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Contents

XXIV Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology

Exploring the Virulence of *Arcobacter butzleri* during Simulated Infection of Human Gut Models

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This PhD thesis research project aims on *Arcobacter butzleri* that is an emerging foodborne pathogen often isolated from pork, chicken and beef meat, which causes different gastrointestinal diseases, such as diarrhoea and abdominal pain, in human and animals due to its invasive behaviour. However, the pathogenicity of *A. butzleri* is still underestimated due to a substantial lack of information on its virulence mechanisms, metabolic and genomic features. In this study we obtain a first description of the mucus role in the infection and different design of analysis protocols for the future test.

Esplorando la virulenza di *Arcobacter butzleri* durante l'infezione simulata di modelli intestinali

Questo progetto di tesi di dottorato si occupa di *Arcobacter butzleri*, un patogeno emergente presente nel cibo frequentemente isolato da carne di suina e bovina e di pollo e che causa differenti sintomi gastrointestinali come diarrea e dolori addominali in uomo e animali a causa del suo comportamento invasivo. Nonostante ciò la patogenicità di *A. butzleri* è sottostimata a causa della penuria di informazioni riguardanti il suo meccanismo di virulenza e aspetti metabolici e genomici. In questo studio abbiamo ottenuto informazioni riguardanti il ruolo del muco nell'infezione e messo appunto differenti protocolli d'analisi per le prove future.

1. State-of-the-Art

Arcobacter butzleri (Vandamme P *et al.*, 1991) is an emerging foodborne pathogen included in Campylobacteraceae family often isolated from pork, chicken, beef meat and water, which causes different gastrointestinal symptoms such as abdominal pain, vomit, diarrhea, in humans and animals due to its invasive behavior. However, the pathogenicity of this gram negative bacteria is still underestimated due to a substantial lack of information on its virulence mechanisms, in bibliography are presents different ecological work about these bacteria but few work on the virulence mechanism, in particular about genomics and transcriptomics data (Ferreira, Queiroz, Oleastro, & Domingues, 2014). The ecological works show the presence of *A. butzleri* in different environmental compartment and host such as waste and marine water, plants, animals (pigs, cattle, poltry) and in clinical cases. In the case of *A. butzleri* infection in addition to the previously cited symptoms has occurred cases of sempticemia. For the wide presence on food and for the pathogenicity of this bacteria specie, new study about virulence mechanism will be useful to denscribe its role in clinical cases (Ramees et al., 2017).

2. PhD Thesis Objectives and Milestones

This study aims to explore, in simulated host-pathogen interactions, colonization and invasion capability of different strains of *A. butzleri*, thanks the use of different intestinal models (mucus producers and not mucus produces cell models). In addition, an initial study virulence gene expression was performed on the type strain, to obtain first data useful for following test, such as genomics and transcriptomics studies on other strains. The aims mentioned for my PhD project can be subdivided into the following activities according to the Gantt diagram (Figure 1).

2.1) Colonization and invasion assay

To determinate the colonization and invasion ability of the bacteria specie in object, after 14 days from the models cellular confluence, a load of 6.5 ± 0.5 Log CFU cm^{-2} was inoculated on three no mucus (Caco-2) and mucus producers (Caco-2/HT29 MTX, 9/1) cell models . After 90 minutes from the inoculum colonization and invasion have been determinated with an omogenelzation (Triton-X 0,25%) and with gentamicin application followed from an omogenelzation respectively.

2.2) Study of bacteria translocation

To determinate the traslocational ability of the bacteria through the cell layer 3D models have been produced and inoculated at the attainment of a TEER (trans epithelial endothelial resistance) of 1000 Ω cm^{-2}. To determinate the bacteria passage, after 1, 2, 4, 6, 24, 30 and 48 hours, microbiological load sampling has been performed in the basolateral compartment. In parallel a TEER mesurament was executed to determinate the cell layer integrity.

2.3) RT-qPCR gene expression study

An *ex novo* primer design (Thornton & Basu, 2011) was performed for nine putative virulence gene (*cadF*, *ciaB*, *cj1349*, *irgA*, *hecA*, *hecB*, *mviN*, *pldA*, *tlyA*) (Douidah et al., 2012) on *A. butzleri* genome (type strain LMG 10828^T), their relative expression was quantified by Reverse Transcriptase (RT)- quantitative (q)PCR under simulated host-pathogen interaction conditions. To collect cDNA to analyzable, *in vitro* gut models of mucus-producing and not producing human cells were inoculated with the pathogen, and total RNA was obtained at different timeframes. The gene expression determination has been calculated with the relative gene expression methods "double delta cq".

2.4) Genomics

The genomes of the strains in object will be sequenced with Illumina Myseq (NGS) to obtain data about different genomes from strains of this specie to link at the colonization and invasion tests.

2.5) Gene annotation

To obtain information about the role of genes sequenced from the previous step, the genomes obtain will be processed with the bioinformatic software Prokka, this to have a gene annotation foundamental to predict the function of *A. butzleri* genes (Seemann, 2014).

2.6) Transcriptomics

RNA collected at different times form the initial inoculum will be study with RNAseq, this to obtain data about the real expression of possible virulence gene. The collecting of RNA will be performed on no mucus and mucus producers cell models, this to botain data about the role of intestinal mucus on *A. butzleri* virulence mechanism.

2.7) Transcriptomics confirmation

The confirmation of trascriptomic data will be performed through different qPCR on the more relevant genes. This adpting the methodology at the point 2.3.

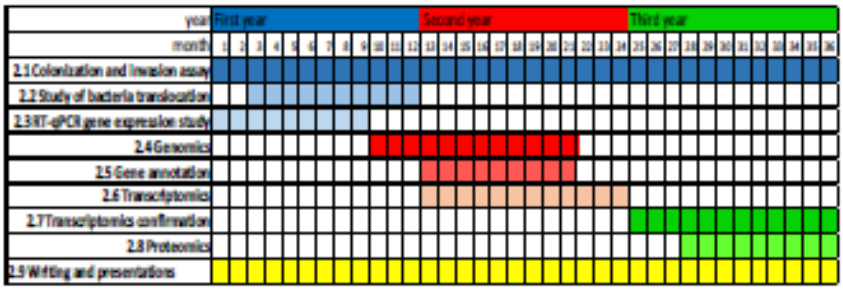
2.8) Proteomics

In conclusion a proteomics study will be executed to obtain data about the most interesting protein in dependence of the gene expression data. This will be important to obtain a further data confirmation and to obtain sequence and structure of the most relevant proteins linked to the *A. butzleri* virulence mechanism.

2.9) Writing and presentations

My PhD work will be presented at different workshop and congress such as IAFP symposium, as well as at the final thesis discussion. The results from my project will be pubblicated on specialized journals.

Table 2 *Gantt diagram for this PhD thesis project.*



3. Selected References

Douidah, L., De Zutter, L., Baré, J., De Vos, P., Vandamme, P., Vandenberg, O., ... Houf, K. (2012). Occurrence of putative virulence genes in Arcobacter species isolated from humans and animals. *Journal of Clinical Microbiology*, *50*, 735–741.

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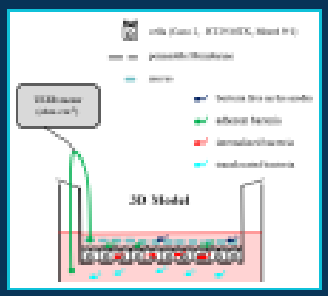
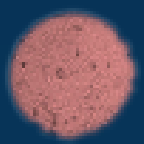
INTRODUCTION

Arcobacter butzleri is an emerging foodborne pathogen of the *Campylobacteraceae* family, often isolated from chicken, pork and bovine **meat**, which causes different **gastrointestinal diseases** in human 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.

M & M/project

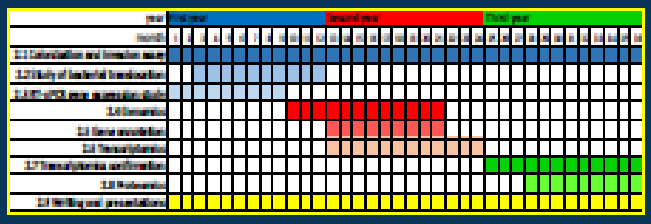




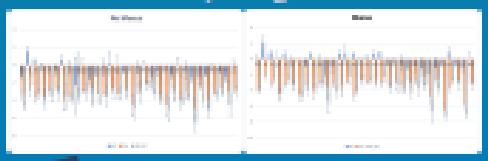
No mucus producer

Mucus producer

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



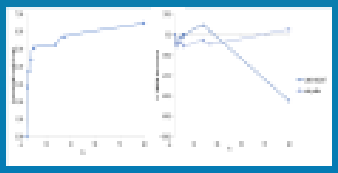
Colonization and invasion ability of 32 *A. butzleri* strains on mucus and not mucus producers cell lines (delta log)



1st year

- ✓ *Higher colonization on mucus producer models*
- ✓ *No significative difference between stains*

Translocation ability of the type strain



- ✓ *Translocation through the epithelial layer before the of the type strain (no cell layer degradation)*

Putative virulence genes expression On type strain Log2 (delta cq)

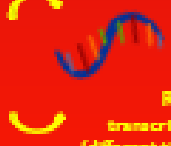
- ✓ *General under-expression*
- ✓ *No significative difference between mucus and not mucus models*

2nd year

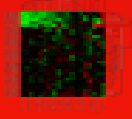
NGS Sequences Cleaning and assembly

Gene annotation (Structural and Functional)

Phylological and genomic data bonds studi



RNAseq transcriptomics studi (different times and models)



3rd year

More relevant expressed genes proteins purification and analysis of structures

Sequence

RNAseq data confirmation

Protein function determination (Study of possible interaction with host biomolecules)

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