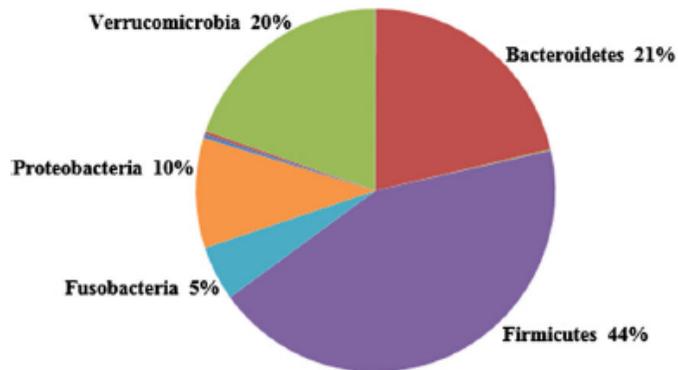
Association between the ABO blood group and the human intestinal microbiota composition

Harri Mäkivuokko^{1*}, Sampo J Lahtinen², Pirjo Wacklin¹, Elina Tuovinen¹, Heli Tenkanen¹, Janne Nikkilä¹, Marika Björklund², Kari Aranko¹, Arthur C Ouwehand² and Jaana Mättö¹



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

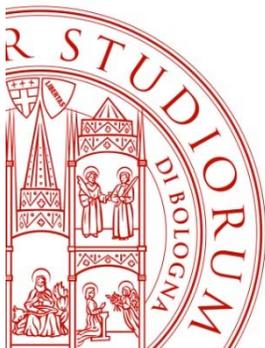
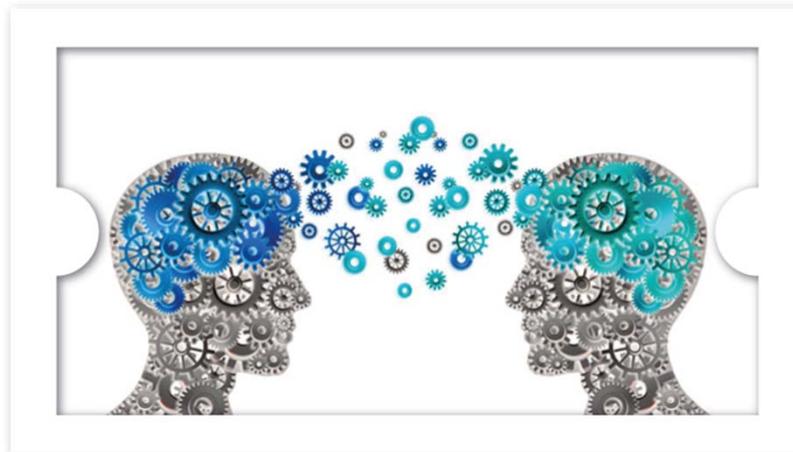


The work is not still finished

A multidisciplinary network to join the pieces

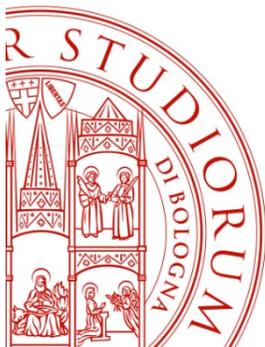
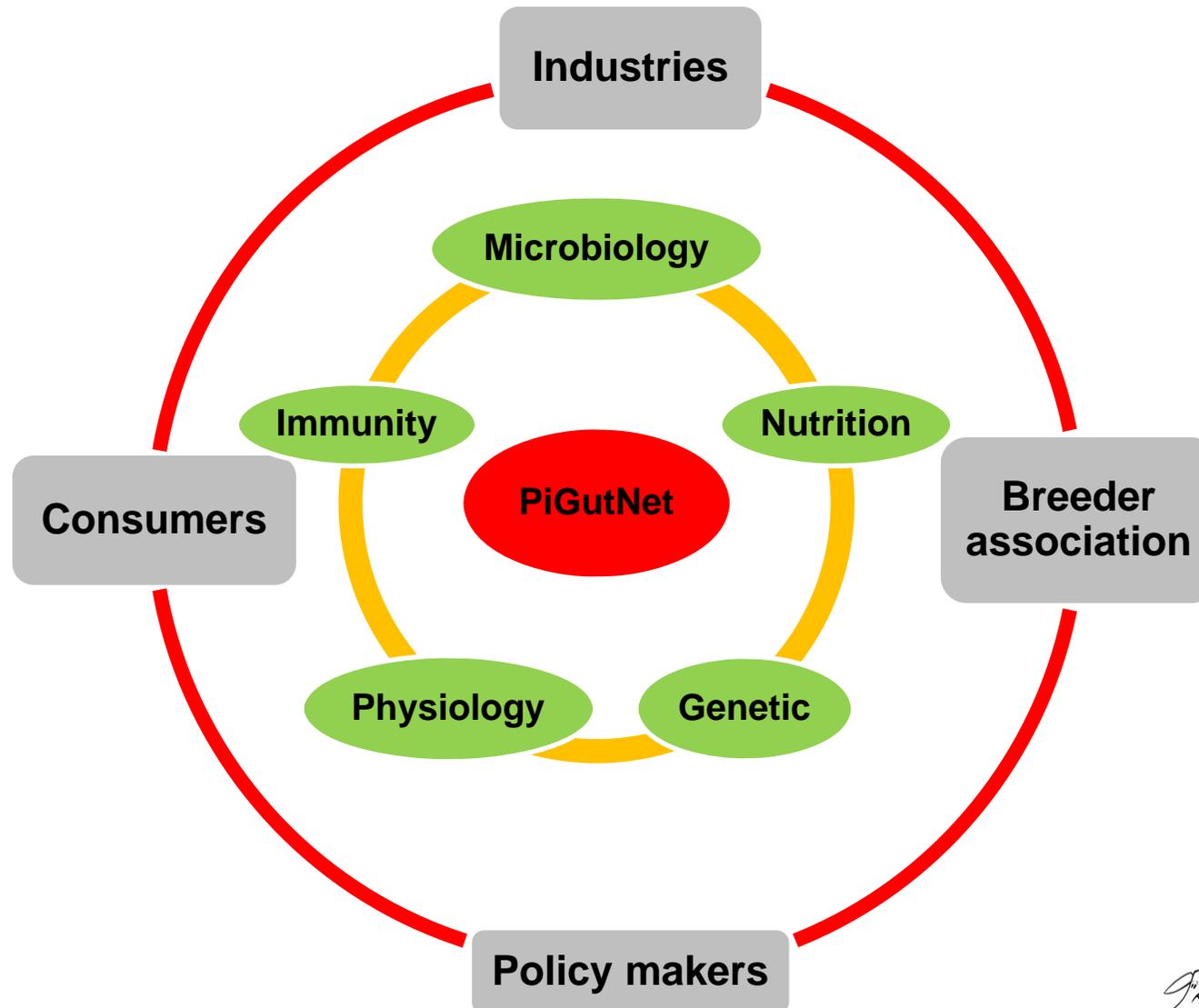


Disseminate the knowledge



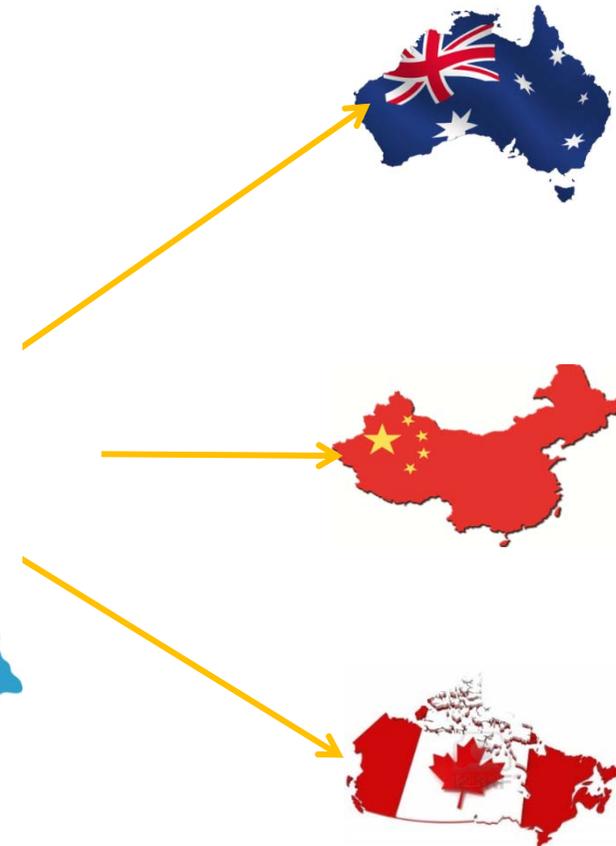
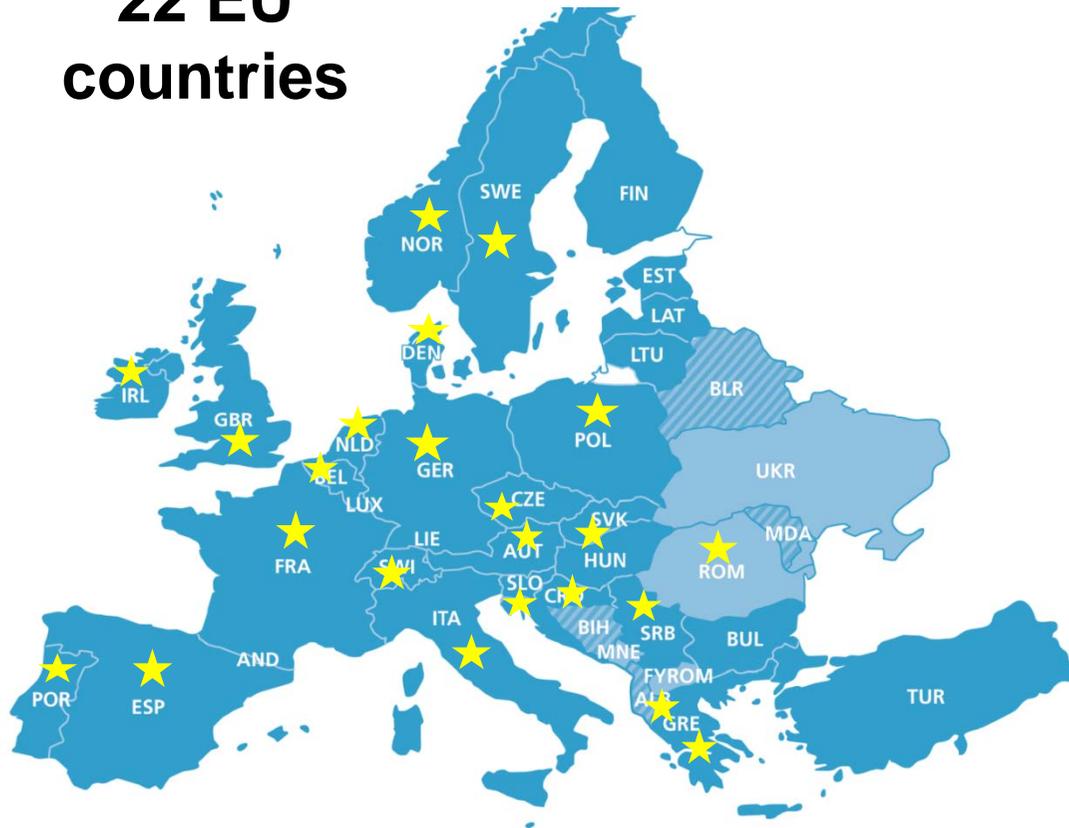
Target groups/end users

Multidisciplinary and Multiplayer Approach



The PiGutNet network

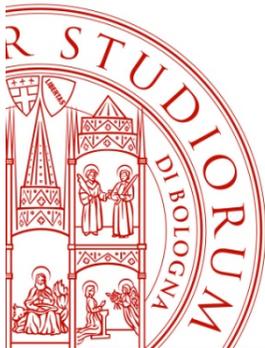
22 EU countries



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

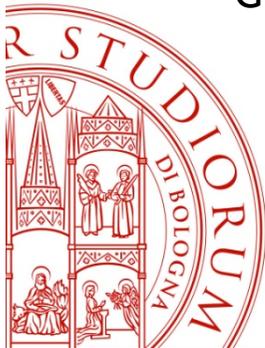
+

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



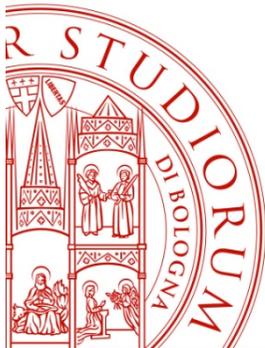
Dimension of the Action

- **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

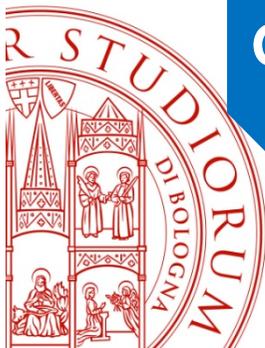
- **Environment and host genetic**

Gap
3

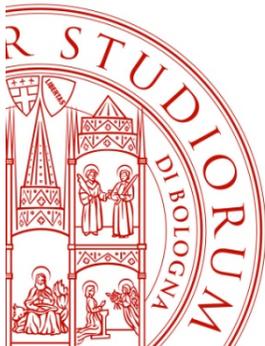
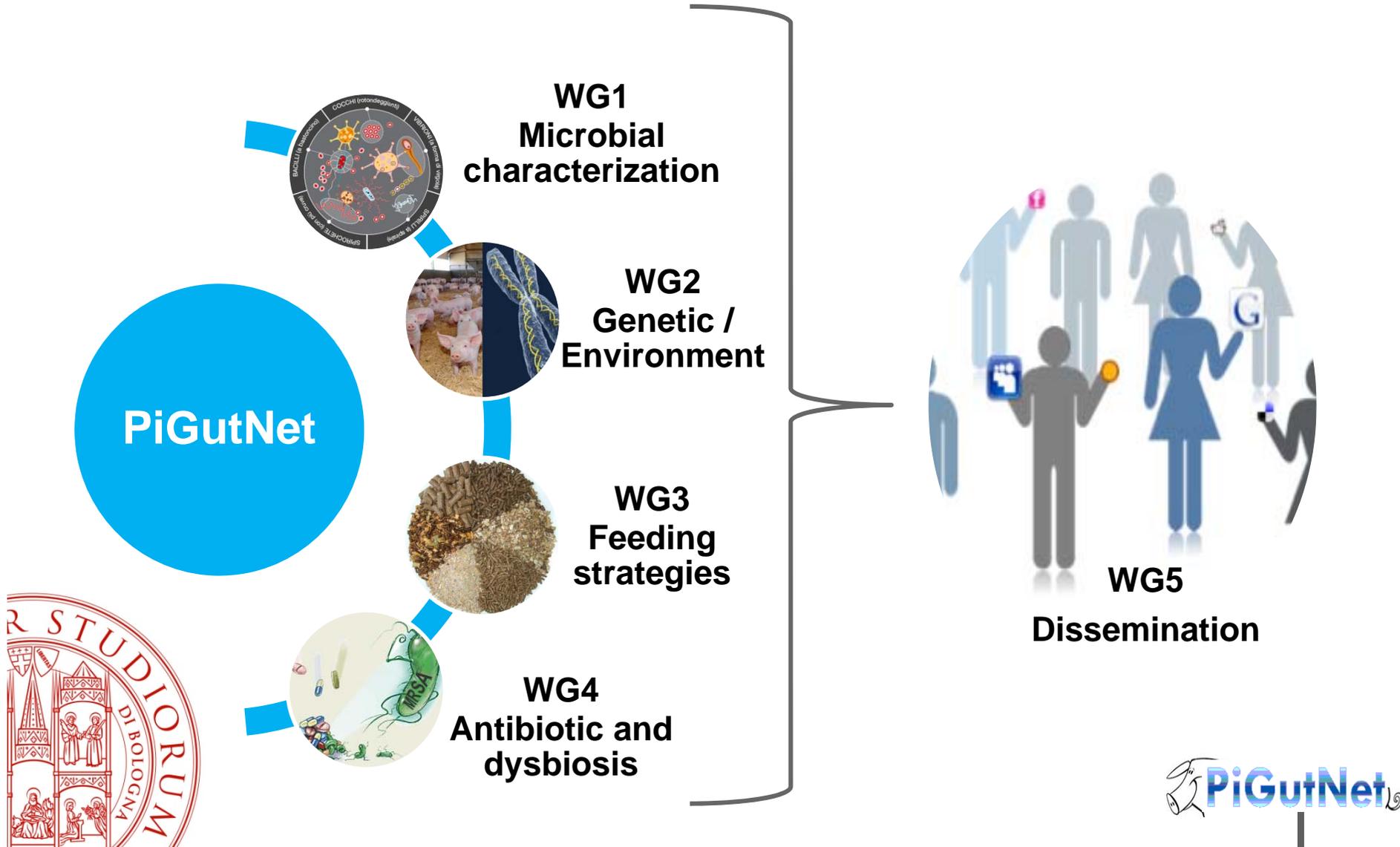
- **Feeding strategies**

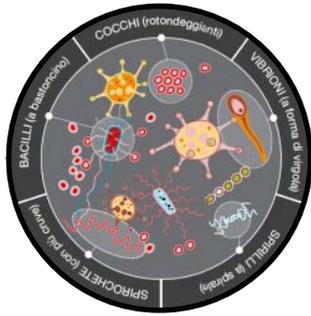
Gap
4

- **Antibiotic resistance**



SCIENTIFIC WORK PLAN





WORKING GROUP 1

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

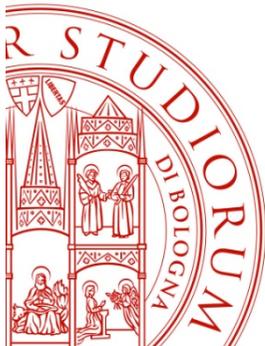
Leader: Dr. Sara Botti (IT)
Co-Leader: Prof. Mick Bailey (UK)



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

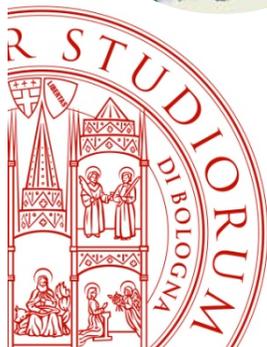
Leader: Prof. J. Francisco Perez (ES)
Co-Leader: Prof. Jan Erik Lindberg (SE)



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

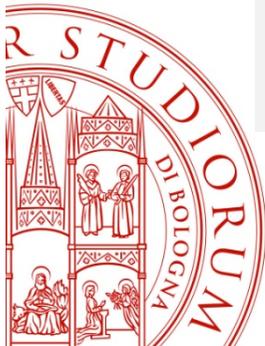
Leader: Prof. Olivera Djuragic(RS)

Co-Leader: Dr. Geert Bruggeman (BE)

Short Term Scientific Mission (STSM)

Leader: Dr. Marianna Roselli (IT)

Co-Leader: Prof. Hermann-Josef Rothkoetter (DE)



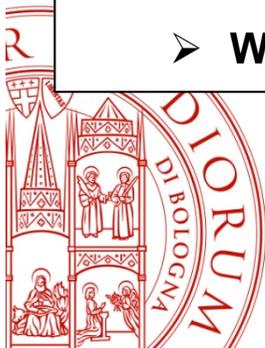
Some questions from the network

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?

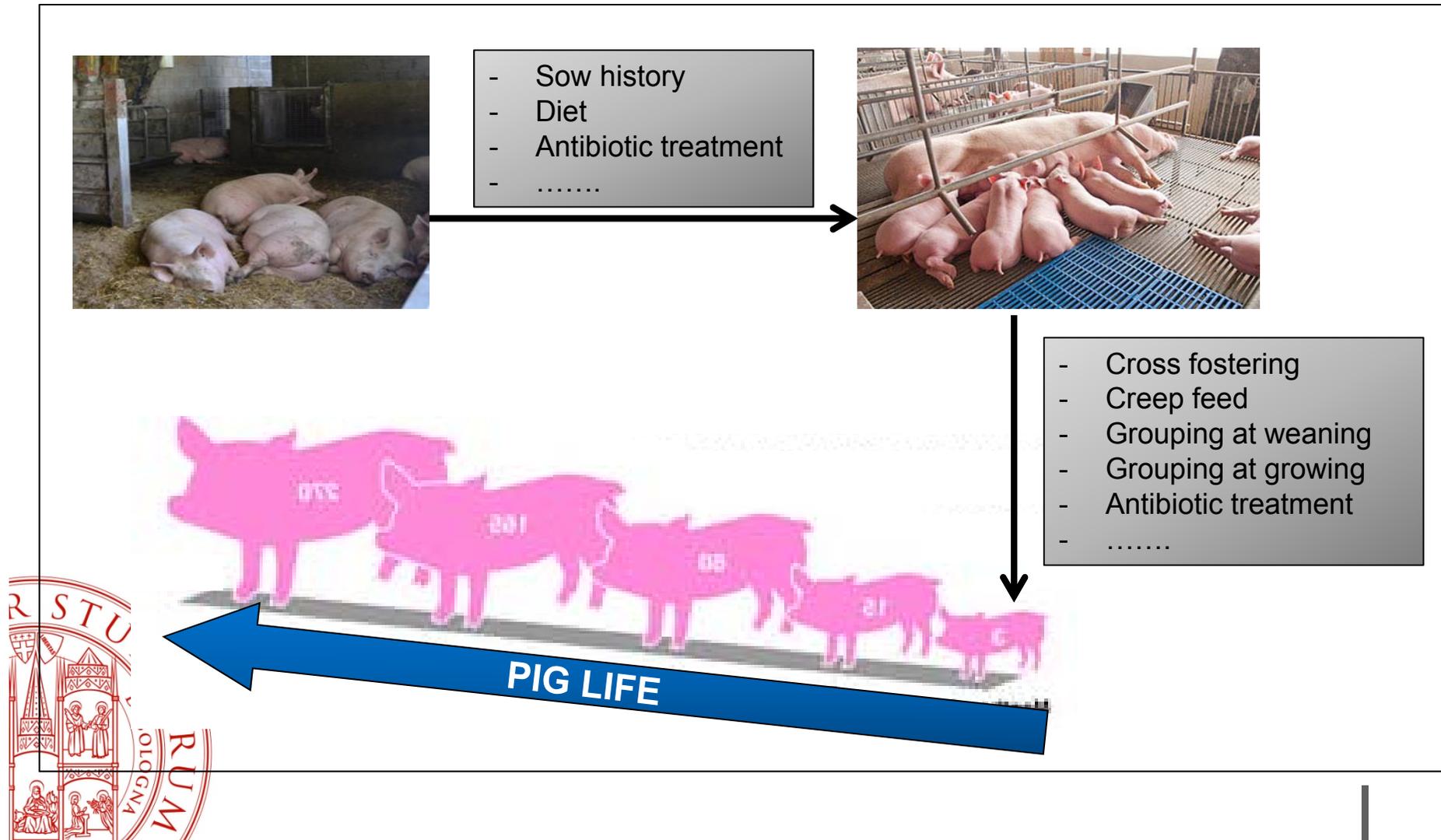
WHICH FACTORS AFFECT IT?

- 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?



Concept discussed between partners in PiGutNet network

The continuity concept



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

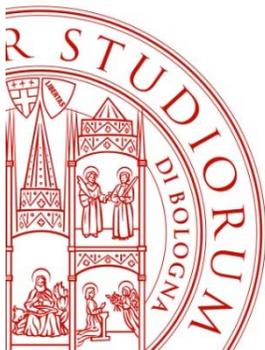


Final Considerations

- 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:

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Project website - www.pigutnet.eu

Follow PiGutNet on Twitter: [@PiGutNet](https://twitter.com/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)

This work is based upon work from COST Action FA1401, supported by COST (European Cooperation in Science and Technology)

