Interaction between *L. monocytogenes* and *P. fluorescens* in dual-species biofilms under simulated dairy processing conditions

Presented by
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Microbial Biofilms

●○○ **Multi-species biofilms**

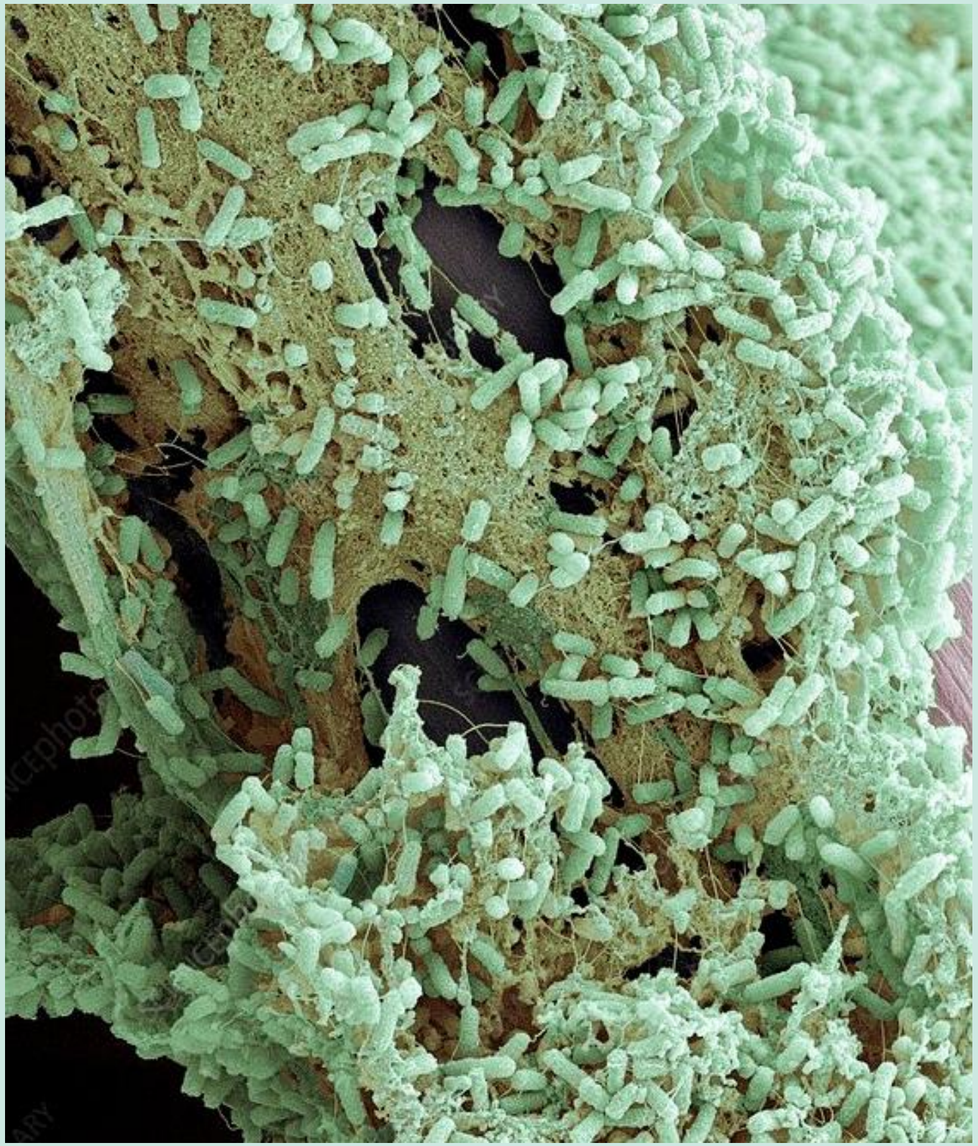
Involve inter-species interactions, such as between *Pseudomonas fluorescens* and *Listeria monocytogenes*

●●○ **Food Plant Management**

Persistent biofilms on dairy plants surfaces

●●● **Food spoilage and Safety**

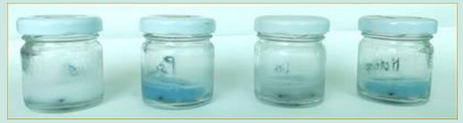
Economic losses

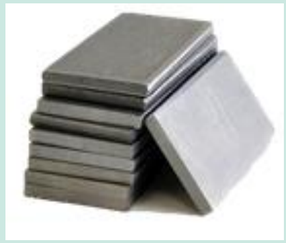


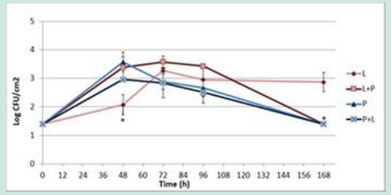
Aim of the study

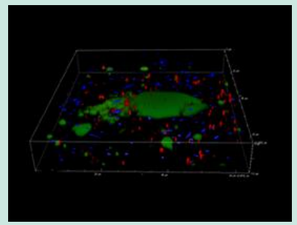
Evaluation of the
interactions between
L. monocytogenes
and
P. fluorescens
in dual-species biofilms simulating
dairy processing conditions,
as well as the
capability of *P. fluorescens* in co-culture
to produce the **blue pigment** in a **cheese model system**

Experimental Plan









L. monocytogenes AND BLUE PIGMENTING ***P. fluorescens***

Single- and co-culture interactions in the Ricotta medium at 12°C for 168 h

Strains isolated from dairy plants and products

BIOFILM ON POLYSTYRENE SURFACE (PS)

Biofilm-forming capability of the strains in mono- and dual-species to select one combination

Crystal violet assay

BIOFILM ON STAINLEES STEEL(SS) COUPONS

L. monocytogenes LM5 and blue pigmenting *P. fluorescens* pf5

Planktonic and sessile cells enumeration; EPS quantification

CLSM ANALYSIS

Mono- and dual-species biofilm structure

LIVE/DEAD BacLight Bacterial Viability kit; observation of pyoverdine fluorescence produced by *P. fluorescens*

Bacterial strains

The most prevalent serotype was the 1/2b, followed by 1/2a, 1/2c and 4b

8 different combined ApaI-AscI pulsotypes recognized

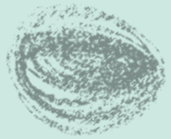
Table 1. *Listeria monocytogenes* and *Pseudomonas fluorescens* strains used in the study.

Species	Strain name	Source of isolation	Serotype	Pulsotype ApaI	Pulsotype AscI
L. monocytogenes	LM1	Gorgonzola cheese	1/2b	GX6A12.0051	GX6A16.0071
L. monocytogenes	LM2	Mozzarella cheese	4b	GX6A12.0073	GX6A16.0010
L. monocytogenes	LM3	Gorgonzola cheese	1/2a	GX6A12.0032	GX6A16.0029
L. monocytogenes	LM4	Caciotta cheese	1/2a	GX6A12.0390	GX6A16.0271
L. monocytogenes	LM5	Environmental	1/2b	GX6A12.0349	GX6A16.0255
L. monocytogenes	LM6	Environmental	1/2b	GX6A12.0005	GX6A16.0009
L. monocytogenes	LM7	Environmental	1/2c	GX6A12.0373	GX6A16.0261
L. monocytogenes	LM8	Environmental	1/2c	GX6A12.0002	GX6A16.0007
P. fluorescens	pf5	Mozzarella cheese			

Adhesion on PS surface

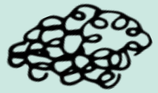
Inoculated Ricotta medium at 12°C for 168 h

L. monocytogenes STRAINS WERE NOT ABLE TO FORM BIOFILMS



P. fluorescens EXHIBITED GOOD BIOFILM-FORMING ABILITY

DIFFERENT BEHAVIOUR FOR THE SPECIES IN COMBINATION

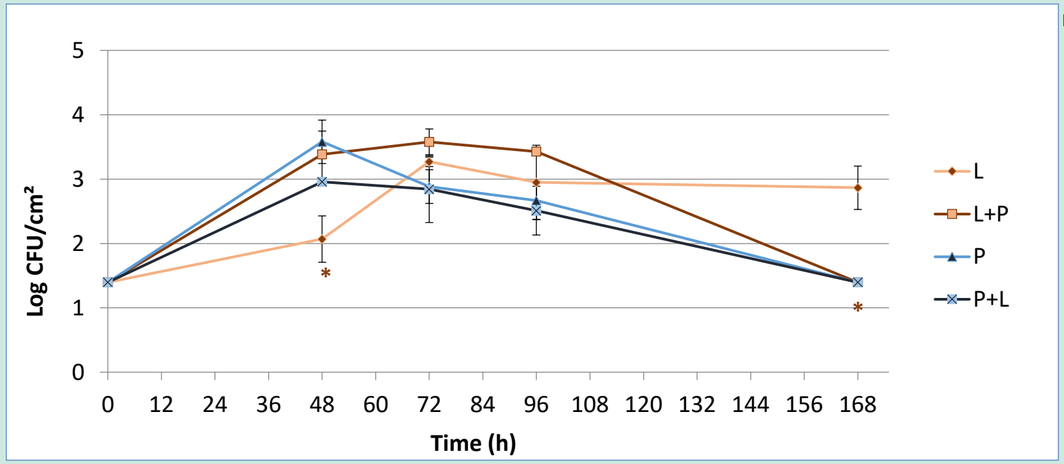


HIGHER BIOFILM BIOMASS FOR *P. fluorescens* pf5 - *L. monocytogenes* LM5 (72h)

SELECTION OF *P. fluorescens* pf5 - *L. monocytogenes* LM5

RESULTS

Sessile cells on SS coupons



L: *L. monocytogenes* in single species;
L + P: *L. monocytogenes* in dual-species;
P: *P. fluorescens* in single species;
P + L: *P. fluorescens* in dual-species

Inoculated Ricotta medium at 12°C for 168 h

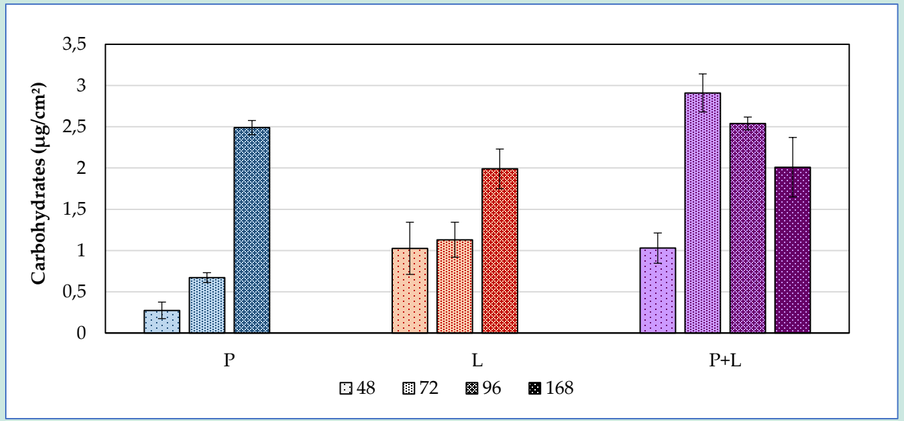
- *L. monocytogenes* LM5 was able to adhere on SS surface
- *P. fluorescens* pf5 increased the population of *L. monocytogenes* in dual-species biofilms (48 h)
- Culture conditions (mono- or dual-species) did not influence *P. fluorescens* pf5 population level

In planktonic status...

- *P. fluorescens* pf5 in single-species showed a greater increase in load over time compared to *L. monocytogenes* LM5
- *P. fluorescens* pf5 determined a slight decrease of *L. monocytogenes* LM5 counts in a dual-species condition (48 h, 96 h)

RESULTS

EPS (carbohydrates) production on SS surface



Inoculated Ricotta medium at 12°C for 168 h

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Behaviour

The total amount of carbohydrates in the biofilms were affected by the time and the species involved in biofilm formation

●●○○

In single-species

No carbohydrates were revealed at 168 h for both strains

●●●○

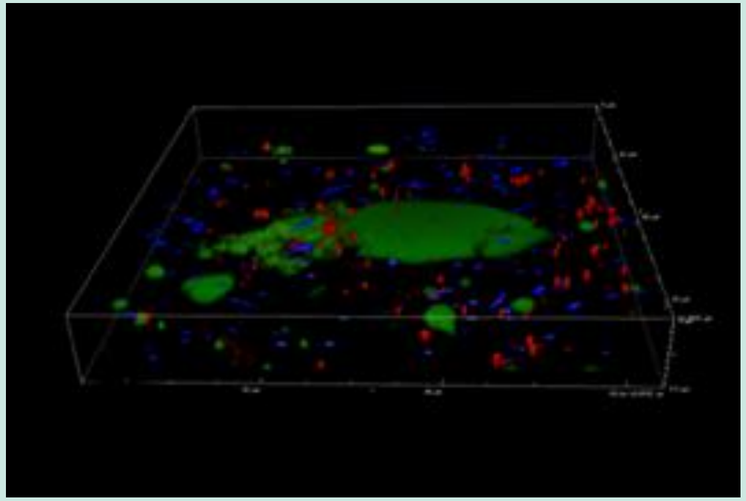
In dual-species

Carbohydrates were revealed at high amount (about 2 µg/cm²) also at the end of the experiment (168 h)

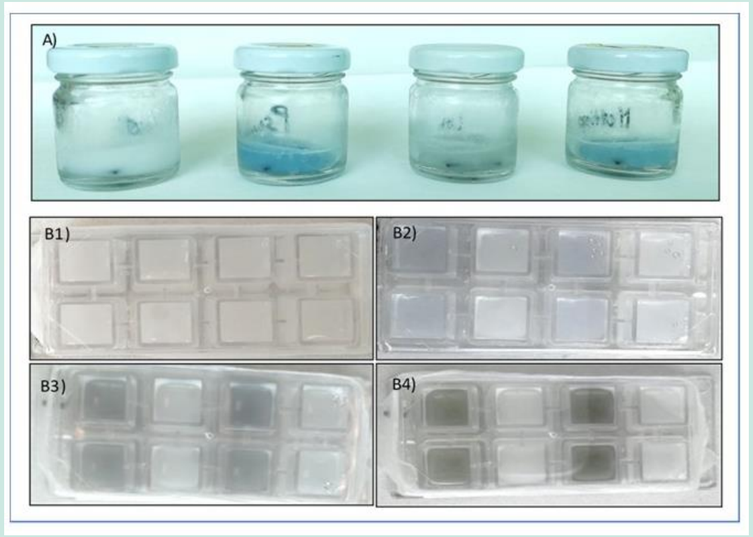
RESULTS

CLSM analysis

- No three-dimensional biofilm architecture was revealed
- Green agglomerates were present only in the samples with *P. fluorescens* pf5, suggesting that they could depend on blue pigment formation
- The production of blue pigment by *P. fluorescens* in the Ricotta medium was revealed both in single- and co-culture starting from 72 h



Blue colour: *P. fluorescens* cells due to the pyoverdine fluorescence; Red colour: damaged cells



A) Blue discoloration in the matrix. From the left: Control, *P. fluorescens* pf5, *L. monocytogenes* LM5 and dual-species samples

B) Change of the blue pigment over time.
B1) 48 h, B2) 72 h, B3) 96 h, B4) 168 h

END

Conclusions

01

BIOFILM-FORMING ABILITY

L. monocytogenes exhibited capability to form biofilms only on SS coupons, unlike *P. fluorescens* that showed this ability on both PS and SS surfaces

02

INTERACTION

The presence of *P. fluorescens* increased *L. monocytogenes* sessile population on SS coupons and total carbohydrates amount

03

IN SUMMARY

The consortium *P. fluorescens* and *L. monocytogenes* could be synergistic

For the future

Comparison of the gene expression between single- and dual- species biofilms and evaluation of interactions in terms of volatilome will be useful to provide more information on the inter-species consortium