UNIVERSITÀ DEGLI STUDI DI TORINO

Genetic Determinants Associated with Biofilm Formation of *Listeria Monocytogenes* from Food and Food Processing Environment

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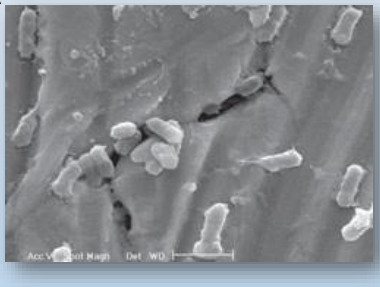
Introduction

Microbial Biofilm

- structured communities of bacterial cells
- attached to a surface (biotic or abiotic)
- embedded in a self-produced matrix of polymeric substances (EPS)
- exhibiting an altered phenotype and gene expression

A



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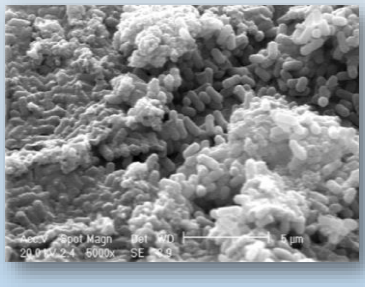
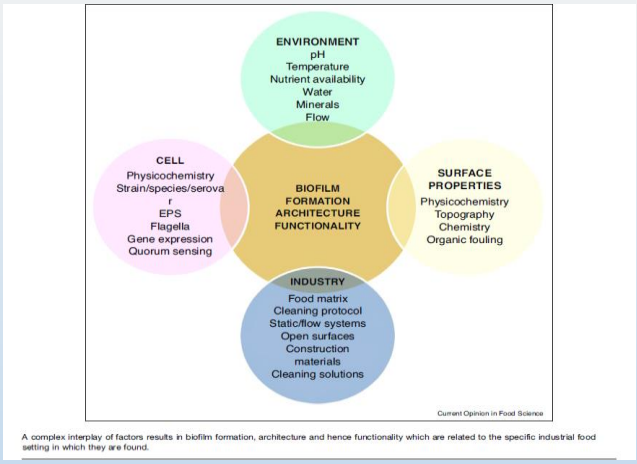


Scanning electron microscopy (SEM) images of *Listeria monocytogenes* in (A) planktonic and (B) biofilm form on stainless steel (A.Ianieri, 2008)

Introduction

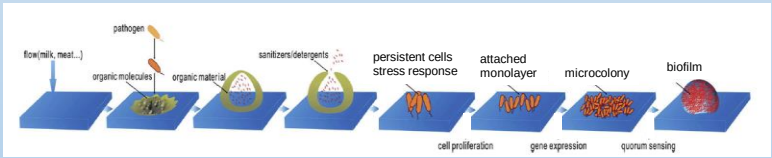


A complex interplay of factors results in biofilm formation, architecture and hence functionality which are related to the specific industrial food setting in which they are found.

Biofilm in the Food processing environment

serious concern:

- can develop on various surfaces: work surfaces, conveyors, pipes…
- more resistant to antimicrobials
- source of contamination of food products



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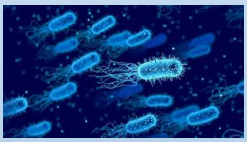
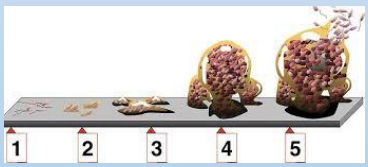
Introduction

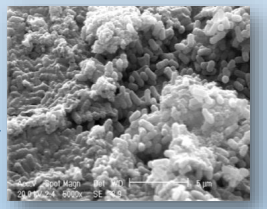
Listeria monocytogenes (L.m) is a psychrotolerant, gram-positive, ubiquitous bacterium

can grow both in planktonic and sessile form, organizing in **Biofilm**

survival and persistence under various stressful environmental conditions in food processing environments







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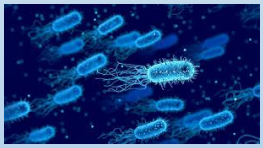
Introduction

L.m is of interest because infection is often associated with a **high mortality rate**, particularly among the elderly (CDC, 2018)

In the EU in 2017, a total of 2,480 confirmed invasive human listeriosis were reported by 28 member states, corresponding to an EU notification rate of 0.48 cases per 100.000 population and a fatality rate of 13.8% (EFSA and ECDC, 2018)

Different types of cheeses (especially fresh-soft and semi-hard varieties) and meat products were often found to be vehicles in listeriosis outbreaks


Introduction

***L.m* Biofilm** in **food-processing environment** is considered to be one of the main sources of repeated food contaminations

Biofilm production of ***L.m*** is a phenotypic character influenced by many genetic and environmental factors such as temperature, surface type, lineage and serotype etc.

In the past, attempts to correlate **Biofilm** phenotype to serotype, origin or lineage gave conflicting results between studies

such comparisons can be, now, addressed at the level of the genome by using **Whole Genome Sequencing** (WGS).

Aim of the study

The purposes of this study were:

- to assess the ability to produce **Biofilm** of ***L.m*** isolates from **food** and **food environment**
- to compare the levels of **Biofilm formation** with the presence/absence or truncations of **Biofilm associated genes**

Materials and Methods

- The biofilm formation of **57** ***L.m*** isolates (previously, whole genome sequenced) selected for their genetic traits (Clonal Complex) and their origins (**28** from **meat origin** and **29** from **dairy origin**) was evaluated according to a previously described method (Stepanovic *et al.*, 2007)

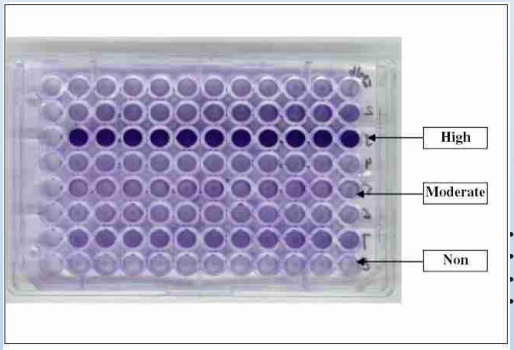


Figure 1: Screening of biofilm producers by TCP method: high, moderate and non slime producers differentiated with crystal violet staining in 96 well tissue culture plate.

Crystal Violet staining

- DO ≤ DOc = no biofilm producers;
- DOc < DO ≤ 2×DOc = weak biofilm producers;
- 2×DOc < DO ≤ 4×DOc = moderate biofilm producers;
- 4×DOc < DO= strong biofilm producers.

Materials and Methods

- A **nucleotide BLAST** of each gene against each genome assembly was performed to determine the presence or absence or truncation of a previously described set of **Biofilm** associated genes

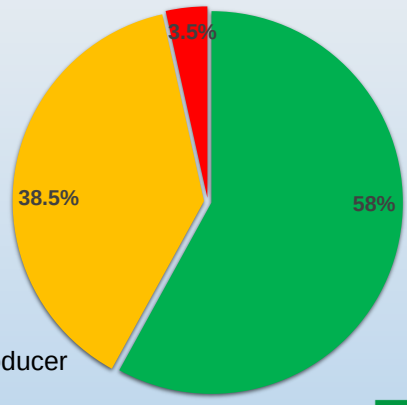
presence or absence (*SSI-1; intL*): significant hits were defined as those with coverage of at least 80% and a percent identity greater than or equal to 80%. (BLAST analysis)
(Pirone-Davies et al., 2018)

truncations (*intA*; *actA*: premature stop codons: PMSCs) were defined as present if a sequence was missing at least ten amino acids from the end of the sequence as compared to the EGD-e reference sequence.
Sequences were translated to amino acids, aligned with MUSCLE, and manually inspected for truncations.

Results
Biofilm formation

- All *L.m* isolates were classified as Biofilm producer

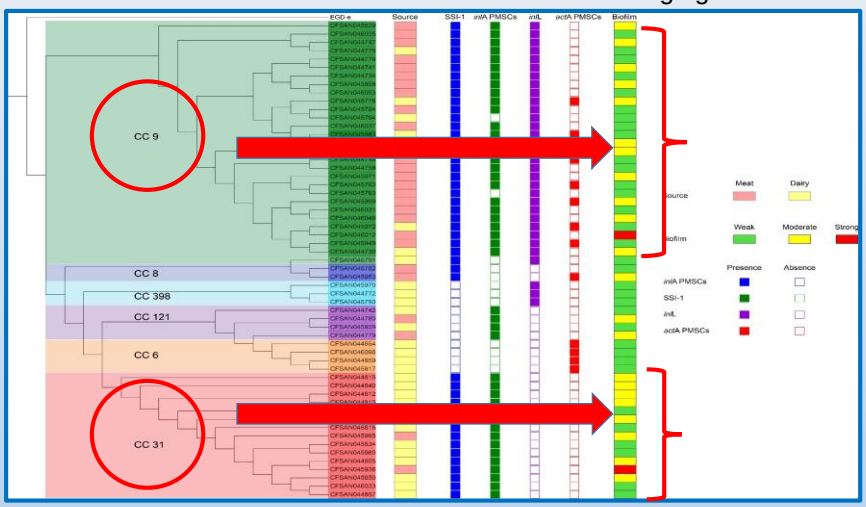
- **58%** weak Biofilm producers
- **38.5%** moderate Biofilm producers
- **3.5%** strong Biofilm producers



- **2/57** isolates from meat origin were strong Biofilm producer

Results
WGS

- A higher Biofilm formation was observed in *L.m* isolates belonging to **CC9** and **CC31**



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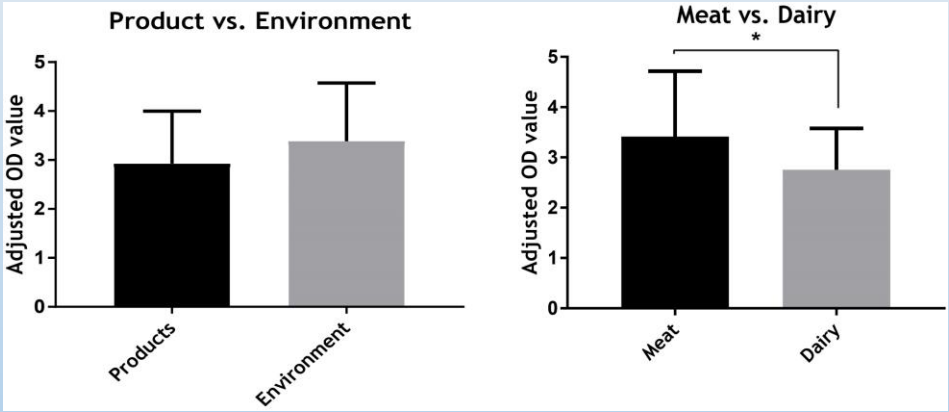
Results
Biofilm formation

- No difference in biofilm production was observed between food and environment isolates
- The percentage of isolates from meat products (**16%**), classified as moderate/strong biofilm producers, was higher than the percentage obtained for isolates from dairy products (**7%**) - ($p < 0,05$)



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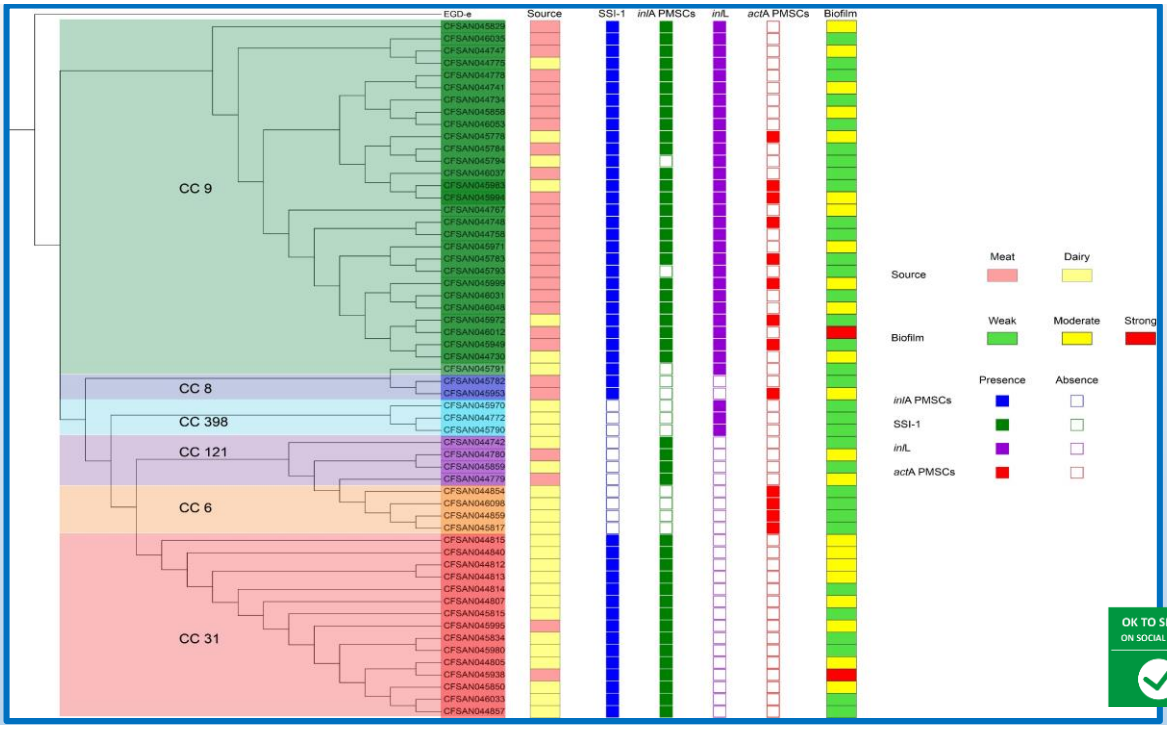
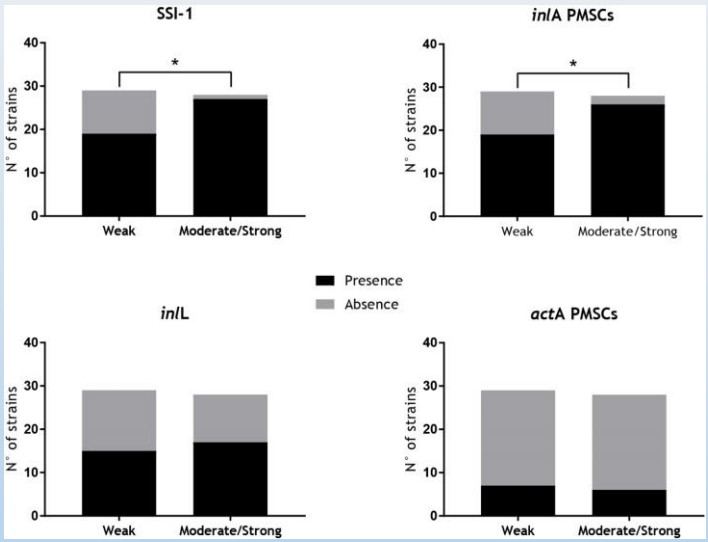


Results

WGS



- The presence of the five genes cluster *lmo0444-lmo0448* (**Stress Survival Islet 1**) and a **truncated *inlA*** protein, was significantly associated with increased levels of **Biofilm**

- The presence of the **inlL** and a **truncated *actA*** protein, was not significantly associated with increased levels of **Biofilm**

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Conclusions

- To date, the most **WGS studies** are carried out on ***L.m*** clinical isolates

L.m isolates from **Food sector** was evaluated in our study

- In accordance with the literature, the presence of ***Stress Survival Islet 1*** and a **truncated *inl*A** protein was associated in the **Biofilm-forming** capacity in *L.m* (Franciosa et al., 2009; Ryan et al., 2010; Keeney at al., 2018)

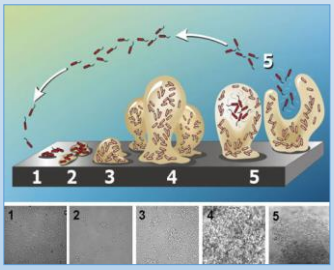
- In literature the ***inlL*** and a **truncated *actA*** protein was associated in the **Biofilm-forming** capacity in *L.m* (Travier et al., 2013; Popowska et al., 2017).

Our finding did not show this evidence

Conclusions

- Associating WGS-genotypes and specific **Biofilm** phenotypes could improve prediction of microbial (***L.m***) behaviors

- The implementation of this information in Hazard Identification and Exposure Assessment processes will open new possibilities to feed quantitative **Microbial Risk Assessment models**



THANK YOU FOR YOUR ATTENTION

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