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July, 8-11, 2024
Fórum Evolución
Conference Centre
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**Comparative genomics of *Arcobacteraceae*
species and insight on *Arcobacter butzleri*
transcriptome during infection of a gut mucus
producer human cell line**

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www.foodmicro2024.com

July, 8-11, 2024
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and Auditorium
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Introduction

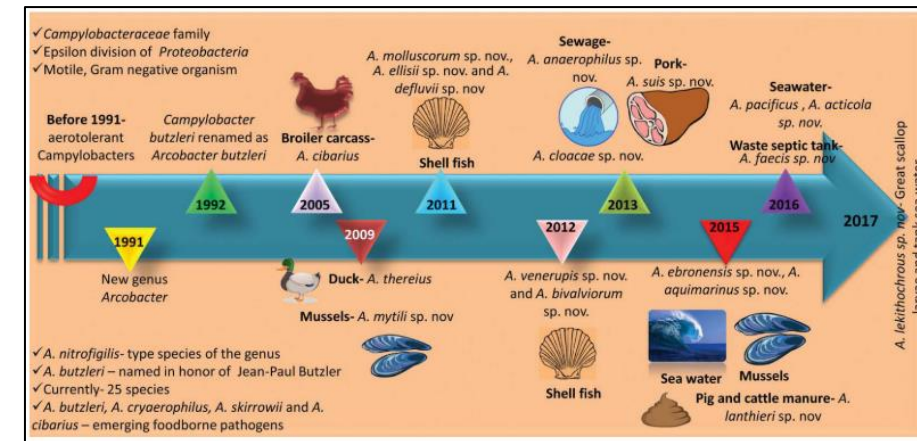
The species belonging to the *Arcobacteraceae* family are Gram negative bacteria isolated from different environments and animals.



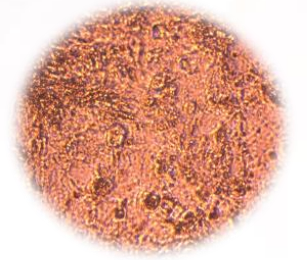
Species isolated from food and animals linked to different **gastrointestinal symptoms** in human and animals.

Animals usually remain asymptomatic.

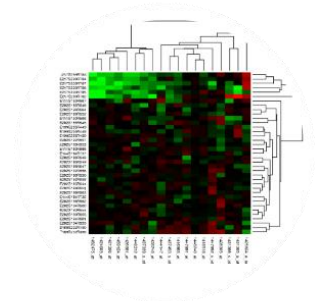
Different strains showed **antibiotic resistance (AR)** and AR genes.



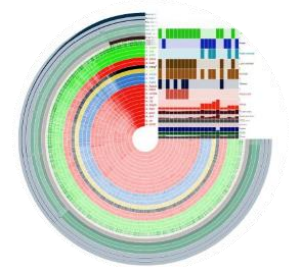
- Evaluation of *A. butzleri* virulence and genomic characteristics



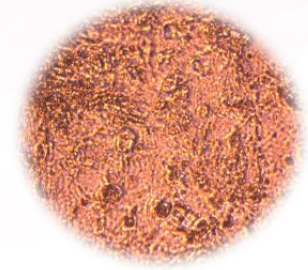
- Evaluation of *A. butzleri* transcriptome



- Study of the *Arcobacteraceae* pangenome



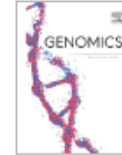
- Evaluation of *A. butzleri* virulence and genomic characteristics



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Genomics

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Original Article


Functional pangenome analysis reveals high virulence plasticity of *Aliarcobacter butzleri* and affinity to human mucus

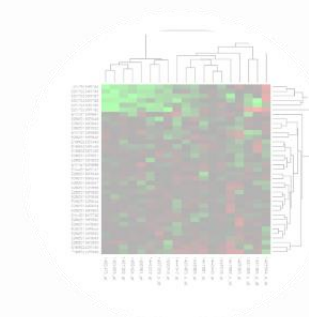
[Davide Buzzanca](#)^{a, b}, [Cristian Botta](#)^a, [Ilario Ferrocino](#)^a, [Valentina Alessandria](#)^a,
[Kurt Houf](#)^b, [Kalliopi Rantsiou](#)^a  

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Evaluation of the relationship between:

Isolation sources,
In vitro virulence,
 Genomes content

o M&M

32 *A. butzleri* strains

isolated from:

- Human (12)
- Pig (15)
- Cow (1)
- Sheep (1)
- Dog (1)
- Chicken (1)
- Horse (1)

+

A. butzleri virulence

properties on *in vitro* intestinal models
 (Colonization and invasion)

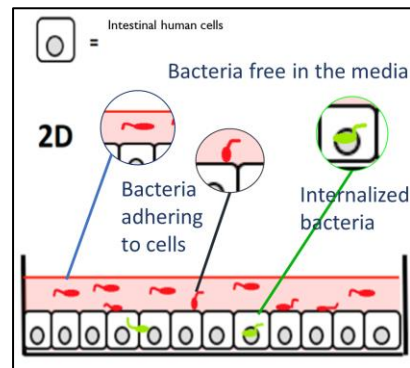
+

Whole genome analysis (Illumina)

- Functional annotation
- Gene enrichment
- Pangenome study

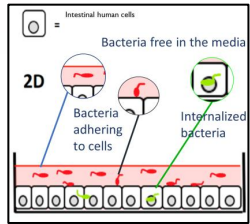
Not mucus producers
 (NMP)
 (Caco-2)
 (Caco-2 + HT29)

Mucus producer (MP)
 (Caco-2 + HT29 MTX)



In vitro cell models

(A) Higher colonization on **mucus** producer cell lines

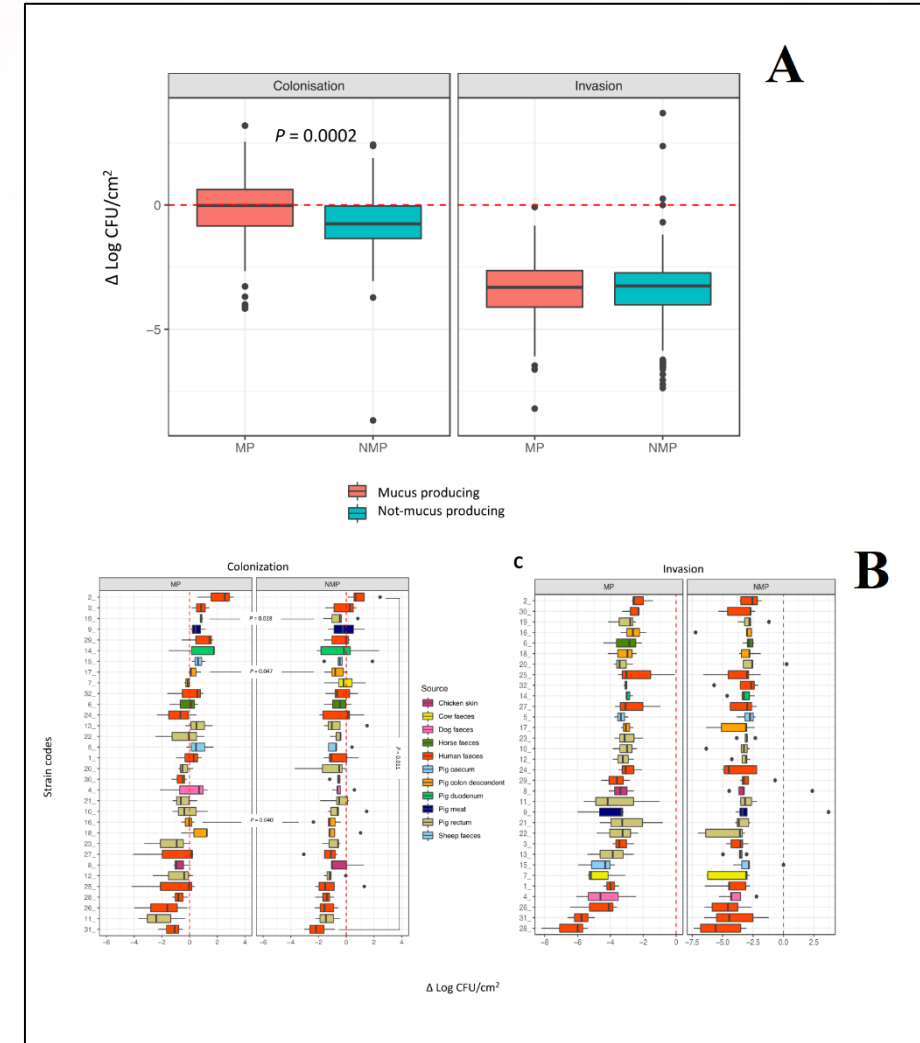


(A, B) COLONIZATION OF ALL STRAINS ON ALL MODELS

7, 26, 28, 31 no invasion on MP

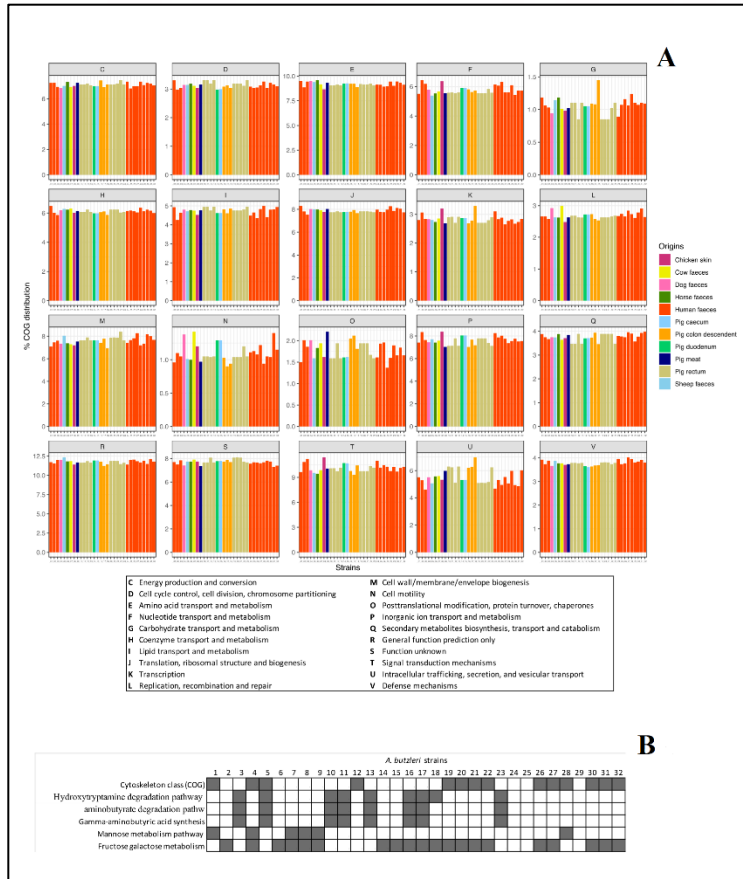
28 no invasion on NMP

(B) Absence significant difference between strains colonization
 (tendence to a higher colonization of the strain 2 from human
 contraposed to the strain 31 from human)



o Pangenome

Metabolic pathways
 (A) (B) Wide presence of gene related to aminoacidic metabolism and transport

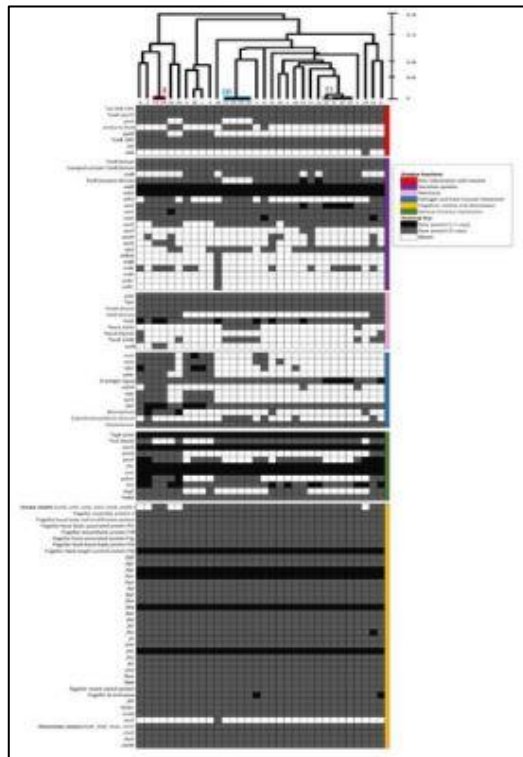


Open pangenome (A, B, C)
 High variability in LPS related genes



114 genes involved in different virulence associated functions:

○ Virulence genes



gene presence absence
1/0 matrix

- **Pili - Flagella (mucus motility)**
- **Adhesion**
- **Invasion**
- **Pathogenesis Regulation**
(e.g. *phoP* and *phoQ*, typical of *Salmonella enterica* and *Escherichia coli*)
- **Bacterial capsule (adhesion)**
- **Secretion systems**
(e.g. Type II protein secretion linked to survive in the host and virulence)
- **Hemolysis**

**O-antigen ligase gene linked to a higher colonization trend
(part of LPS antigen production)**



Transcriptome Analysis of *Arcobacter butzleri* Infection in a Mucus-Producing Human Intestinal *In Vitro* Model

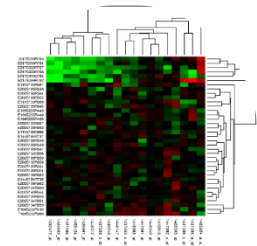
Davide Buzzanca,^{a,b} Valentina Alessandria,^a Cristian Botta,^a Negin Seif Zadeh,^a Ilario Ferrocino,^a Kurt Houf,^b Luca Cocolin,^a Kalliopi Rantsiou^a

^aDepartment of Agricultural, Forest and Food Sciences, University of Turin, Turin, Italy

^bDepartment of Veterinary and Biosciences, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

- Evaluation of *A. butzleri* transcriptome

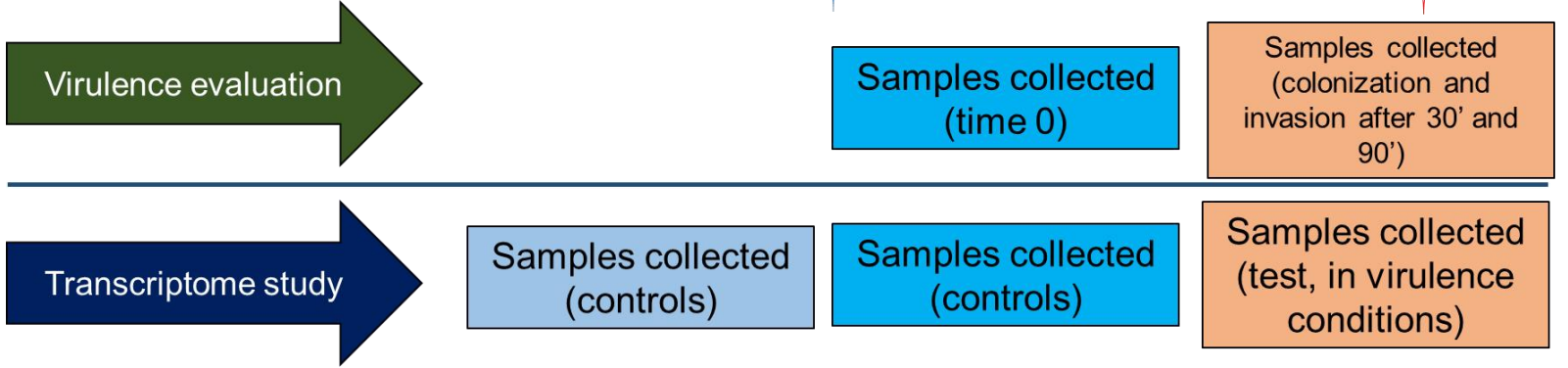
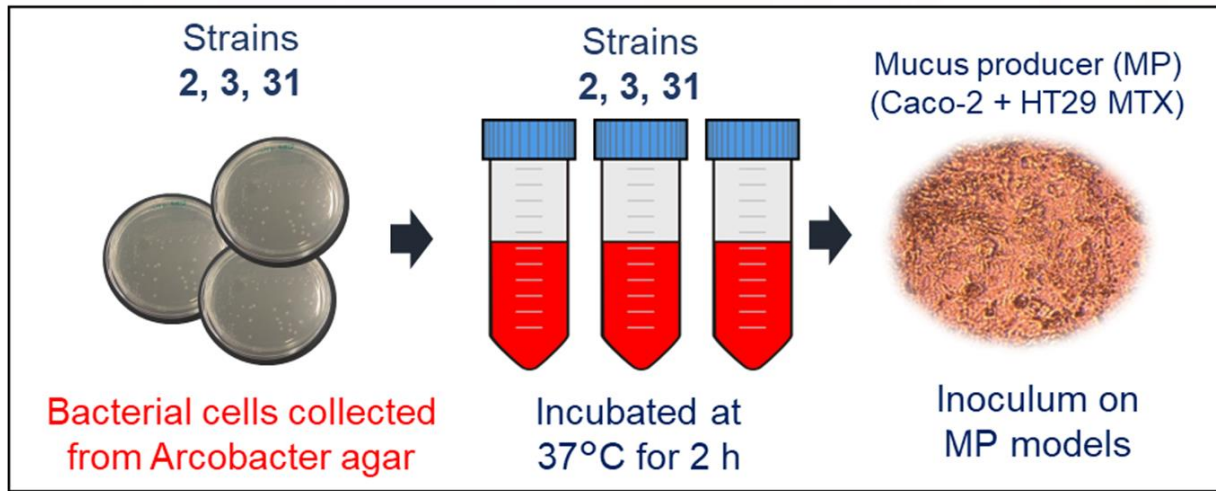
- Study of the *Arcobacteraceae* pangenome



○ RNAseq - strains selection

Selection of 3 *A. butzleri* strains from the previous whole genomes studies and *in vitro* studies:

- LMG 11119 (human; Italy; work code 2)
- LMG 10828^T (human; U.S.A; work code 3)
- 31 (human; Belgium; work code 31)

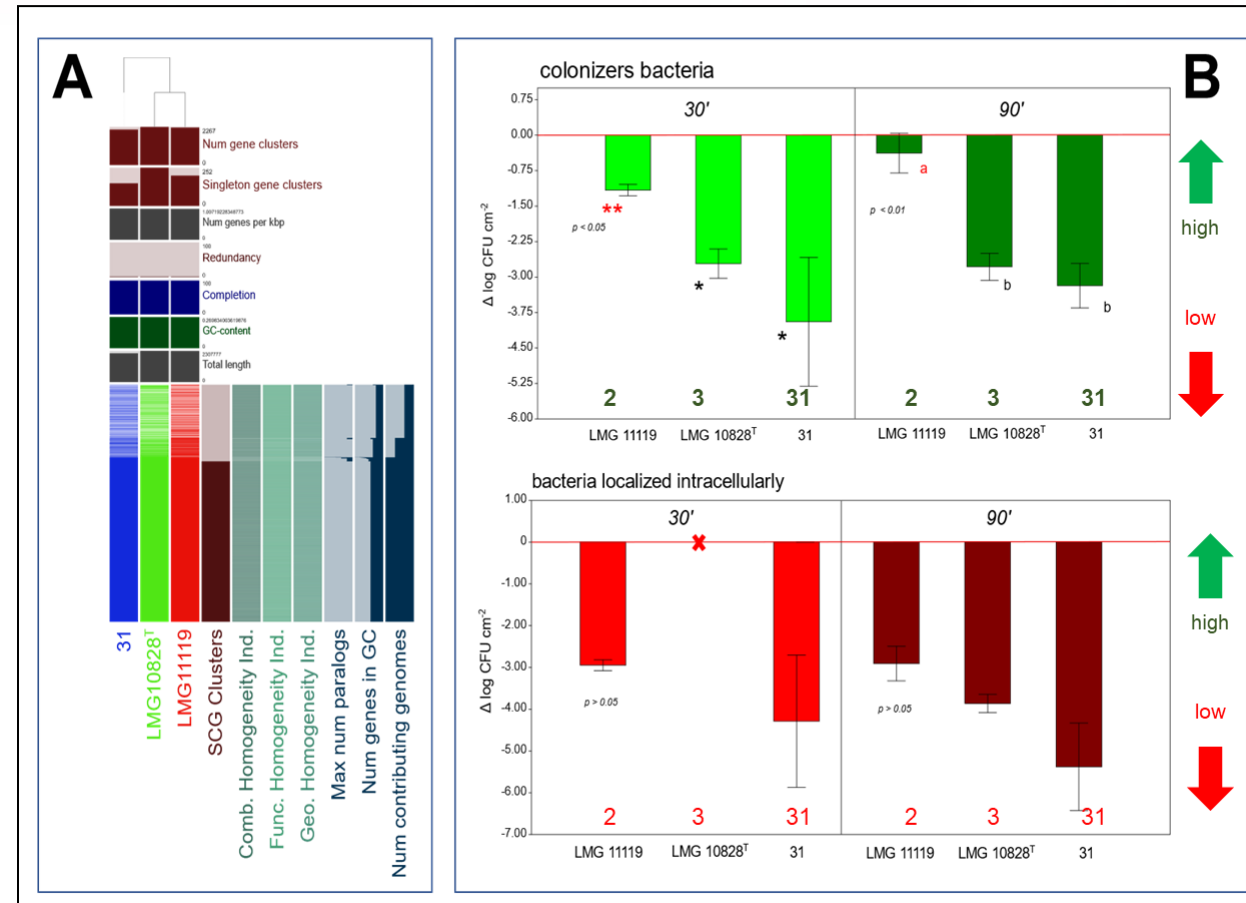


o *In vitro* colonization

(A). The three strains showed differences at genome level

(B). The strains showed difference during *in vitro* test:

- Higher colonization of the strain LMG 11119 (work code; 2)
- LMG 10828^T (work code; 3) was not invasive at 30'



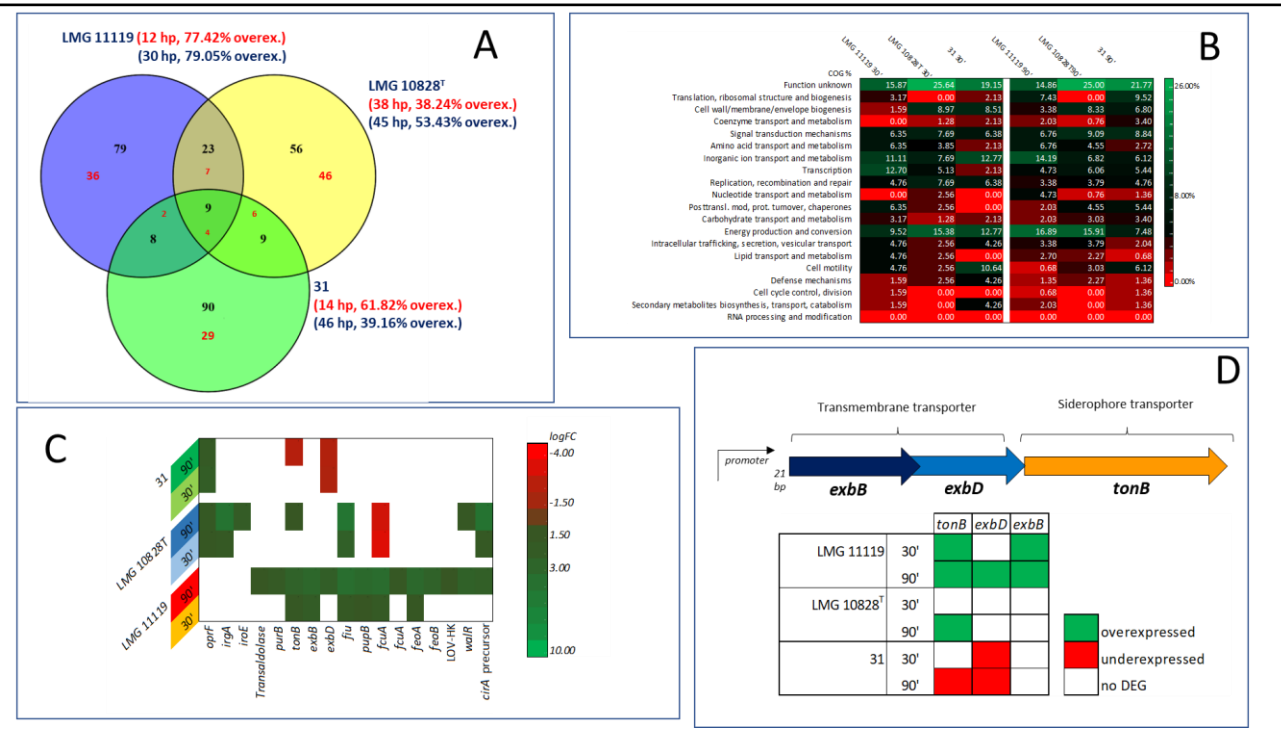
oDEGs

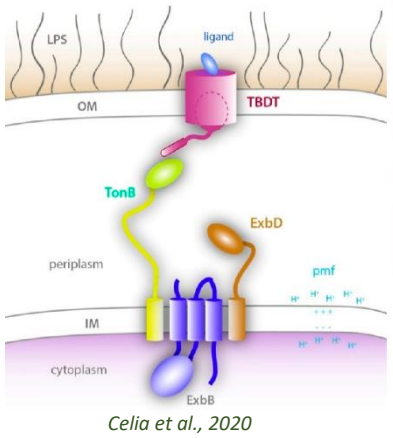
(A). Strain LMG 11119 (higher colonization) showed a higher ratio of overexpressed DEGs (48/62 at 30', 128/161 at 90')

High number of DEGs with functions unknown (B)

3 currently considered putative virulence genes overexpressed:
 LMG 10828^T: *cadF*, *irgA* (30', 90'); *iroE* (90')
 31: *cadF* (30', 90')

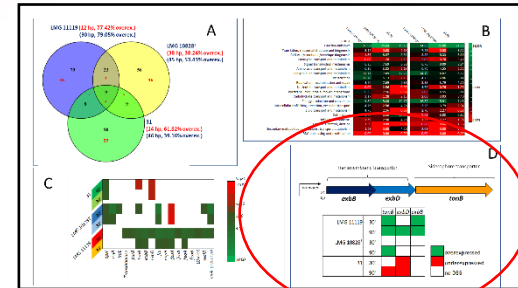
Comparison between bacteria in DMEM (120' minute of incubation) and in contact with *in vitro* cell models (logFC < - 1.5 > 1.5; p-value < 0.05, FDR corrected)





TonB system (TonB, ExbB and ExbD proteins)

linked to the bacterial inner membrane involved in iron transport and assimilation

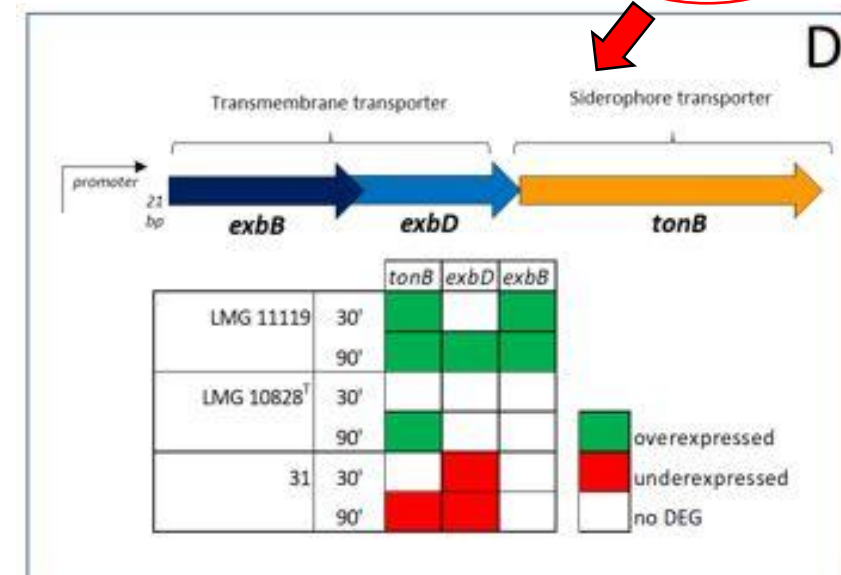


(D). TonB system genes were overexpressed in the strain that showed **higher colonization (LMG 11119)** together with other iron related genes:

(*fiu* (Catechol siderophore receptor Fiu), *pupB* (receptor for the siderophores ferric pseudobactin BN8), *fcuA* (ferrichrome receptor), *feoA*, *feoB* (putative Fe²⁺ uptake protein))

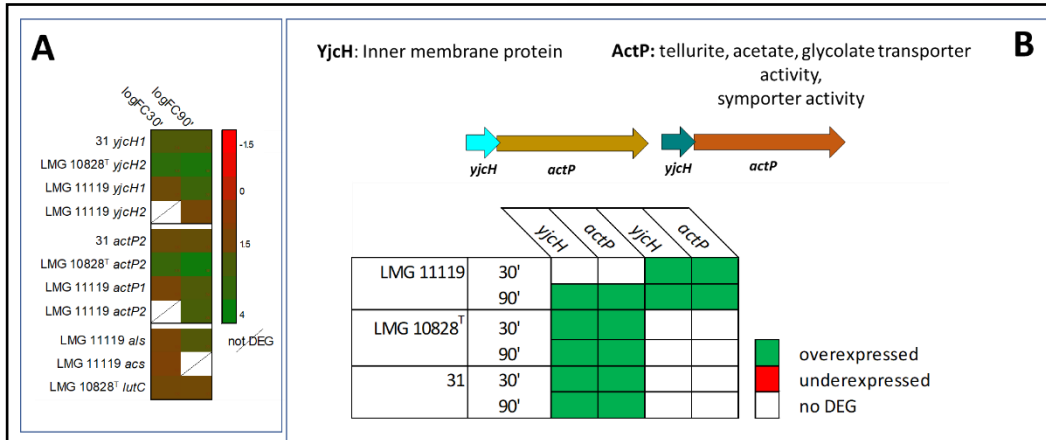
The reference strain (LMG 10828^T) showed only the overexpression of ***tonB***

Underexpression of the TonB related genes in the strain **31**

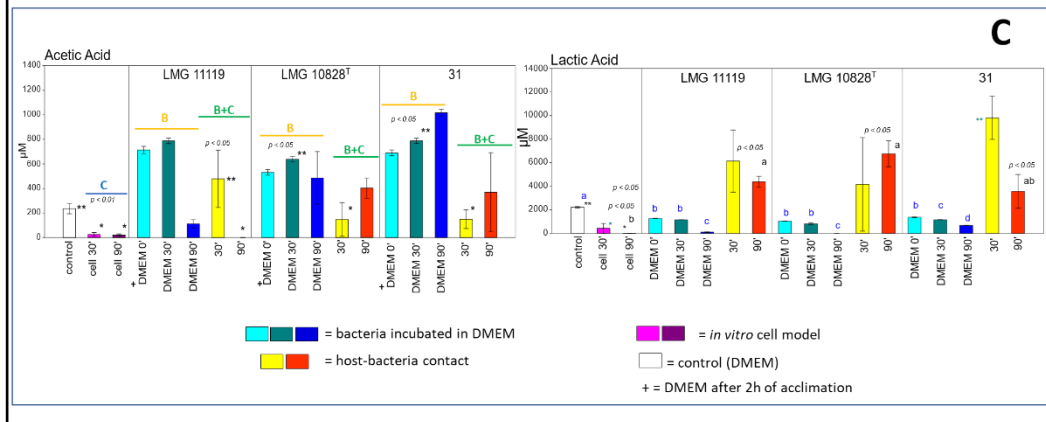


o DEGs - organic acids

In the three *A. butzleri* strains was observed the differential expression of organic acid related genes (A, B) and a dynamics in the organic acid concentration (C).



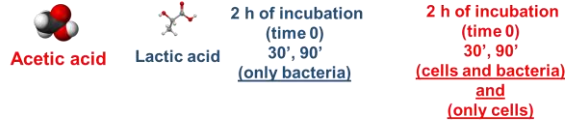
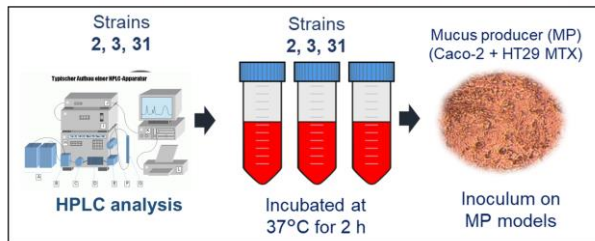
(A, B). Overexpression of *yjch* and *actP* genes
 Deletions of this operon in *Escherichia coli* = virulence and colonization capability decrease in avian lungs (Zhige et al., 2019).



(A, B). Two copies overexpressed in the strain LMG 11119 (higher colonization)

(A). LMG 11119 showed the overexpression of Acetyl-CoA synthetase

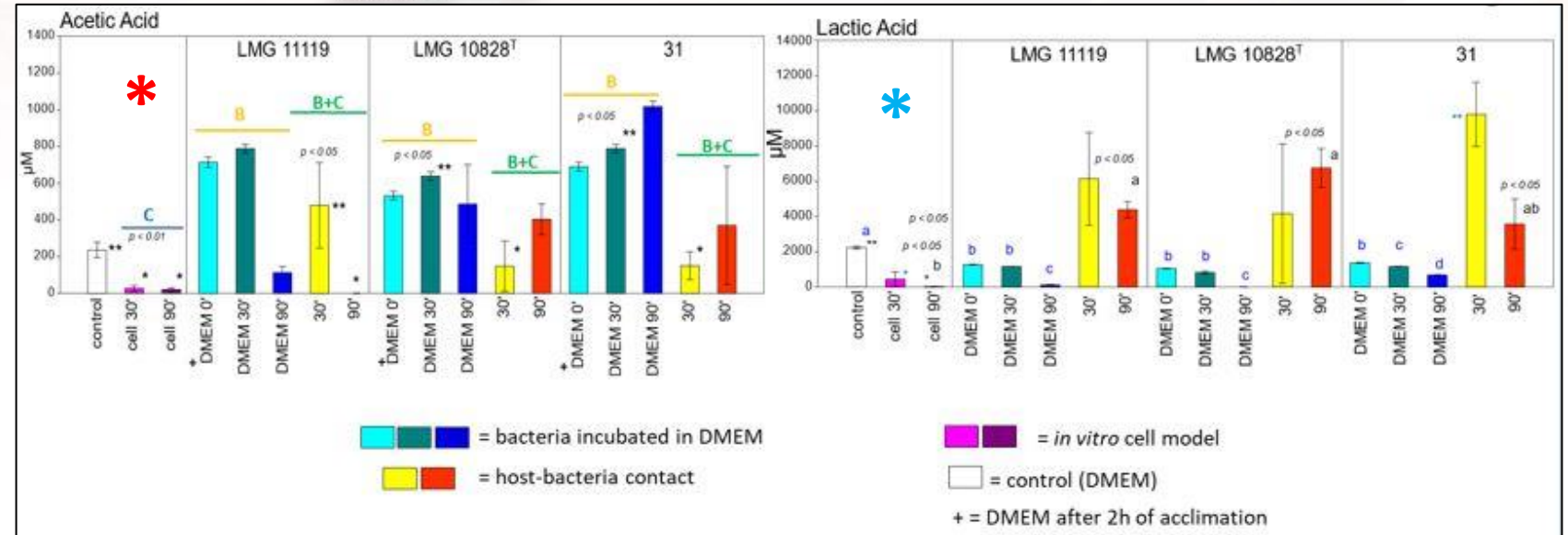
Organic acids



Acetic acid *

The comparison between DMEM and DMEM with *A. butzleri* after 150' showed the increase in acetic acid concentration.

Not detectable acetic acid for the strain LMG 11119 (90' of infection) (higher colonization)



Lactic acid *

Increase of DMEM lactic acid concentration of *A. butzleri* in contact with host cell LMG 10828^T and LMG 11119 (90'), 31 (30')

Increase of lactic acid concentration without related *A. butzleri* DEGs suggests the production of this molecule by host cell line (stress response).

This aspect has been observed in Caco-2 as a response to *E. coli* infection (He et al., 2013).

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Arcobacteraceae comparative genome analysis demonstrates genome heterogeneity and reduction in species isolated from animals and associated with human illness

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^b Department of Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy

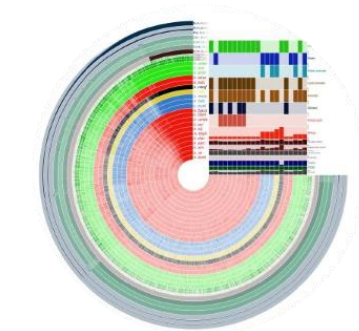
^c Laboratory of Microbiology, Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, Karel Lodewijk Ledeganckstraat 35, 9000 Ghent, Belgium

- Study of the *Arcobacteraceae* pangenome

characteristics



1e

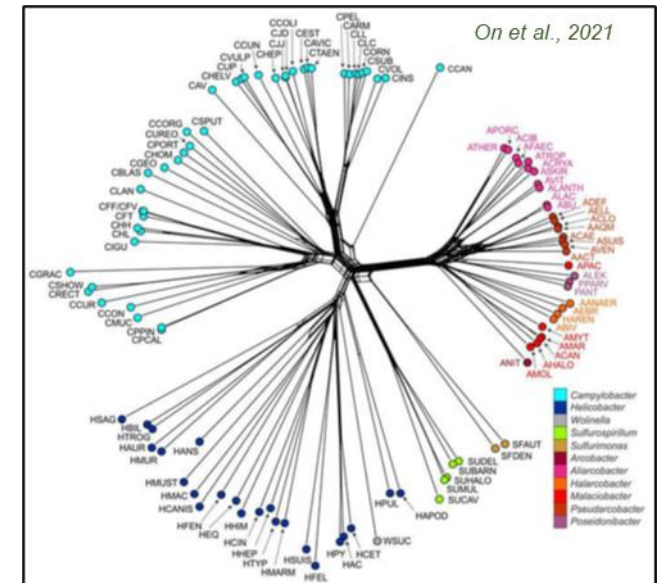
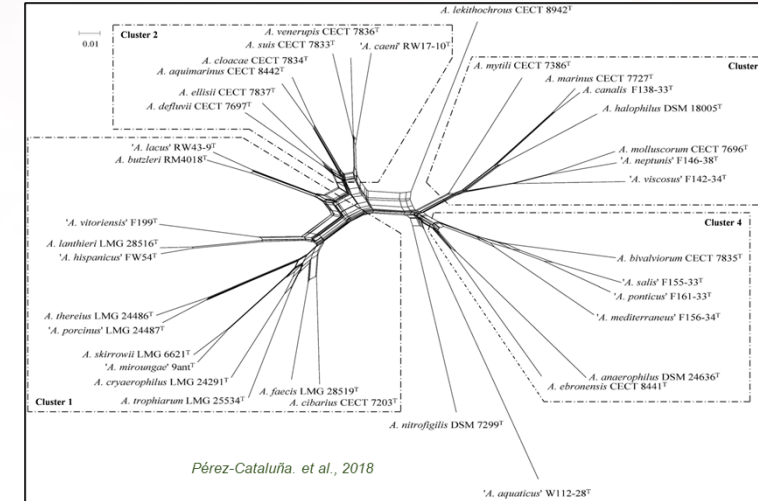


Pangenome - type strains selection

Pangenome study of *Arcobacteraceae* to evaluate the presence of different bacterial genera and relationships between the species

Arcobacteraceae species have been isolated from different environments and animals.

Species	Strain	Group	Run accession
<i>Arcobacter butzleri</i>	LMG 10828 ^T	1	SRR18076128
<i>Arcobacter cibarius</i>	LMG 21996 ^T	1	SRR3664169*
<i>Arcobacter cryaerophilus</i>	LMG 24291 ^T	1	SRR7985382*
<i>Arcobacter cryaerophilus</i>	LMG 10829	1	SRR7985571*
<i>Arcobacter porcinus</i>	LMG 24487 ^T	1	SRR18076131
<i>Arcobacter skirrovii</i>	LMG 6621 ^T	1	SRR18076130
<i>Arcobacter thereus</i>	LMG 24486 ^T	1	SRR18076129
<i>Arcobacter trophiarum</i>	LMG 25534 ^T	1	SRR18076127
<i>Arcobacter vandammei</i>	LMG 31429 ^T	1	SRR18076126
<i>Arcobacter vitoriensis</i>	LMG 30050 ^T	1	SRR18076123
<i>Arcobacter faecis</i>	LMG 28519 ^T	1	SRR18076124
<i>Arcobacter lacus</i>	LMG 29062 ^T	1	SRR5221256*
<i>Arcobacter lanthieri</i>	LMG 28516 ^T	1	SRR18076125
<i>Arcobacter ellisii</i>	LMG 26155 ^T	2	SRR7588928*
<i>Arcobacter venerupis</i>	LMG 26156 ^T	2	SRR5914676*
<i>Arcobacter suis</i>	LMG 26152 ^T	2	SRR7591528*
<i>Arcobacter halophilus</i>	CCUG 53805 ^T	3	SRR7587110*
<i>Arcobacter molluscorum</i>	LMG 25693 ^T	3	SRR7591199*
<i>Arcobacter mytili</i>	LMG 24559 ^T	3	SRR7588217*
<i>Arcobacter bivalviorum</i>	LMG 26154 ^T	4	SRR7586655*
<i>Arcobacter nitrofigilis</i>	LMG 7704 ^T	5	NC_014166.1*
<i>Campylobacter jejuni</i>	NCTC 11168 ^T	outgroup	NC_002163.1*
<i>Helicobacter pylori</i>	MT 5135 ^T	outgroup	NZ_CP071982.1*



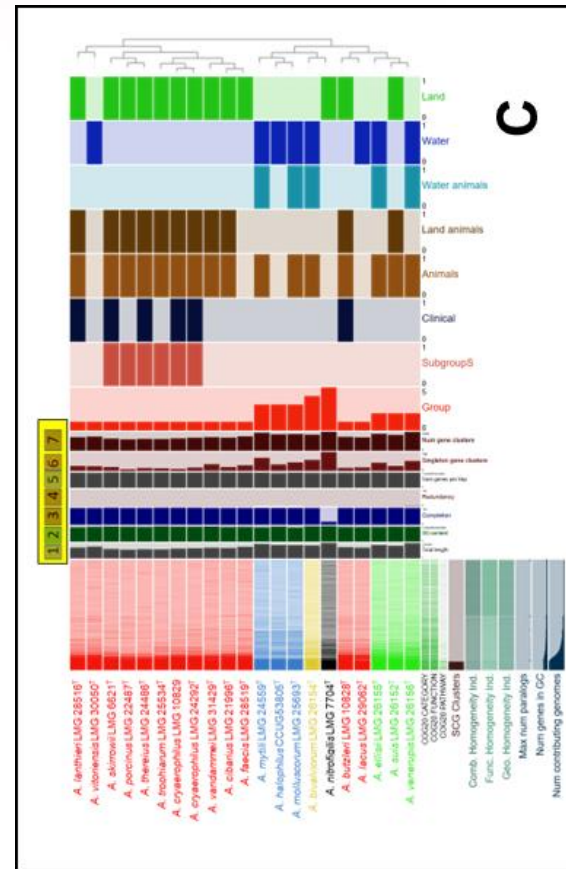
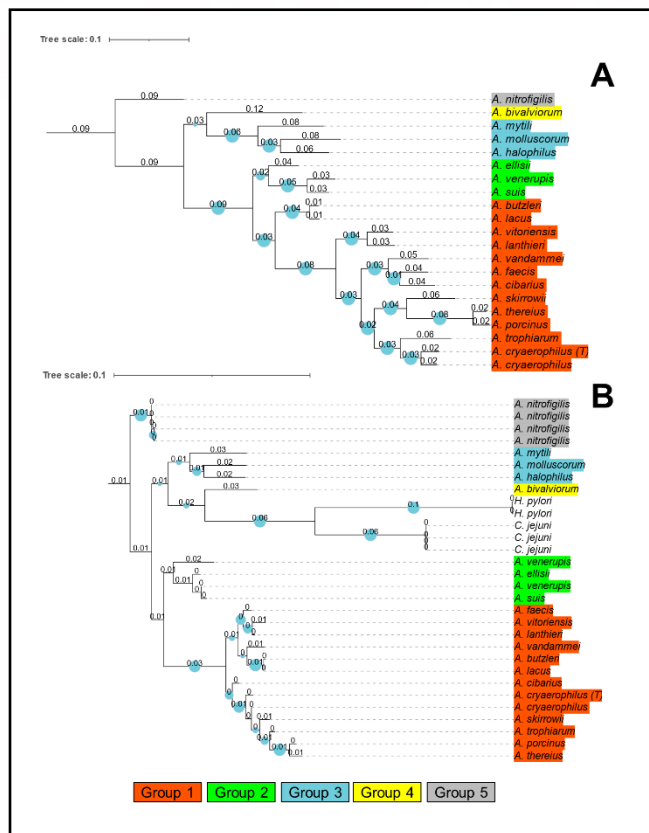
Genome size

Smaller genome size of group 1 genomes
 (2.12 Mb, st. dev \pm 0.2 Mb, p-value < 0.05)

Further reduction of genome size in subgroup S
 (part of group 1);
 (*A. cryaerophilus*, *A. porcinus*, *A. skirrowii*, *A. thereius*
 and *A. trophiarum*)

Absence of a clear separation between the members of
 the 5 proposed groups and genera
 (different distances between and internal to the groups).

The genomic distance in some cases was higher within
 the same group than between different groups.

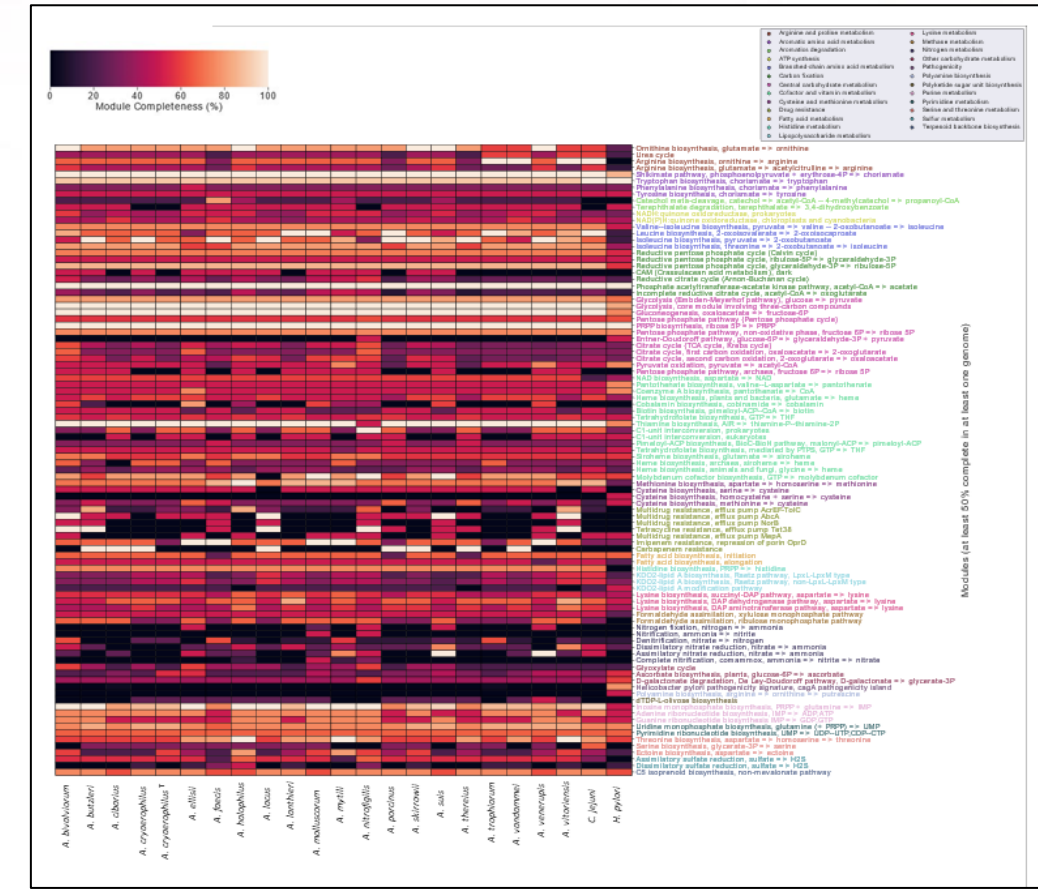


(A). bcgTree (107 core sequences) (B). 16S rRNA . C.) Anvi'o

Orthogroups

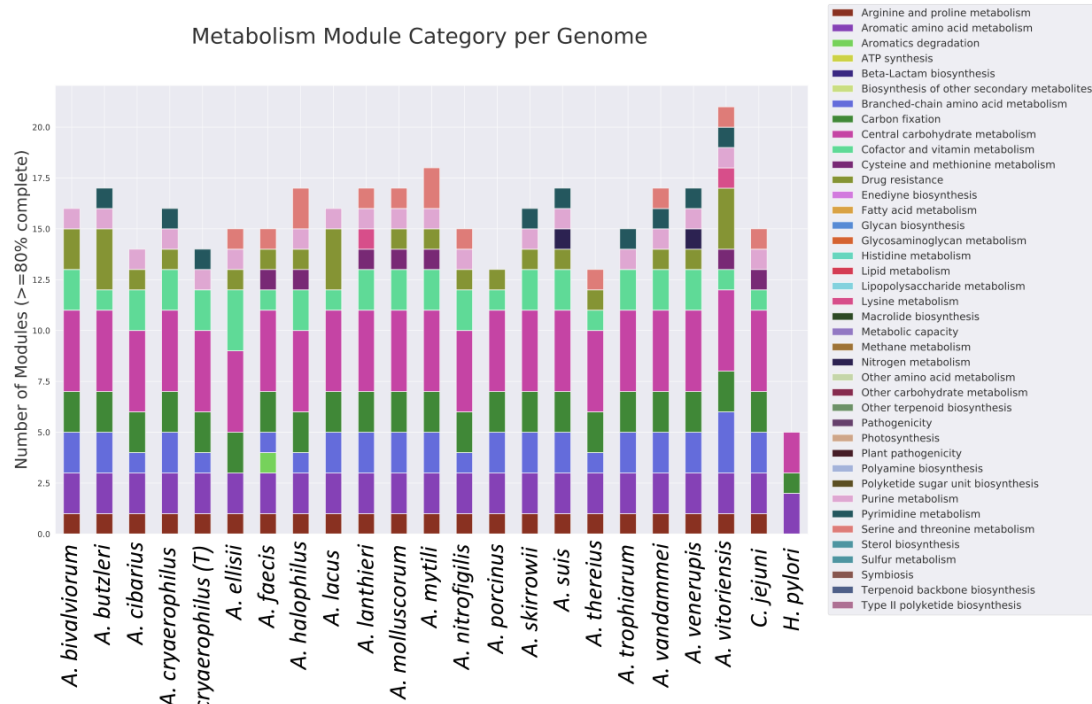
Higher Ratio of some orthogroups in group 1
 (% of specific classes on COGs total, EggNOG annotated)
**lipid transport and metabolism, cell motility, cell wall-
 membrane-envelope biogenesis, translation, cell division,
 chromosome partitioning and ribosomal structure,
 biogenesis, and cell cycle control.**

Comparison with groups 2-5 (*p-value* < 0.05)



Gene classes

Metabolism Module Category per Genome

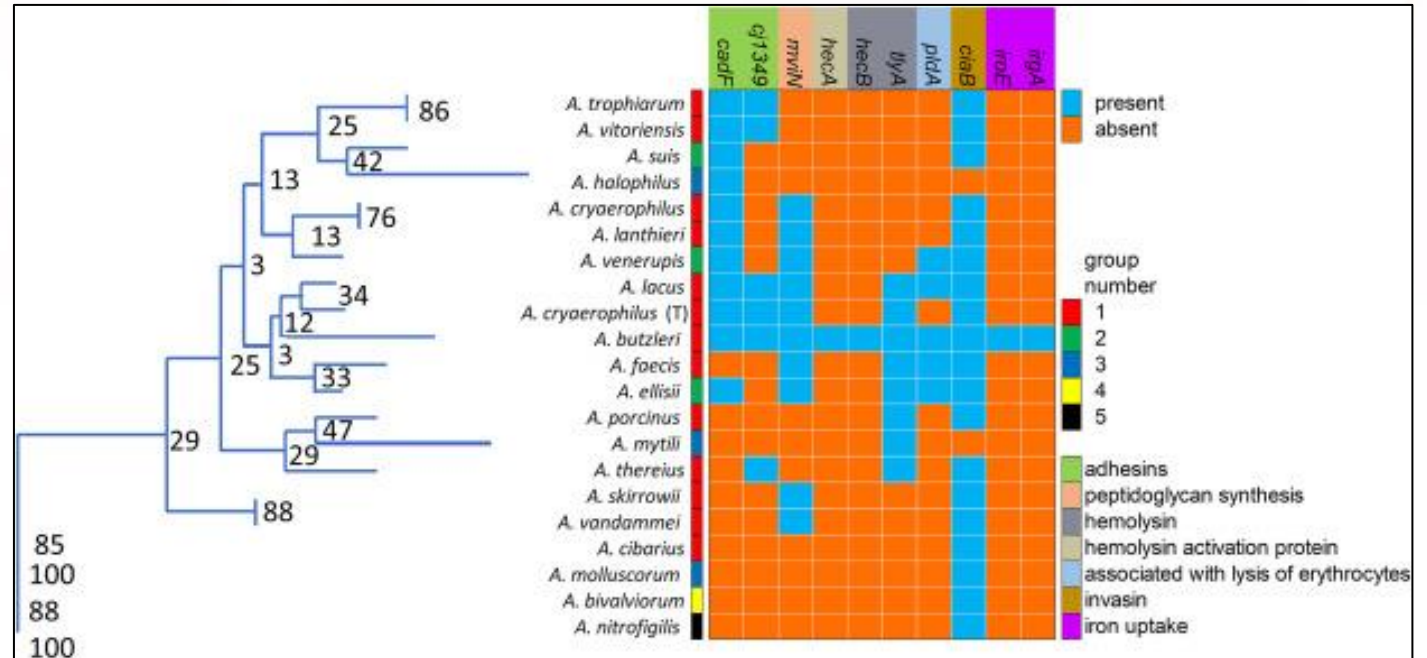


Group 1. positive correlation to antibiotic resistance genes

Group 2. cobalamin biosynthesis and assimilatory nitrate reduction

Group 3. positive correlation ectoine (osmotic resistance) (group related to water environment)

○ Putative virulence genes



Positive corr: Group 1: *cj1349* (fibronectin-binding protein). **Clinical: *ciaB*** (invasin)

mviN negative correlation to group 3 (water-water animals)

Specific orthogroups related to **TonB function** (iron transport and virulence) are **negatively and positively correlated** to group 1 (specific orthogroups in the different groups)

○ Conclusions

- The *in vitro* host-colonization of *A. butzleri* has been **confirmed** with a **favorable role of mucus**

- *A. butzleri* shows an **open pangenome**

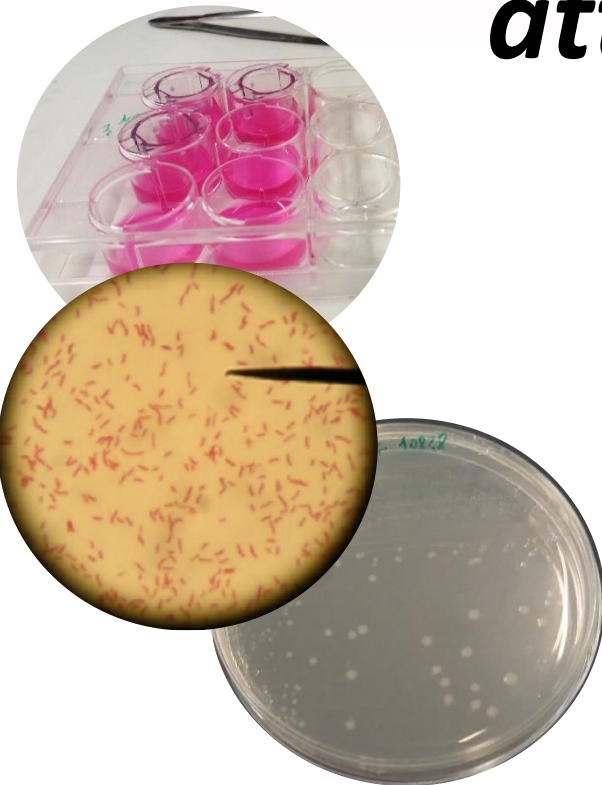
(relevant the variability of **LPS-related region**)

- Different **virulence-related genes** were **detected** in *A. butzleri* with different functions (adhesins, transcription regulation, cell wall biogenesis, biofilm formation)

- The **RNAseq data** suggest an important role of **organic acid** and **iron related genes** during *A. butzleri* infections.
- Part of the putative **virulence genes** detected with **functional annotation** were **confirmed by transcriptomic data**

- **Genome partitions** suggest the existence of a **single genus** related to *Arcobacteraceae* family (*Arcobacter* spp.)
- The **species** linked to **clinical samples and animal hosts** showed a **smaller genome size**
- The **functional annotation** showed the presence of **genes linked to the source of isolation**

***Thanks for your
attention!***



***Davide Buzzanca*,
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Ilario Ferrocino,
Luca Cocolin,
Kalliopi Rantsiou,
Kurt Houf,
Valentina Alessandria***



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