

**Antimicrobial peptides of *Lactobacillus plantarum*  
UTNCys3.4 strain isolated from native fruits of  
Ecuadorian Amazonia inhibit the growth of  
foodborne pathogens**

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# Outline

- Introduction
- Aiming and Workflow
- Results and Discussions
- Conclusions
- Acknowledgments

# Introduction

- **Lactic acid bacteria (LAB)** are known as the most versatile microorganisms used as probiotic or functional foods.
  - Ubiquitous gram positive, catalase-negative, non-sporulating, aero tolerant
  - Fermentative organisms that produce lactic acid as the major end product of carbohydrate metabolism
  - Among LAB species, several are producing antimicrobial substances.

# Introduction

- LAB are known as inhibiting invading Gram-negative bacteria due to the presence of several active components such as short-chain fatty acids or hydrogen peroxide, proteins such bacteriocin-like inhibitory substances (Smith et al. 2007).
  - **Bacteriocins**
    - Proteinaceous antibacterial substances
    - Ribosomally synthesized
- In the recent years, lactobacillus species were investigated to select strains with greater antimicrobial capacity to be used in bio-preservation of food products.

# Introduction

- In Ecuador, the presence of pathogens in food was reported (Ministry of Public Health, Ec, 2013).
- Due to defective storage condition, poor manufacturing practices, most artisanal typical dishes (i.e. mote, chicha, chocho) contains a significant number of pathogenic and spoilage microorganism, therefore the risk of developing diseases is elevated; so, currently the authorities are implementing new strategies to reduce the contamination by pathogenic microorganism.
- Accordingly, the research was centered on identification of natural ingredients to be used in preservation.

# What we are interested for?

- According to the new territorial redistribution several zones of Ecuador known as undeveloped natural areas were included in the governmental policy as important resources to be considered.
- In this context our current work relates to isolation, characterization and evaluation the probiotic capacity of lactic acid bacteria isolated from native un-exploited ecological niches originated from Ecuador.
- Previously, we identify several LAB strains of fruits collected from rainforest of Santo Domingo de Los Tsachilas Provence, which showed probiotic potential(Benavidez et al., 2016)

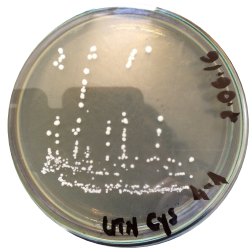
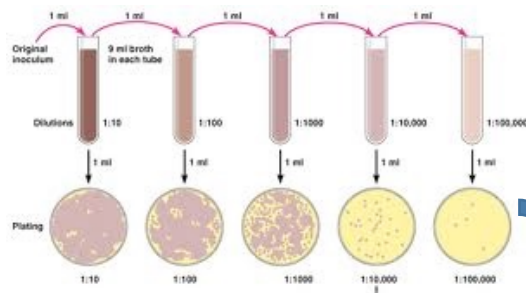
## **Aiming of the study**

- To isolate, identify and characterized novel bacteriocinogenic lactic acid bacteria of native microbiota of Ecuador.
- We proposed large scale experiments to search for lactic acid bacteria with potential probiotic capacity in Amazon, Sucumbíos Provence.

# *Solanum stramonifolium*

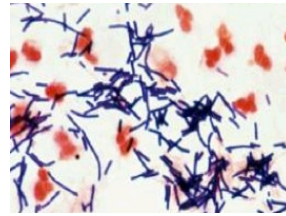


1. **Samples collection:** native fruits, flowers, soil

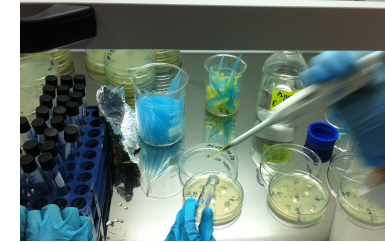


2. **Strain selected** from MRS plates, purification of single colonies

## Workflow



3. **Morphological and Biochemical Studies, well-agar diffusion assay,**



Well-agar diffusion assay

5. **Spectrum of activity** against food pathogens: *E.coli*, *Salmonella*, *Shigella*

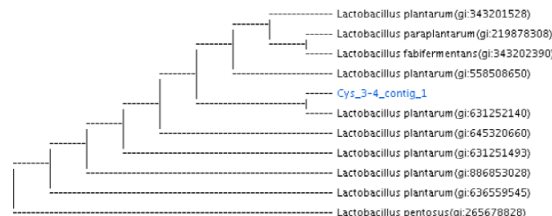
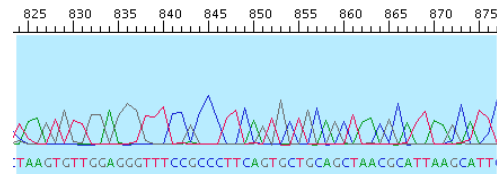


6. **Antimicrobial components characterization**

- Enzymatic sensibility
- pH, temperature stability

7. **Bacteriocin production**

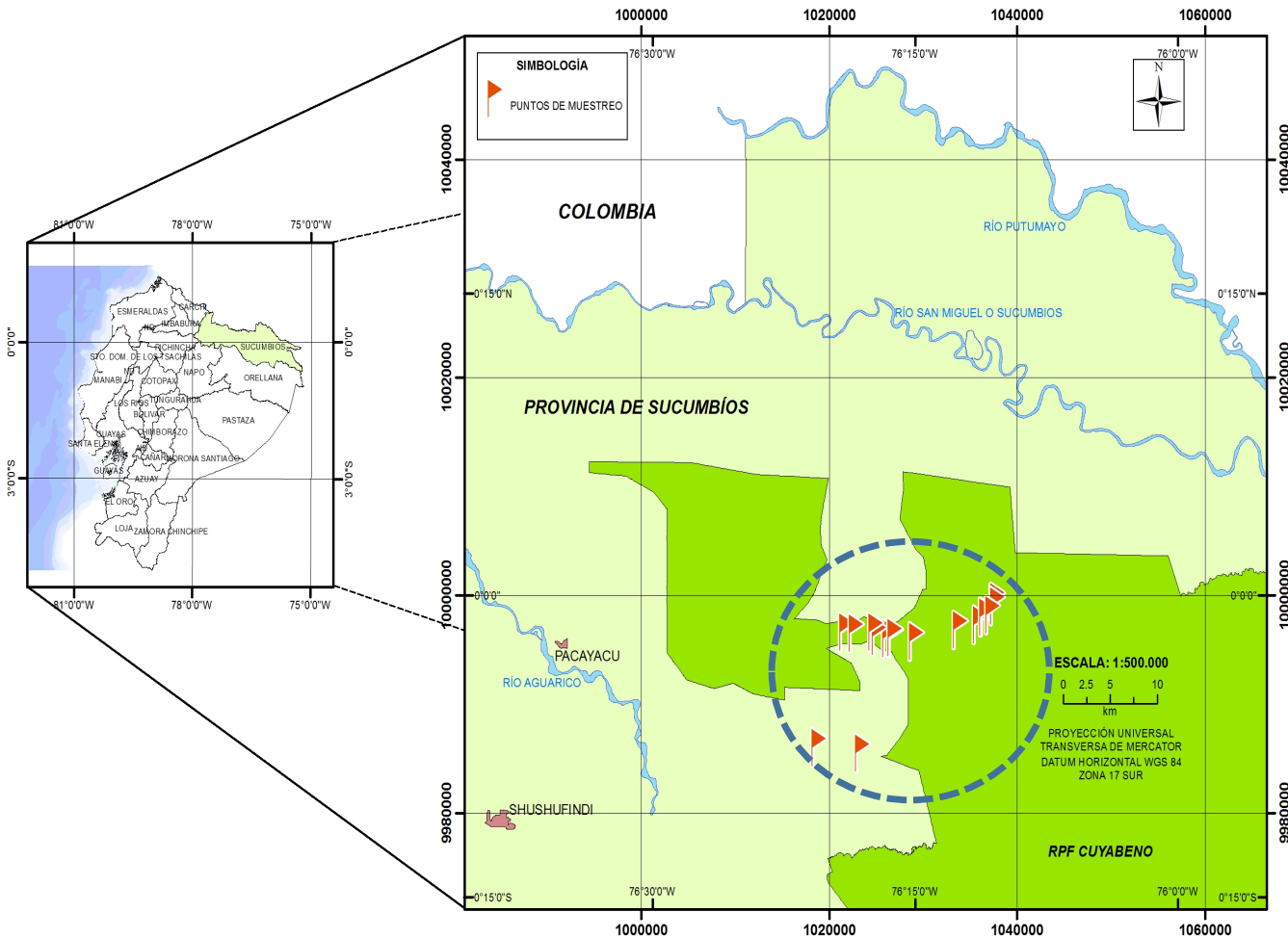
- Kinetics of bacteriocin production
- Medium optimization



4. **16s rRNA sequencing** (custom service, Macrogen, Inc. Korea)



# Results and discussions



15 native fruits were collected.

100 cells per each sample were  
LAB related

identification

50% *Lactobacillus* sp.

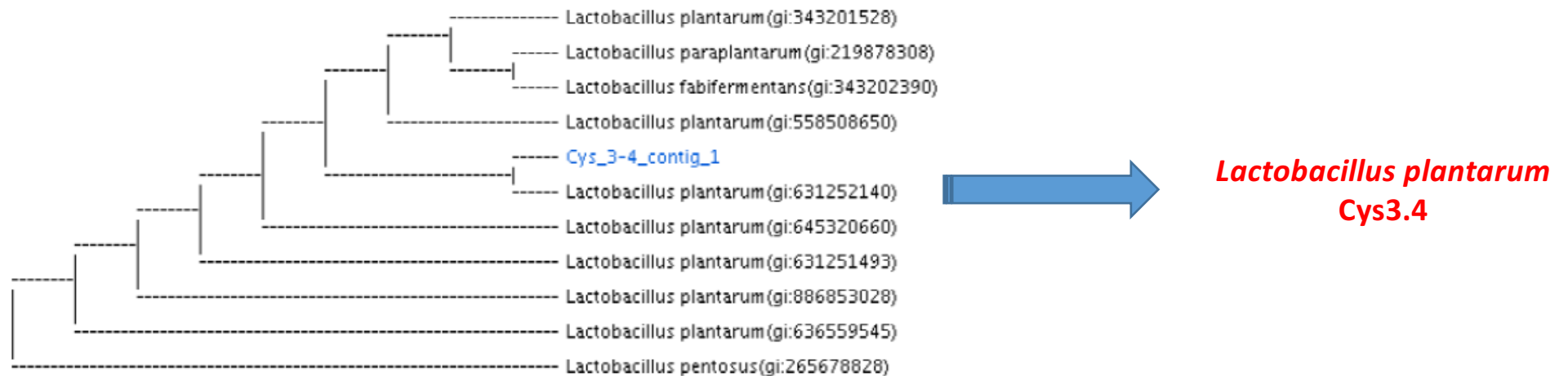
20% *Lactococcus* sp.

30% *Enterococcus* sp

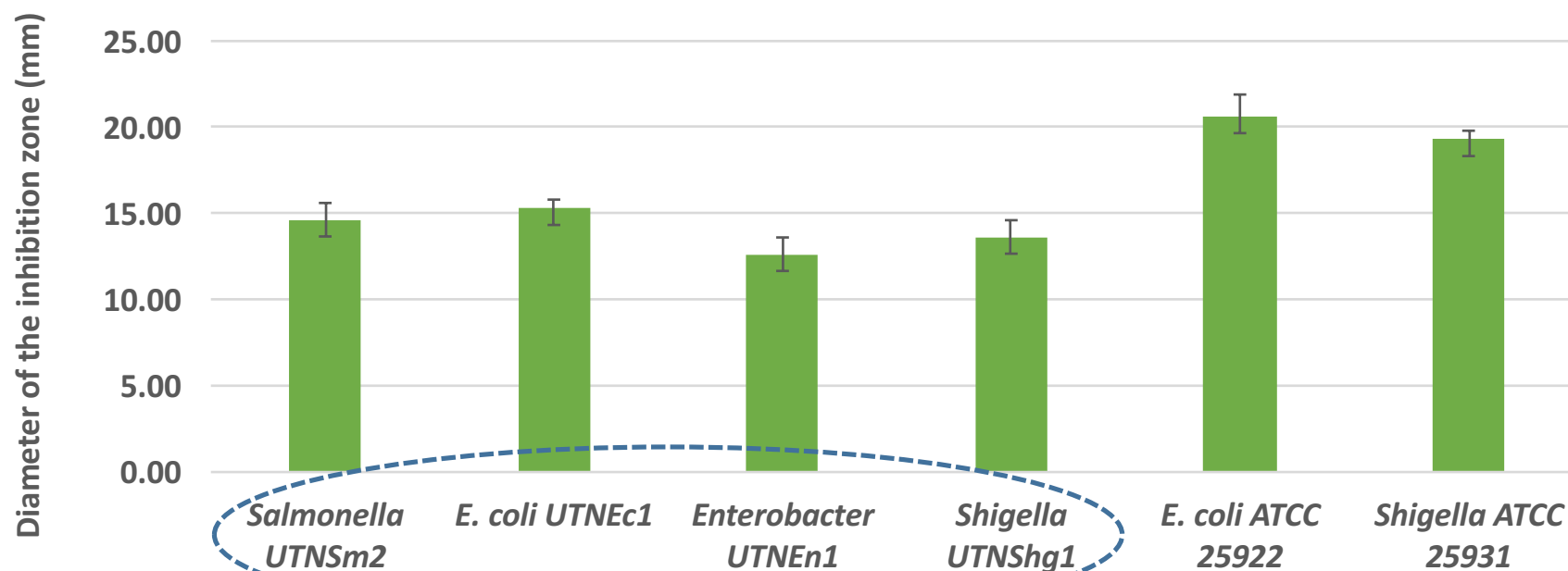
Selection of *Lactobacillus*  
strains with elevated  
antimicrobial activity

# Identification of Cys3.4 strain

- API50CHL carbohydrate profile and 16S rRNA sequencing demonstrated that the isolated was *L. plantarum* assigned and Cys3.4 strain with 99% identity.
- The strain was deposited at GenBank with accession number KY110685.



# Broad spectrum of antimicrobial activity of Cys3.4 strain



100% contamination  
in traditional dishes

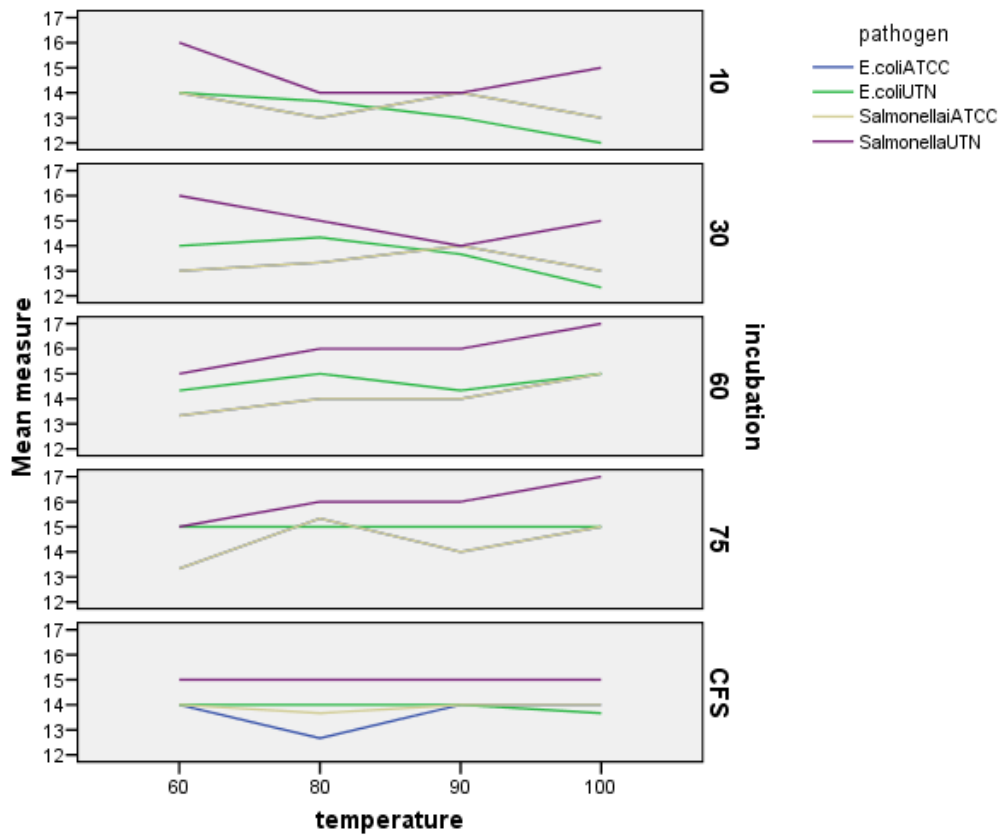
# Enzyme, temperature and pH sensibility

Treatment	Activity (AU/ml)	Component
	<i>E. coli</i> ATCC25922	
Enzymes (1mg/ml)		
NCFS + Proteinase K	-	Proteinaceous
NCFS + Trypsin	-	Proteinaceous
NCFS + Pepsin	-	Proteinaceous
NCFS + Lysozyme	6400 AU/ml	Active/ non-lipid
NCFS + Lipase	6400 AU/ml	Active/ non-lipid and carbohydrate moiety
NCFS	3200 AU/ml	Active
TFS	6400 AU/ml	Active
pH		
2.0	12800 AU/ml	Active
4.0	6400 AU/ml	Active
6.0	3200 AU/ml	Active
10.0	800 AU/ml	Active
Heat*		
60°C	6400 AU/ml	Active
80°C	6400 AU/ml	Active
90°C	6400 AU/ml	Active
100°C	6400 AU/ml	Active
121°C	3200 AU/ml	Active
CFS	6400 AU/ml	Active

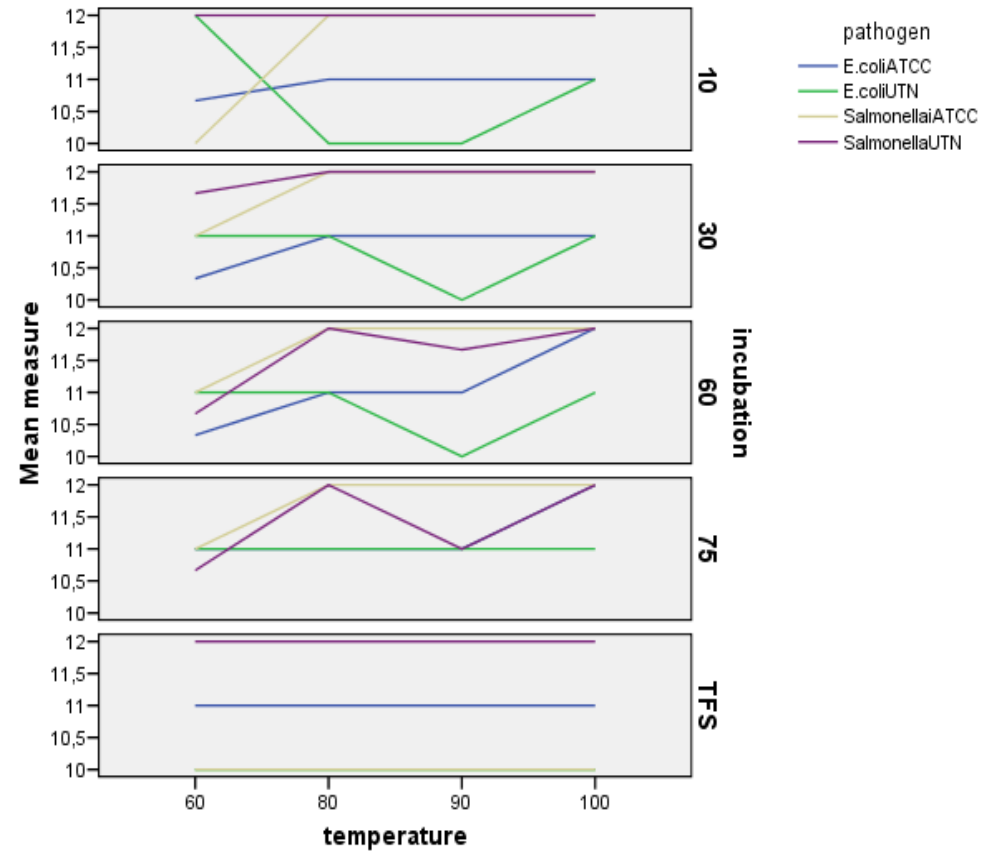
Results are means of 3 measurements  $\pm$  standard deviation of three replicates. CFS-crude fluid supernatant; TFS-neutralized CFS (pH 6.0); NCFS: neutralized CFS and hydrogen peroxide eliminated.

# Heat stability

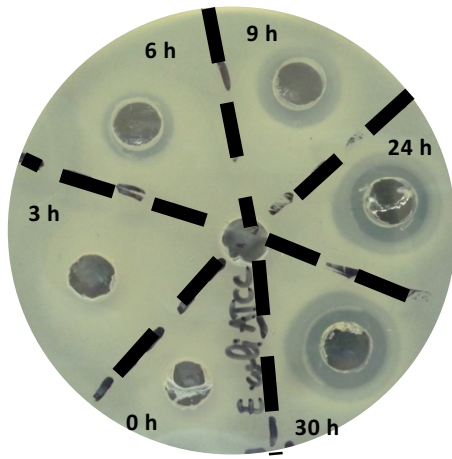
## CFS Cys3.4



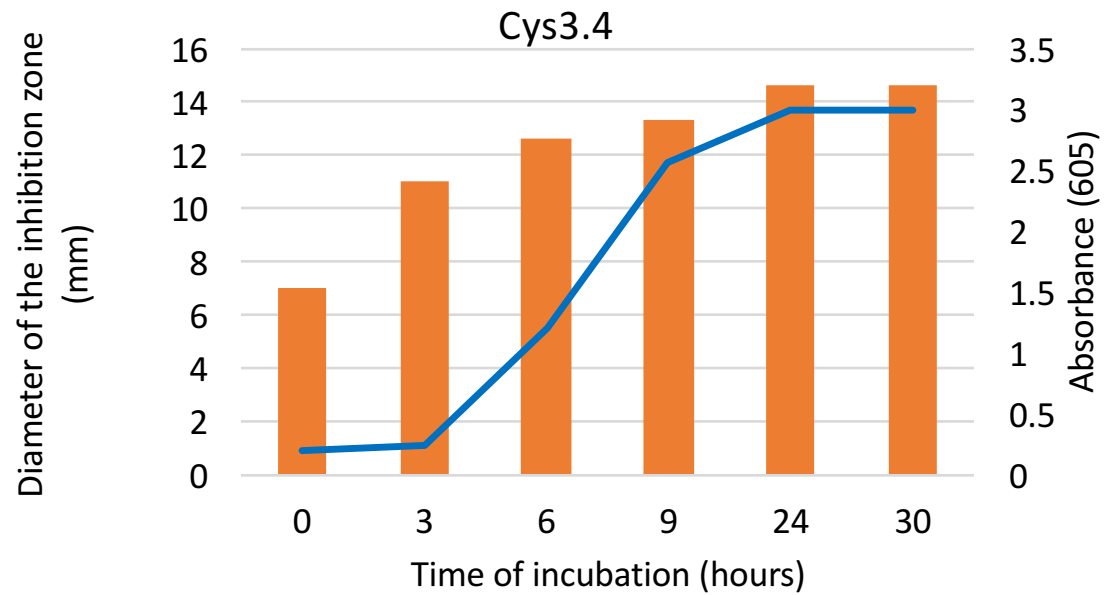
## NCFS Cys3.4



# Bacteriocin production

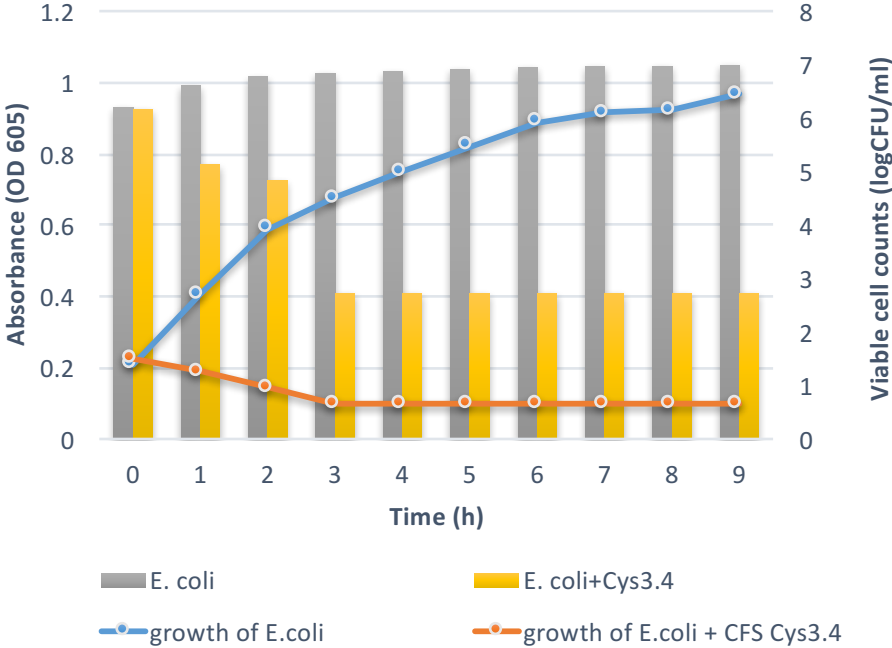
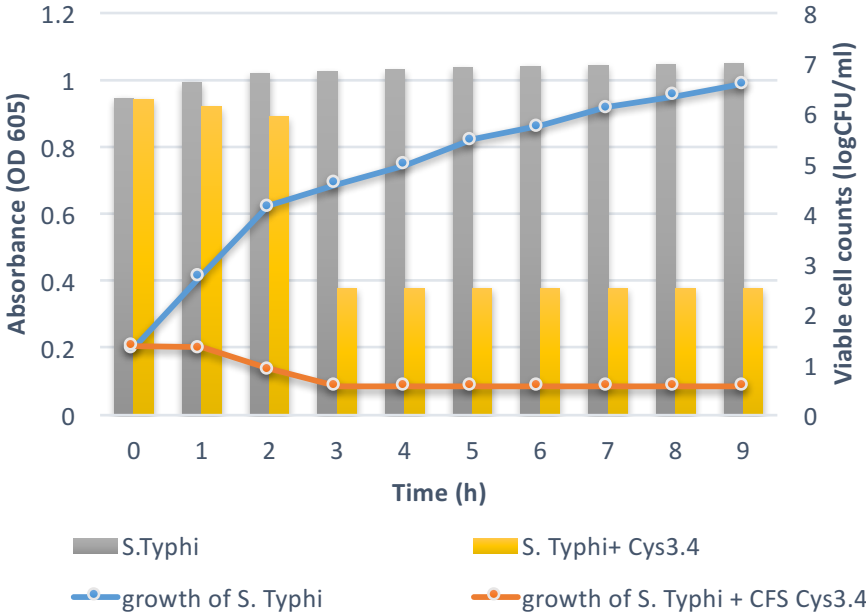


Antimicrobial activity against *E. coli* ATCC25922 with time of *L. plantarum* Cys3.4 growth



Bacteriocins production of *L. plantarum* Cys3.4 with time

# Effect of BLIS produced Cys3.4 strain on indicator pathogens



Effect of bacteriocin-derived Cys5-4 strain on growth and viability of the indicator cells. A. *E. coli* UTNEc1; B. *Salmonella* UTNSm2 (bars, represent the viable cell counts with and without bacteriocin Cys5-4, lines-growth registered as optical density at 605).

# Effect of pH, temperature and chemicals in adsorption cellular

Adsorption cellular of Cys3.4 (%)		
Treatment	E. coli Ec1	Salmonella Sm2
<b>Effect of pH</b>		
2.0	90.38	87.87
4.0	94.47	95.3
6.0	96	95.9
<b>Effect of temperature (°C)</b>		
4	74	69
15	98	87
30	95	100
37	91	91
45	94	95
<b>Effect of chemicals (1%), pH 6.5</b>		
NaCl	92	88
Triton X-100	99	71
EDTA	25	41
SDS	55	52
CFS	94	94



# Conclusions

- We identified *L. plantarum* Cys3.4 strain with greater capacity to suppress several pathogenic bacteria.
- The inhibitory activity *in vitro* was highly related with the presence of bacteriocin-like molecules and depends at least in part, by lowering pH and/ or the presence of organic acids.
- We showed that the bacteriocin producing Cys3.4 strains has a bacteriocidal mode of action.
- We shall further test the biopreservative potential *in vivo*.

# Acknowledgements

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