

PhD Workshop  
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and Bio-Technologies  
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**Omics and molecular technologies for the analysis of complex microbial communities**

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# INTRODUCTION

## Characteristics

Gram positive
Catalase negative
Anaerobic facultative
G+C content < 50%
Optimum temperature 20-30 °C
Optimum pH 4-5
Heterofermentative metabolism

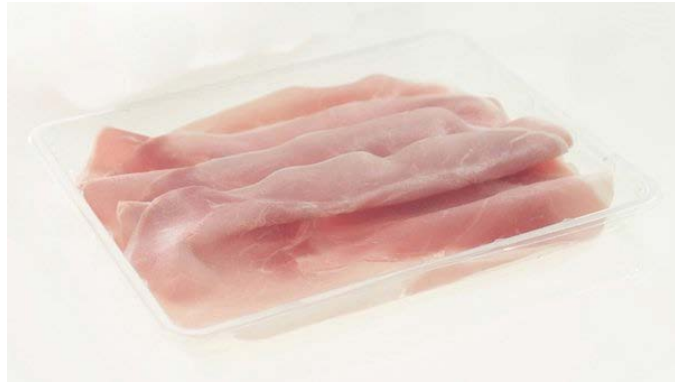
## *LEUCONOSTOC CARNOSUM*

- Highly widespread species in the food microbiota;
- Able to extend the shelf life and safety of food products;
- Spoilage bacterium in vacuum-packaged meat.



<b>Kingdom</b>	Bacteria
<b>Phylum</b>	Firmicutes
<b>Class</b>	Bacilli
<b>Order</b>	Lactobacillales
<b>Family</b>	Leuconostocaceae
<b>Genus</b>	Leuconostoc
<b>Species</b>	L.carnosum

# AIM OF PROJECT



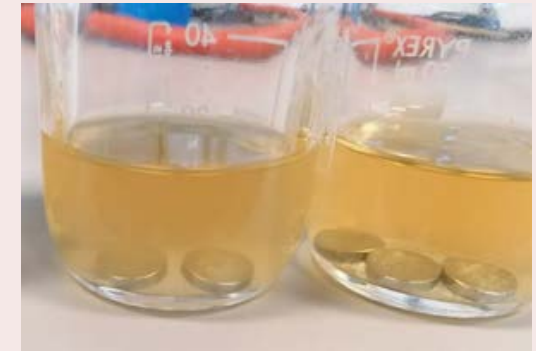
The aim of this work was to analyse various strains of *Leuconostoc carnosum* in terms of biofilm formation and bacteriocins production in the context of food processing.



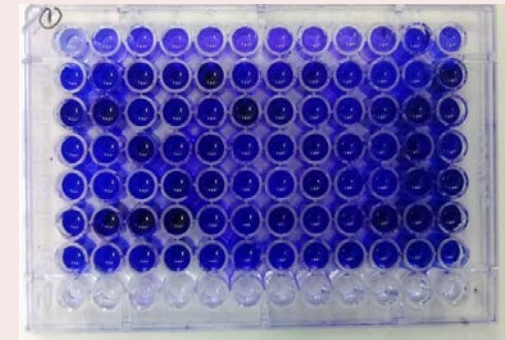
# MATERIAL AND METHODS

We have tested 12 *L.canosum* strains:

- Stainless steel coupons
- biofilm formation in Microtiter plates 96 wells
- Cross immunity
- Auto-aggregation
- Curve growth of bacteriocin

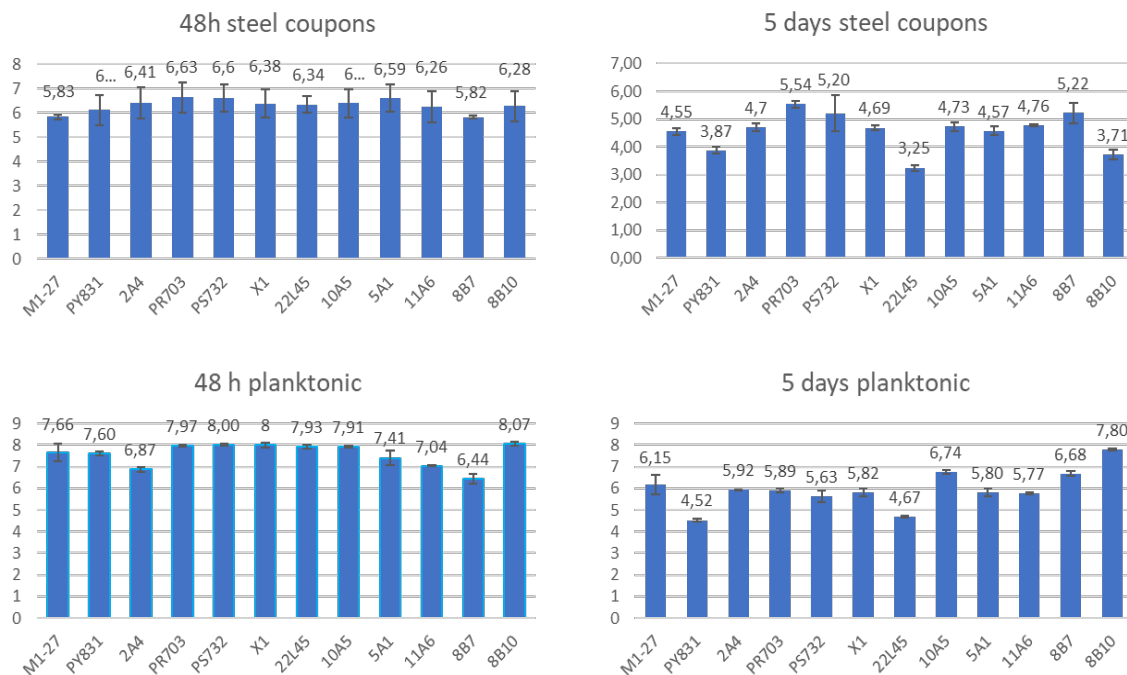


Biofilm formation onto the surfaces of stainless steel coupons.



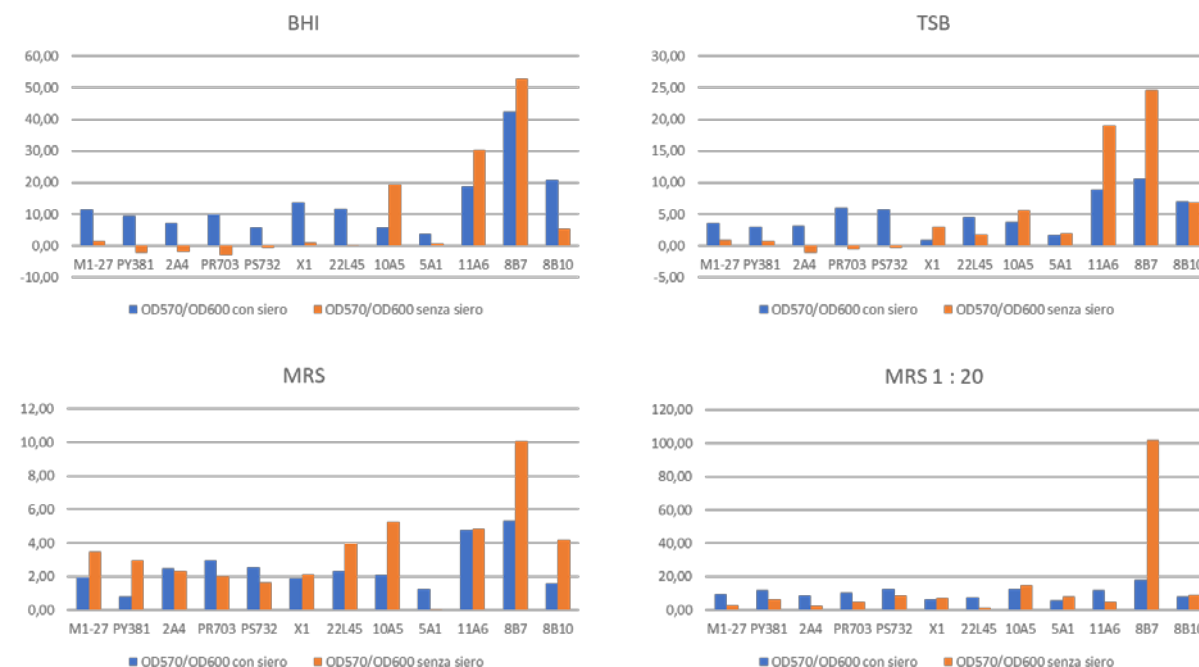
Biofilm formation in microtiter plate after add acetic acid 20%

## Stainless steel coupons



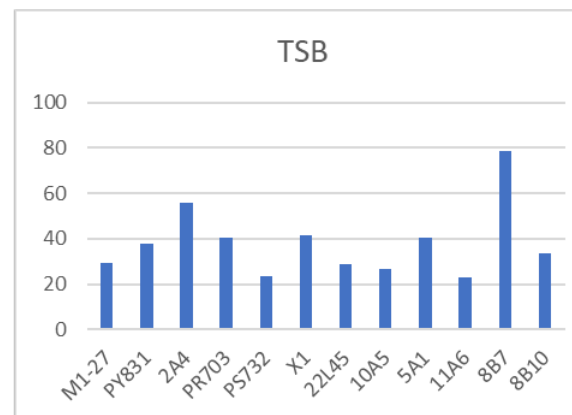
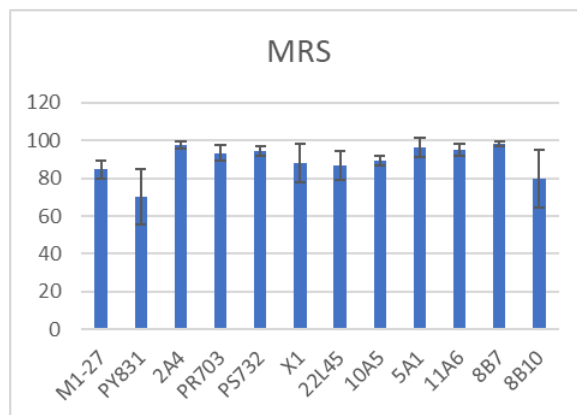
In the graph above each column indicates the average value of microbial concentration (cfu / mL) calculated on three replicates for each strain

## Biofilm formation in microtiter plates



Comparison of biofilm formation using 4 different media and plates with a pre-treatment of pure serum

## Auto-aggregation



The results show the difference in percentage of auto-aggregation in MRS and TSB

## Cross-immunity

Spot →	M1-27	PY831	2A4	PR703	PS732	X1	22L45	10A5	5A1	11A6	8B7	8B10
Plate ↓												
M1-27	-	-	-	-	-	-	-	-	-	+	+	-
PY831	-	-	-	-	-	-	-	-	-	-	-	-
2A4	-	-	-	-	-	-	-	-	-	+	-	-
PR703	-	-	-	-	-	-	-	-	-	+	-	-
PS732	-	-	-	-	-	-	-	-	-	+	-	-
X1	-	-	-	-	-	-	-	-	-	+	+	-
22L45	-	-	-	-	-	-	-	-	-	+	-	-
10A5	-	-	-	-	-	-	-	-	-	+	-	-
5A1	-	-	-	-	-	-	-	-	-	+	-	-
11A6	-	-	-	-	-	-	-	-	-	-	-	-
8B7	-	-	-	-	-	-	-	-	-	-	-	-
8B10	-	-	-	-	-	-	-	-	-	+	+	-

Clarifying halo diameter	11A6	8B7
M1-27	2,5 mm	2 mm
PY831	0 mm	0 mm
2A4	1 mm	0 mm
PR703	2,5 mm	0 mm
PS732	3 mm	0 mm
X1	11 mm	4 mm
22L45	1 mm	0 mm
10A5	8 mm	0 mm
5A1	2 mm	0 mm
11A6	0 mm	0 mm
8B7	0 mm	0 mm
8B10	4,5 mm	2 mm

In these tables are been reported the effect of Leucocin B produced by 11A6 and 8B7 against the other strains and the respectively diameter of clarifying halo



- The selected strains of *Leuconostoc carnosum* resulted able to form biofilms both in stainless steel coupons and in microplates. In this case, we obtained a higher production of biofilm for all the strains on MRS medium, whereas only in BHI and TSB media the microplates pre-treated with serum for 30 minutes had a positive outcome compared to untreated microplates, except strains *L. carnosum* 10A5, 11A6, and 8B7.
- The curve growth of Leucocin B excluded the production of the bacteriocin, in these conditions, both during growth and at the stationary phase.

The future perspective will be to repeat the curve growth of the bacteriocin and to see the biofilm formation of *L. carnosum* strains using the confocal

# Thank you for the attention

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