

# MICROBIAL DIVERSITY 2019

MICROBIAL DIVERSITY AS A SOURCE OF NOVELTY:  
FUNCTION, ADAPTATION AND EXPLOITATION



MD 2019

**Study of *Arcobacter butzleri* invasion during simulated infection of different intestinal models**

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**Introduction:** *Arcobacter butzleri* is an emerging foodborne pathogen included in *Campylobacteraceae* family often isolated from pork, chicken and beef meat, which causes different gastrointestinal symptoms in humans due to its invasive behavior. However, its pathogenicity is still underestimated due to a substantial lack of information on its virulence mechanisms. This work aims at exploring the virulence potential of this bacterial species.

**Materials and Methods:** Two-dimensional (2D) mucus-secreting (HT29 MTX/Caco-2 cells) and not mucus-secreting (Caco-2 cells) models of human gut were subjected to a simulated invasion with *A. butzleri* LMG 10828T. Total bacterial RNA was recovered at 90 and 210 minutes, and concurrently from the bacteria alone (control). At the same time, bacterial counts were performed to describe its colonization and invasion capabilities. Moreover, tests were performed on three-dimensional (3D) models to study the bacteria translocation through the cell layer by measuring the Transepithelial/endothelial Electrical Resistance (TEER) and by quantifying the bacterial presence in the basolateral compartment of the model. Primers for the nine genes (*cadF*, *ciaB*, *cj1349*, *irgA*, *hecA*, *hecB*, *mviN*, *pldA*, *tlyA*) at present considered linked at the *Arcobacter* spp. pathogenesis were designed ex novo. Reverse Transcriptase-(RT)-

quantitative (q)PCR under simulated host-pathogen interaction was performed to quantify gene expression.

**Results:** As first outcome, a RT-qPCR protocol suitable to quantify the relative expression of the nine virulence genes in human simulated infections was optimized. These genes were differentially ( $p < 0.01$ ) expressed during the simulated infection of both gut models in comparison to the bacteria alone. Besides, we observed a remarkable downregulation of genes encoding proteins responsible for agglutination and hemolysis (*hecA*, *pldA*), rather than those directly correlated to adhesion and invasion potentialities (*cadF*, *ciaB*, *cj1349*). In addition, *A. butzleri* cells colonized and invaded both models at 90 minutes, but we observed a favorable role of the mucus for the pathogen colonization. Moreover, the data from 3D models suggested an intracellular passage of *A. butzleri* through the epithelial barrier, with a complete passage occurring within 24 hours.

**Conclusions:** In conclusion *A. butzleri* LMG 10828T was able to invade both human gut models but showed a greater colonization capability in presence of mucus. In parallel, part of the virulence genes investigated were downregulated, indicating the need of further deepen the topic.

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## MD 2019

More than 200 scientists, coming from 30 countries, will meet in Sicily to participate to the "Microbial Diversity Conference 2019". They are driven by a strong passion that share them together: the applied microbiology. These scientists are "microorganisms hunters" that are looking all over the world for new microbial species, new strains and their metabolites or functions able to improve the quality of our life from the point of view of health, food, environmental and agricultural sustainability, energy resources and waste management. All the scientific researches presented by the participants to this Conference are included in this volume that collects abstracts of keynote, plenary, selected lectures and posters presented from September 25<sup>th</sup> to 27<sup>th</sup>, at the **Microbial Diversity Conference** in Catania, Italy. This is the fifth edition of the event organized by the **Italian Society of Agricultural, Food and Environmental Microbiology (SIMTREA)**. As the Conference subtitle suggests "**Microbial diversity as a source of novelty: function, adaptation and exploitation**" this book offers different point of view of the microbial diversity: from Omics to culturomics, the exploitation of microbial diversity in ecological systems, the mechanisms of microbial resistance, the microbiota and host, the association and interaction in natural ecosystems and the microbiome adaptation, assembly and functioning. Inside these scientific sessions we find a variety of interesting researches that could represent in the future a key to reading to solve some environmental concerns discussed in Rio de Janeiro Earth Summit in 1992.

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**Study of *Arcobacter butzleri* invasion during simulated infection of different intestinal models**

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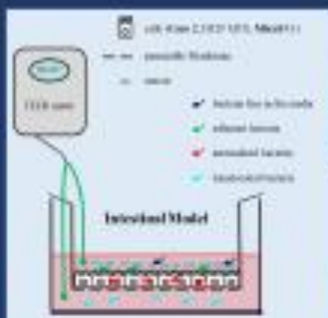
INTRODUCTION

*Arcobacter butzleri* is an emerging foodborne pathogen of the *Campylobacteraceae* family, often isolated from chicken, pork and bovine meat and water which cause different gastrointestinal diseases in humans and animals. However, pathogenicity of *A. butzleri* is still underestimated due to a substantial lack of information on its virulence mechanisms, metabolic and genomic features.

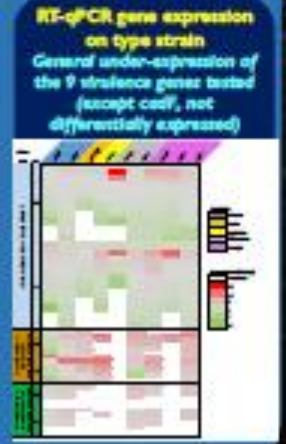
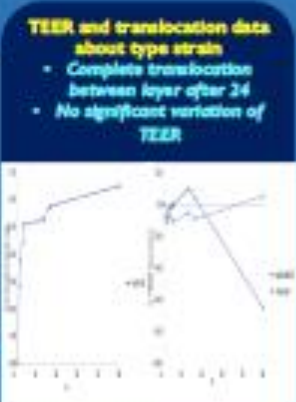
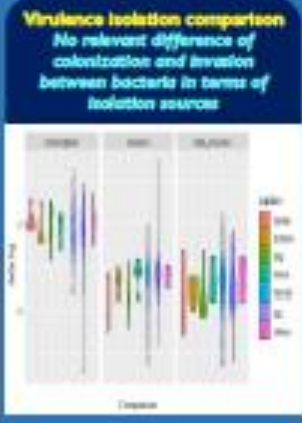
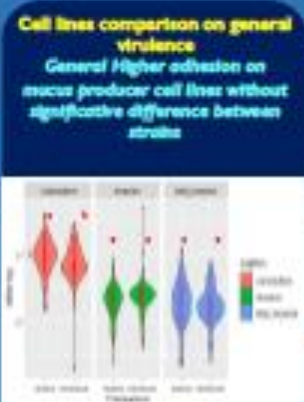


Contact between intestinal cell lines and 32 *Arcobacter butzleri* strains (from different samples) to simulate virulence conditions

Physiological and gene expression study  
(nine putative virulence genes)  
Similarity with *Campylobacter jejuni*



RESULTS



CONCLUSIONS

- ✓ The presence mucus is favorable at the bacteria colonization
  - ✓ No significant difference between strains virulence behavior
  - ✓ No significant difference between strains in terms of isolation source
  - ✓ TEER and translocation data suggest an intracellular passage of the bacteria
  - ✓ All the nine virulence genes present in bibliography are under-expressed or not differentially expressed
- ↓
- Possible under-expression in the first virulence phase or in vivo
  - Possible action of other genes

FUTURE PROSPECTS

Sequencing and genomes annotation (software Prokka)

New RT-qPCR at different timing

RNAseq to study the transcriptome in virulence conditions