***Τμήμα Μοριακής Βιολογίας και Γενετικής***
***Σχολή Επιστημών Υγείας***
***Δημοκρίτειο Πανεπιστήμιο Θράκης***

ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ
ΜΕΤΑΦΡΑΣΤΙΚΗ ΕΡΕΥΝΑ ΣΤΗΝ ΒΙΟΙΑΤΡΙΚΗ
Μοριακή Διαγνωστική, Βιοδείκτες και Στοχευμένες Θεραπείες

**Αλέξης Γαλάνης**
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*Τμήμα Μοριακής Βιολογίας και Γενετικής, ΔΠΘ*
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# *Gut Bless Probiotics!*

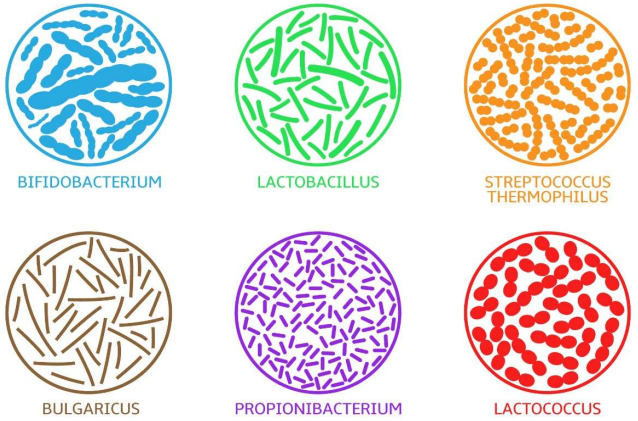
# Pro (latin) + Biotic (greek) = For Life

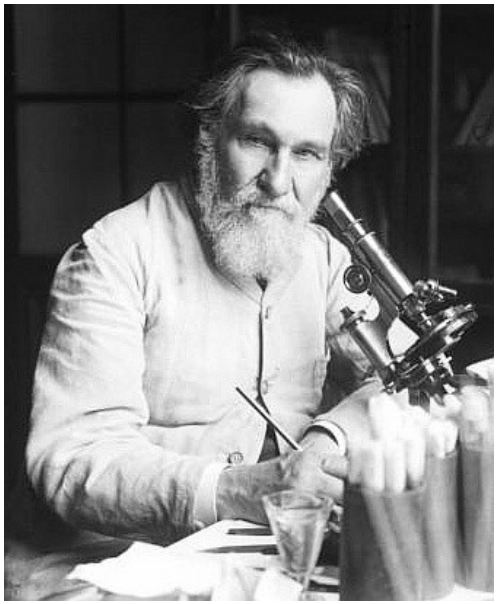
The term was first used in 1965, by **Lilly and Stillwell**, to describe substances secreted by one organism which stimulate the growth of another.

The theory that regular consumption of lactic acid bacteria in fermented dairy products may contribute to enhanced health and longevity was originally developed by the Russian immunologist and Nobel Laureate in Medicine, **Elie Metchnikoff** and presented in his book *"The prolongation of life"* published in 1907).

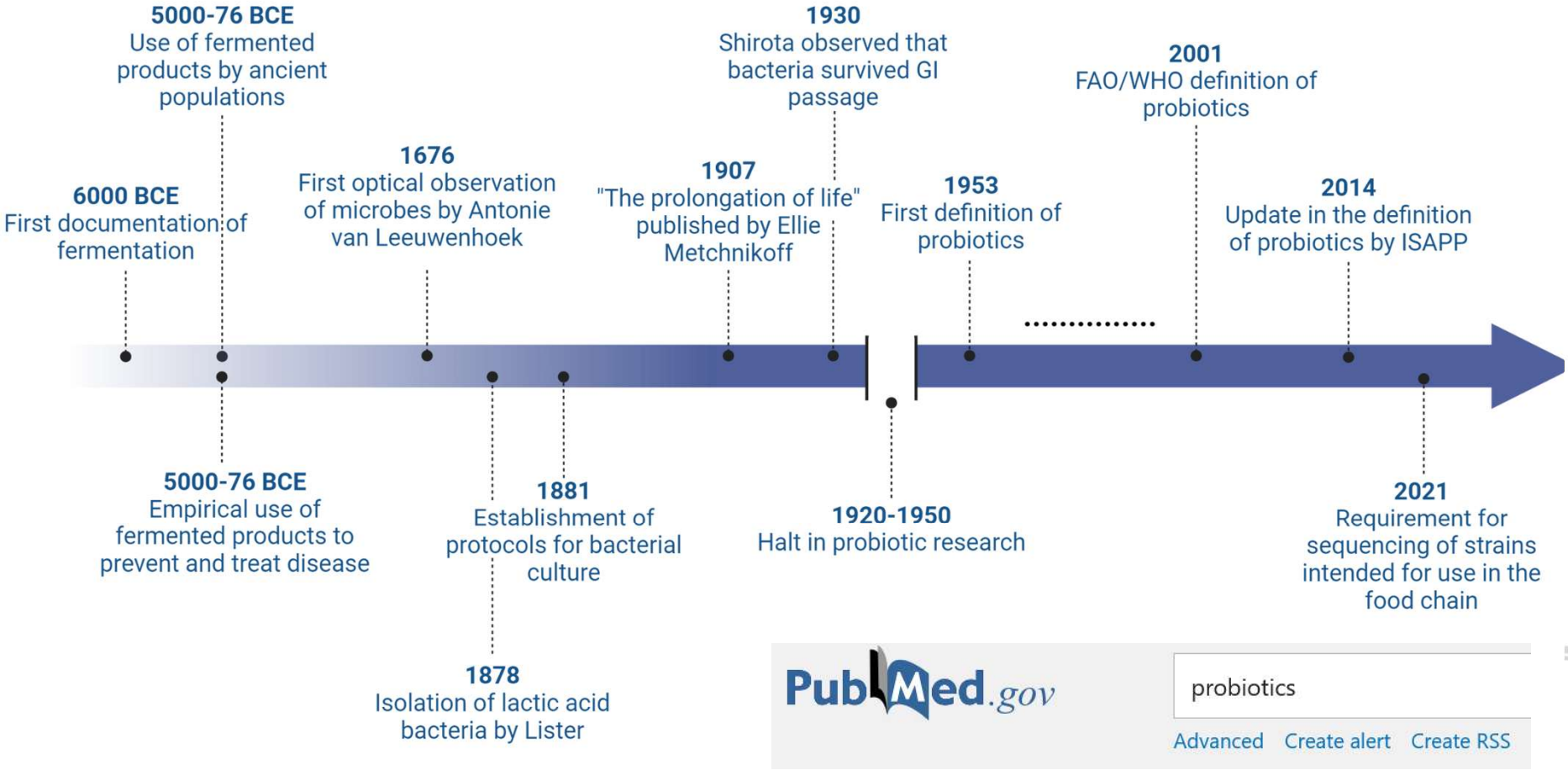
In 1989, **R. Füller** defined probiotics as *"a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance".*

**Probiotics** are defined as "*live microorganisms that, when administered in adequate amounts, confer a* ***health benefit on the host***" *Food and Agriculture Organization and the World Health Organization (FAO/WHO), 2002.*



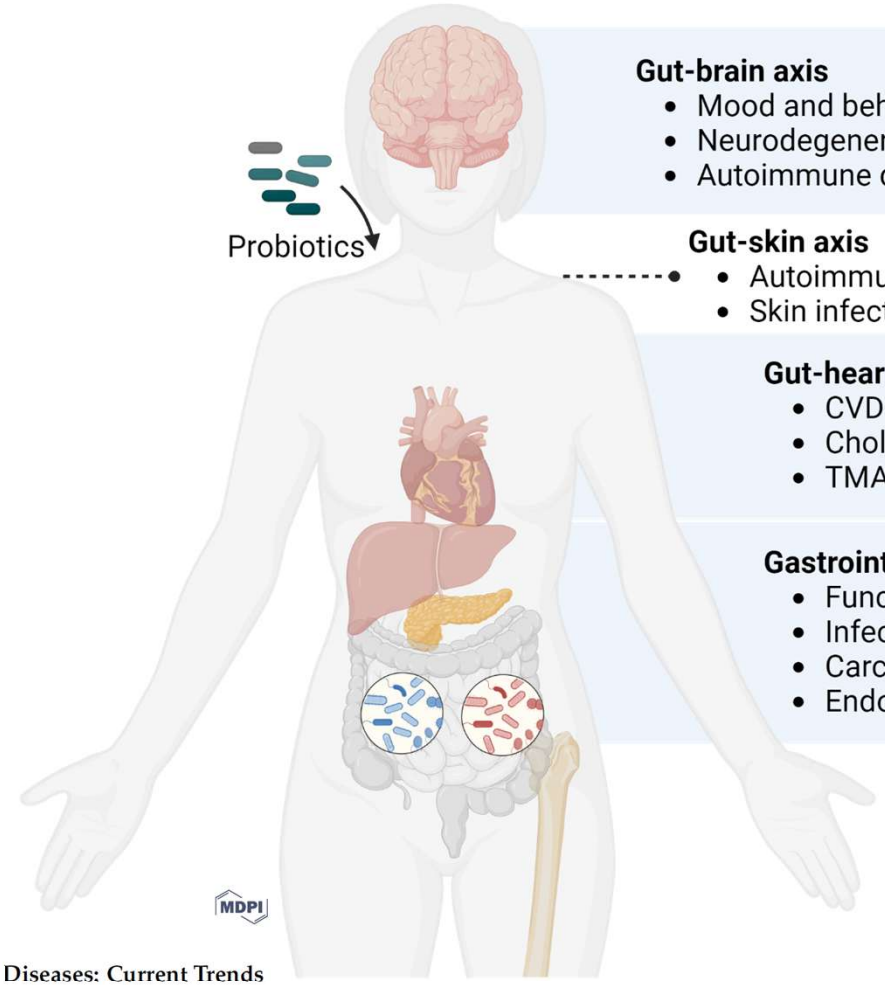
# Probiotics: from ancient murals to contemporary applications







# Health benefits of probiotics




**Gut-brain axis**
- Mood and behavior disorders
- Neurodegenerative disorders
- Autoimmune disorders

**Gut-skin axis**
- Autoimmune skin disorders
- Skin infections

**Gut-heart axis**
- CVD
- Cholesterol metabolism
- TMAO metabolism

**Gastrointestinal tract**
- Functional disorders
- Infections
- Carcinogenesis
- Endocrine disorders

*nutrients*

MDPI

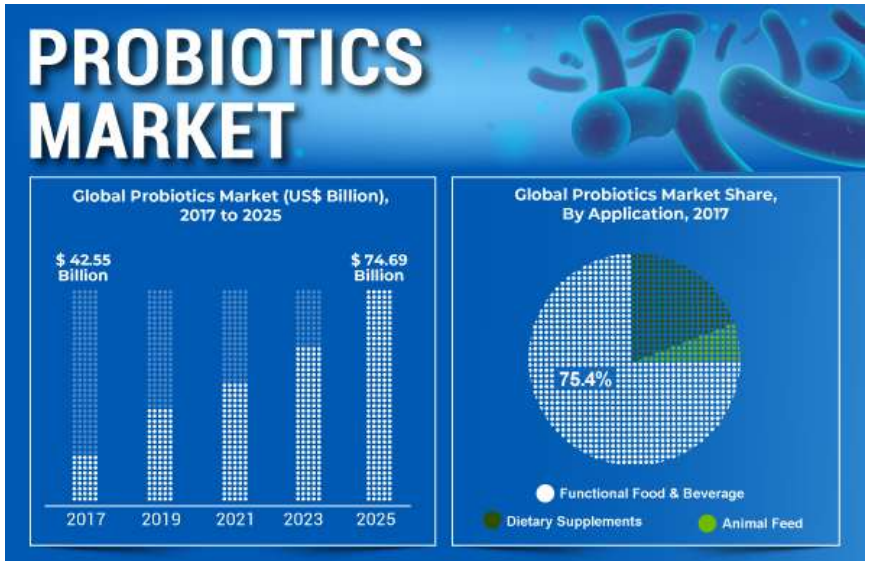
*Review*

**Probiotics in Extraintestinal Diseases: Current Trends and New Directions**

Despoina E. Kiousi [1], Athanasios Karapetsas [1,†], Kyriaki Karolidou [1], Mihalis I. Panayiotidis [2], Aglaia Pappa [1] and Alex Galanis [1,*]

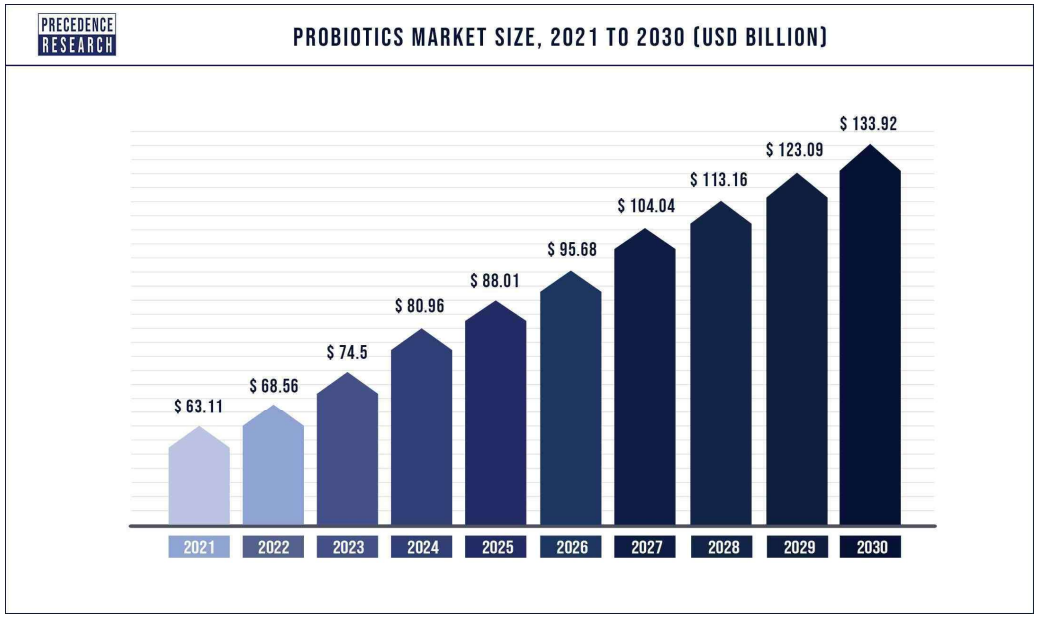
*Nutrients* **2019**, *11*(4), 788; https://doi.org/10.3390/nu11040788

- ✓ Strain-specific
- ✓ Host-specific
- ✓ Diet-specific
- ✓ Disease-specific
- ✓ Microbiome-specific




The global demand for probiotics is increasing significantly due to **health benefits associated with probiotic food products**, and the increasing use of probiotics in foods due to the rising consumer awareness related to healthy diets.

The probiotics market is projected to **grow from USD 83.11 billion in 2021 to 133.92 billion by 2030**, at a Compound Annual Growth Rate of 9.5% from 2021 to 2030.



**No health claims have been approved for ‘probiotic’ and therefore terms that imply a probiotic function are not permitted.**

efsa
European Food Safety Authority

CONTAINS PROBIOTICS

# HEALTH CLAIMS MADE ON FOODS: LEGAL FRAMEWORK

## *Regulation (EC) No 1924/2006*

Health claims should only be authorised in the EU after **a scientific assessment of the highest possible standard**

**Claims substantiated by**

- **generally accepted scientific evidence**
- **totality of the available scientific data**
- **weighing the evidence**

**EFSA NDA Panel adopts scientific opinions**

**AUTHORISATION: by Commission/Member States, European Parliament scrutiny**

**EFSA remit & role: with focus on scientific substantiation of Health Claims made on foods**

**EFSA meeting with IPA Europe**

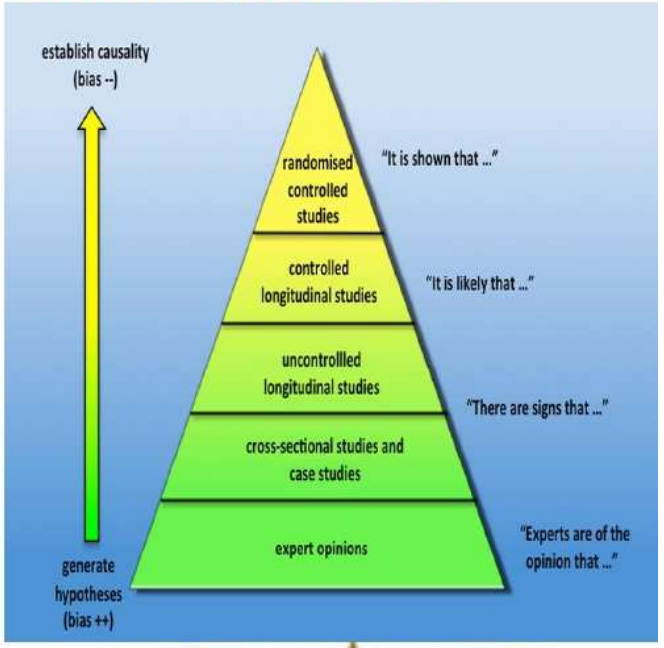
Parma, 18 January 2019

efsa

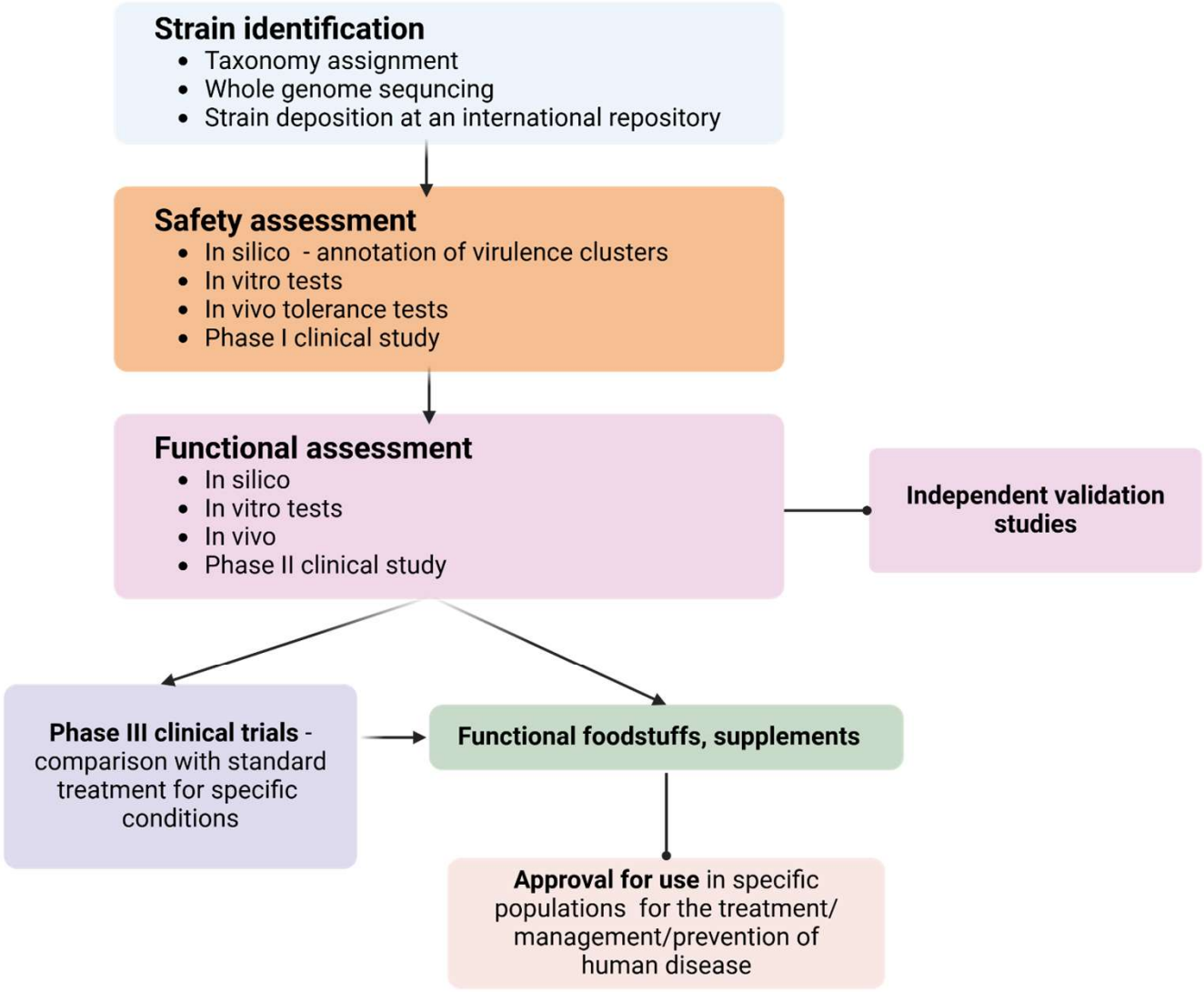
# PRINCIPLES FOR SCIENTIFIC SUBSTANTIATION (cont.)

## *General scientific guidance for stakeholders on health claim applications*

- **Pertinent human efficacy studies (central for substantiation) – hierarchy of evidence**
  - ✓ carried out with **the food/constituent for the claim**?
  - ✓ **appropriate outcome measure(s)** for the claimed effect?
  - ✓ **study group** is representative of the target population?
  - ✓ **the design and quality of the study** in relation to the risk of bias?
  - ✓ **conditions for human studies** vs. conditions of use for the claim?
- **Supportive studies:** Efficacy studies in animals, non-efficacy studies in humans, animals/*in vitro* (e.g. mechanisms that explain the effect of the food)
- **Weighing the evidence:** combining human efficacy studies +supportive studies +biological plausibility of the effect to conclude on substantiation

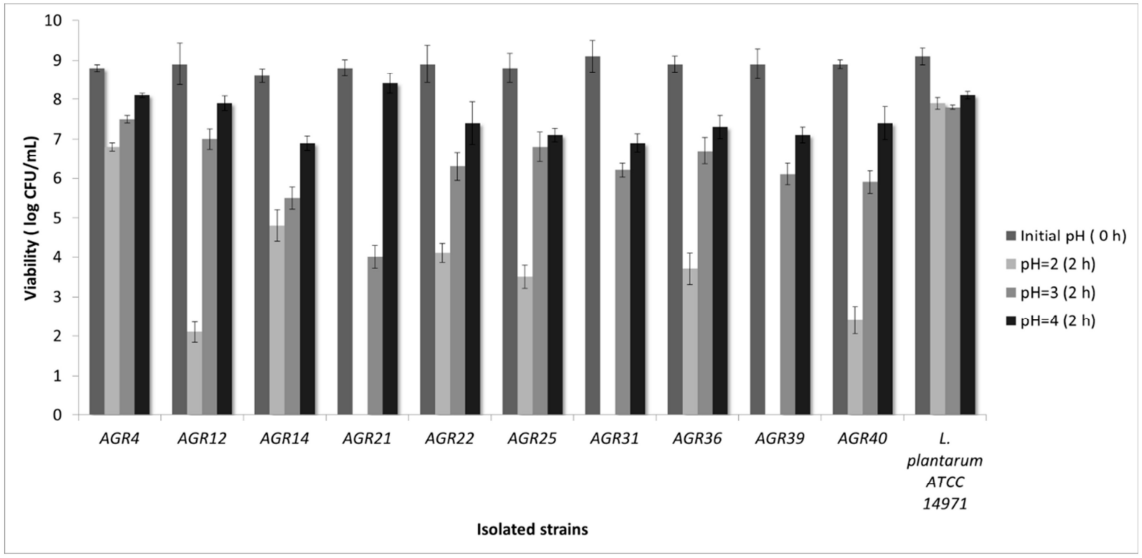


# Criteria for probiotic selection

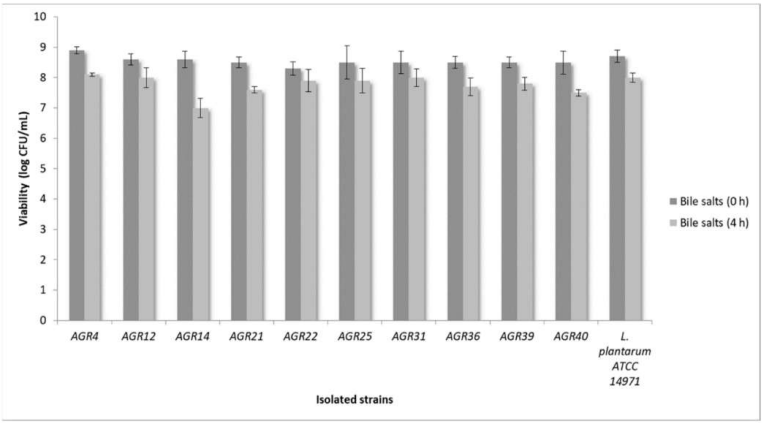


# In vitro tests simulating the human gastrointestinal tract

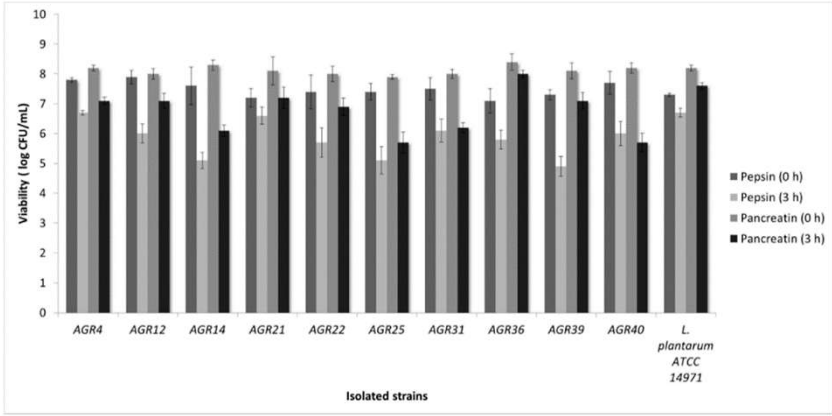
## Resistance to low pH




## Tolerance to bile salts




## Resistance to pepsin and pancreatin




**Isolation of a *Lactobacillus paracasei* Strain with Probiotic Attributes from Kefir Grains**

by Stavros Plessas [1,*], Despoina Eugenia Kiousi [2], Marina Rathosi [2], Athanasios Alexopoulos [1], Yiannis Kourkoutas [3], Ioanna Mantzourani [1], Alex Galanis [2] and Eugenia Bezirtzoglou [4]

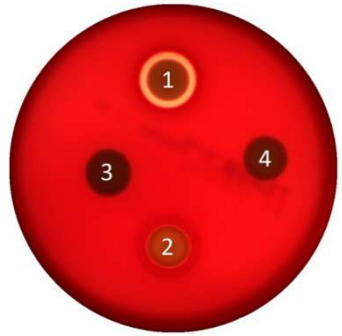
*Biomedicines* **2020**, 8, 594.

# In vitro tests simulating the human gastrointestinal tract

**Bacterial haemolytic activity** is the ability of bacteria to break down red blood cells, a process also known as hemolysis. Hemolytic activity can be determined using in vitro methods like the blood agar plate assay, where a clear zone around microbial colonies indicates hemolysis.

Bacteria produce substances called hemolysins that damage the red blood cell membrane, releasing hemoglobin.

Blood agar plates were examined for signs of ***β*-haemolysis (1)** (appeared as clear zones around colonies), ***α*-haemolysis (2)** (green zones around colonies), or ***γ*-haemolysis,** also called non-hemolysis, as no lysis of red blood cells occurs (no zones around colonies) **(3, 4).**




**Mapping the Key Technological and Functional Characteristics of Indigenous Lactic Acid Bacteria Isolated from Greek Traditional Dairy Products**

by Christina S. Kamarinou [1,2], Olga S. Papadopoulou [1], Agapi I. Doulgeraki [1], Chrysoula C. Tassou [1], Alex Galanis [2], Nikos G. Chorianopoulos [1,*] and Anthoula A. Argyri [1,*]

*Microorganisms* **2022**, 10, 246.

# In vitro tests simulating the human gastrointestinal tract

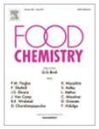
**Antibiotic susceptibility expressed as minimum inhibitory concentration (MIC)**

**Tests included the following antibiotics:**

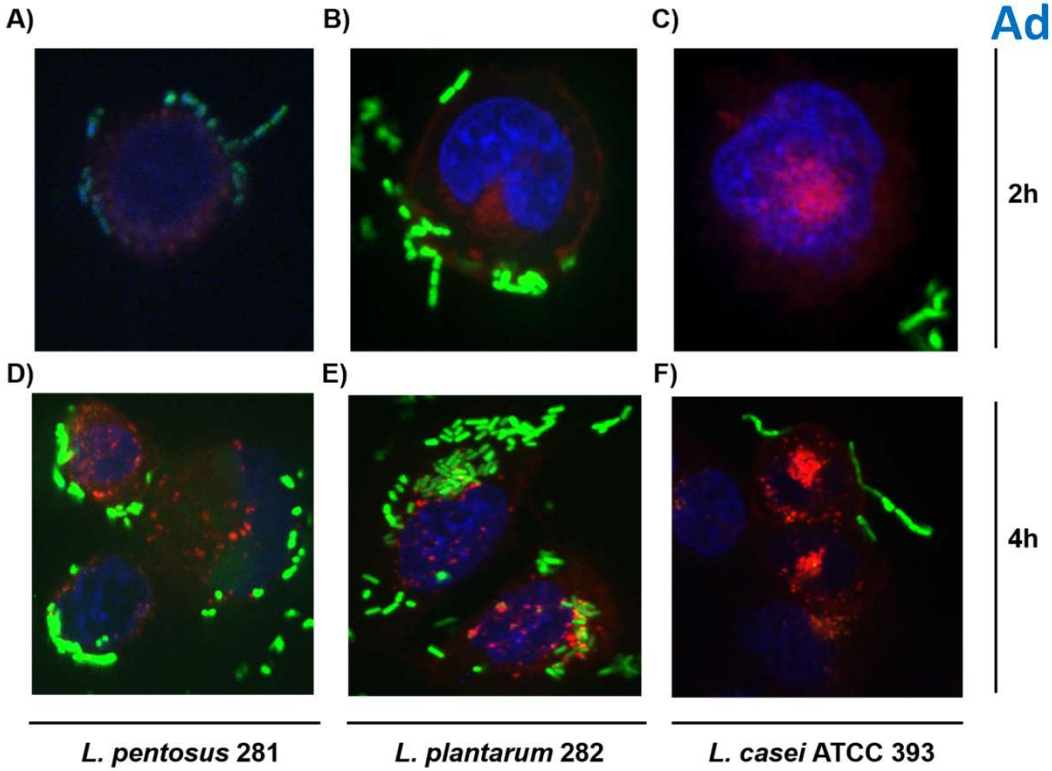
amoxycillin (256–0.015 g/mL),
amoxycillin + clavulanic acid (256–0.015 g/mL),
ampicillin (256–0.015 g/mL),
clindamycin (256–0.015 g/mL),
erythromycin (256–0.015 g/mL),
gentamycin (1024–0.06 g/mL),
metronidazole (256–0.015 g/mL),
tetracycline (256–0.015 g/mL),
tigecycline (256–0.015 g/mL)
vancomycin (256–0.015 g/mL)

Plessas S, Nouska C, Karapetsas A, Kazakos S, Alexopoulos A, Mantzourani I, Chondrou P, Fournomiti M, Galanis A, Bezirtzoglou E. Isolation, characterization and evaluation of the probiotic potential of a novel *Lactobacillus* strain isolated from Feta-type cheese.

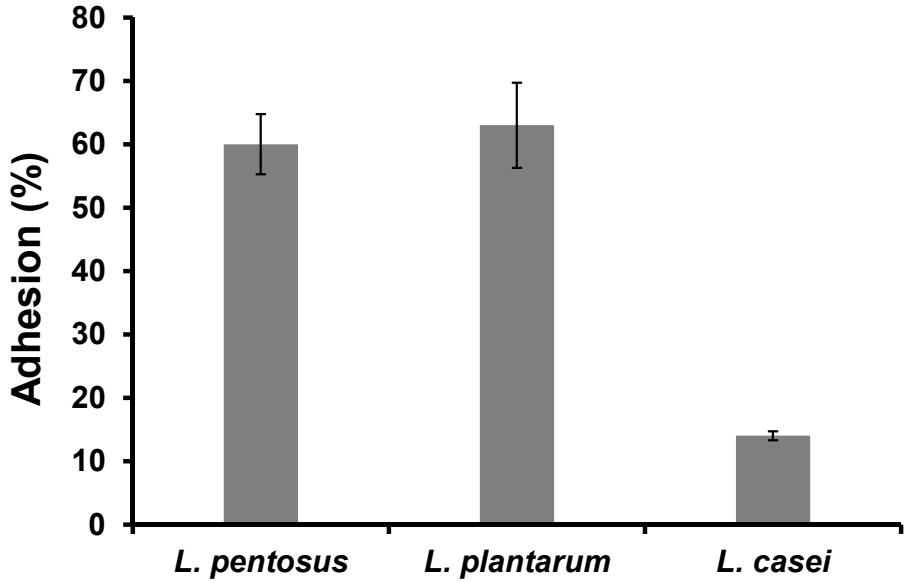
Food Chemistry
Volume 226, 1 July 2017, Pages 102-108



# In vitro tests simulating the human gastrointestinal tract




## Adherence capacity



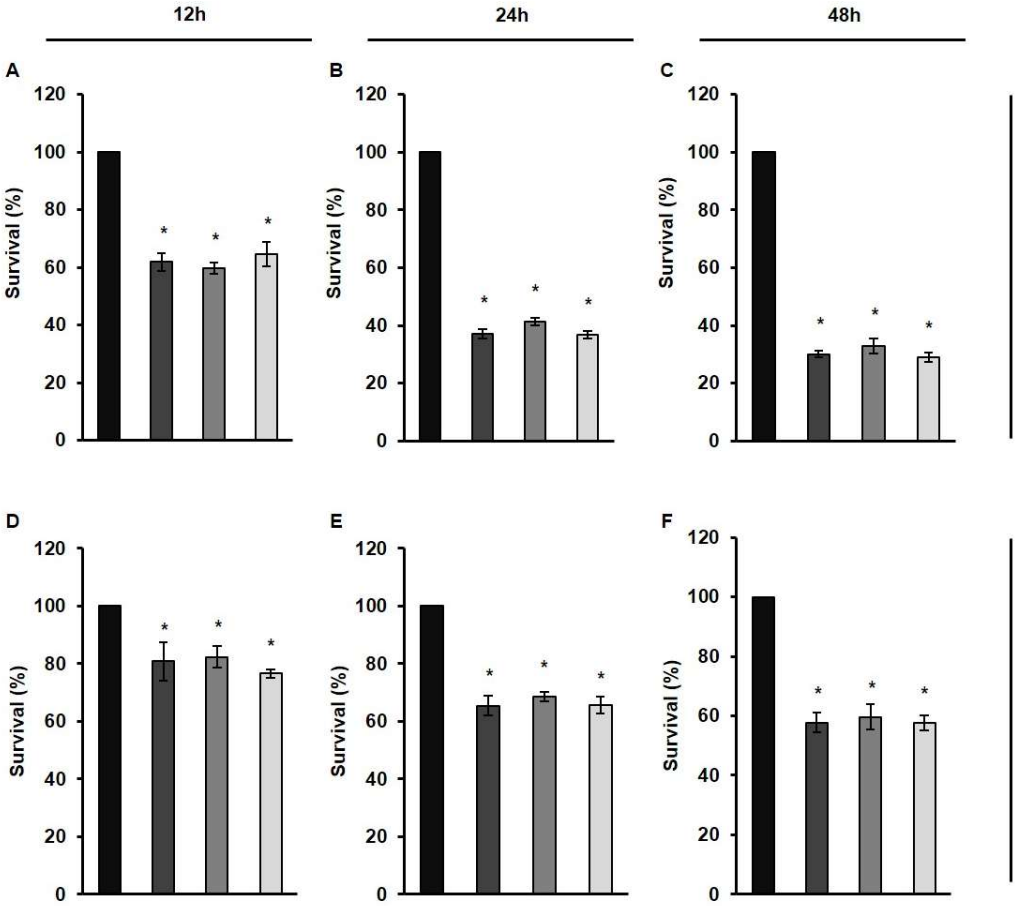

**Two potential probiotic lactobacillus strains isolated from olive microbiota exhibit adhesion and anti-proliferative effects in cancer cell lines**

*Georgia Saxami* [a], *Athanasios Karapetsas* [a], *Eleftheria Lamprianidou* [b], *Ioannis Kotsianidis* [b], *Aikaterini Chlichlia* [a], *Chrysoula Tassou* [c], *Vassilis Zoumpourlis* [d], *Alex Galanis* [a,*]

*Journal of Functional Foods* **2016**, 24, 461-471.

# Health promoting properties of probiotic bacteria

## Anti-proliferative activity (viable cells)




***Sulforhodamine B colorimetric assay***

**_Lactobacillus paracasei_ K5 displays adhesion, anti-proliferative activity and apoptotic effects in human colon cancer cells**

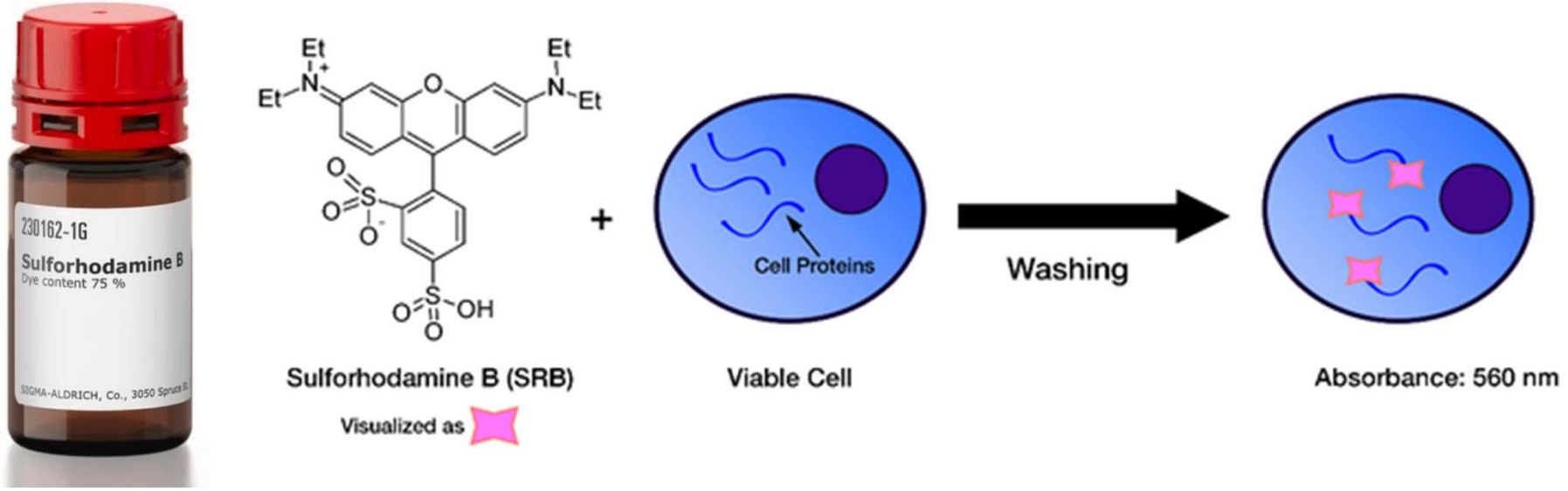
P. Chondrou, A. Karapetsas, D.E. Kiousi, D. Tsela, A. Tiptiri-Kourpeti, I. Anestopoulos Kotsianidis, E. Bezirtzoglou, A. Pappa, A. Galanis
*Corresponding author: agalanis@mbg.duth.gr

*Beneficial Microbes* **2018**, 9, 975-983.

# Health promoting properties of probiotic bacteria

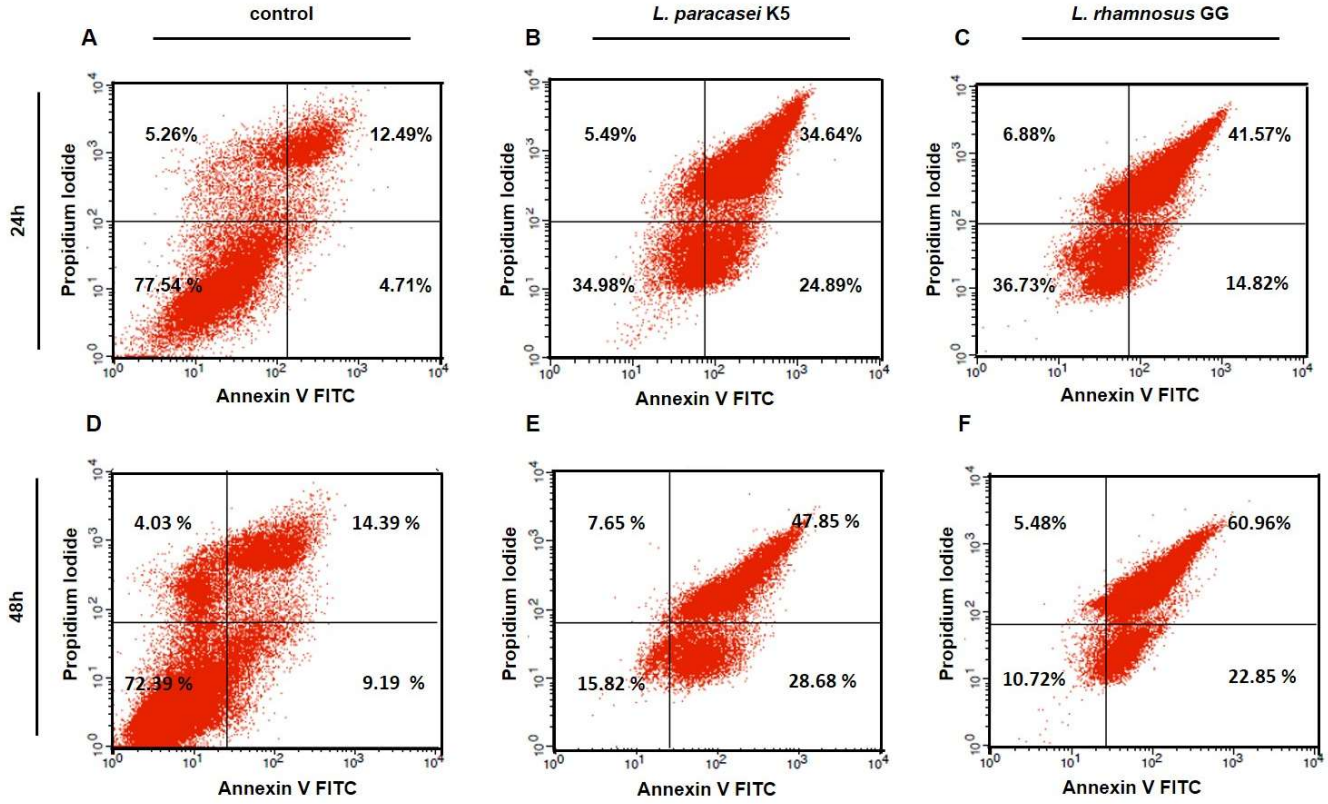
The **Sulforhodamine B (SRB)** colorimetric assay is a method for measuring cell viability, proliferation, and protein content by staining cellular proteins with SRB dye, which is then solubilized and measured using a spectrophotometer.

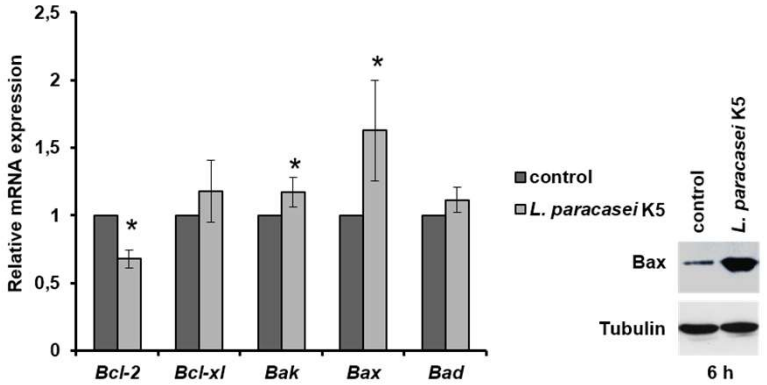
The amount of dye bound to the protein is directly proportional to the cell number, making it a useful and cost-effective tool for drug screening and cytotoxicity testing. The assay involves fixing cells with trichloroacetic acid (TCA), staining them with SRB, washing away excess dye, and then dissolving the bound dye in a basic solution for optical density (OD) measurement at 510nm.



# Health promoting properties of probiotic bacteria

## Anti-proliferative activity (Induction of apoptosis)

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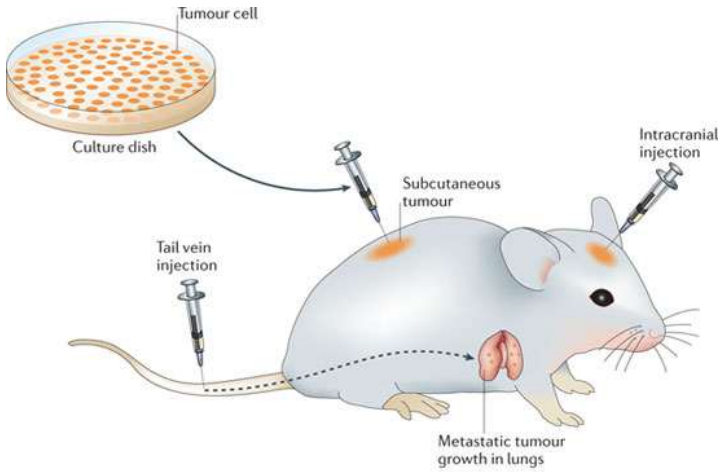
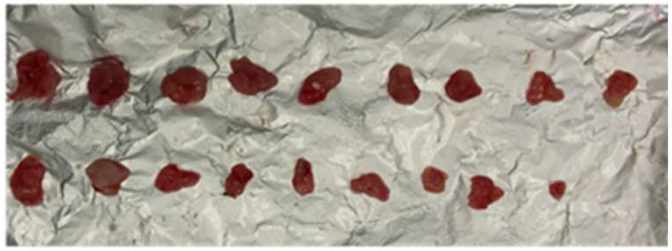
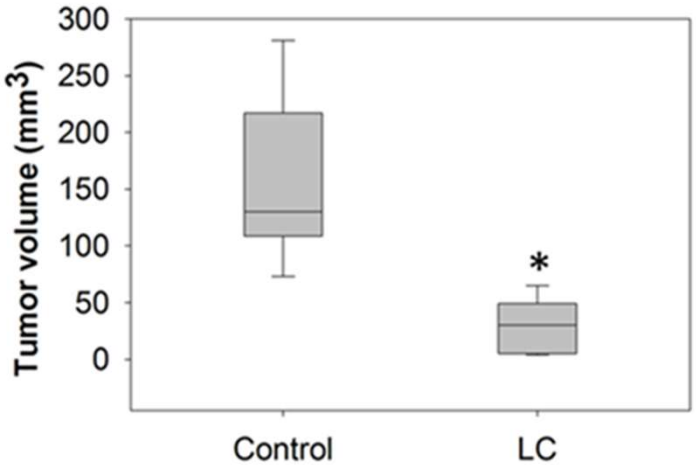
*Beneficial Microbes* **2018**, 9, 975-983.

# Health promoting properties of probiotic bacteria

## Anti-proliferative activity (in vivo)






***murine colon carcinoma model***

*Lactobacillus casei* Exerts Anti-Proliferative Effects Accompanied by Apoptotic Cell Death and Up-Regulation of TRAIL in Colon Carcinoma Cells

Angeliki Tiptiri-Kourpeti, Katerina Spyridopoulou, Valentina Santarmaki, Georgios Aindelis, Evgenia Tompoulidou, Eleftheria E. Lamprianidou, Georgia Saxami, Petros Ypsilantis, Evangeli S. Lampri, Constantinos Simopoulos, Ioannis Kotsianidis, Alex Galanis, Yiannis Kourkoutas, Dimitra Dimitrellou, Katerina Chlichlia

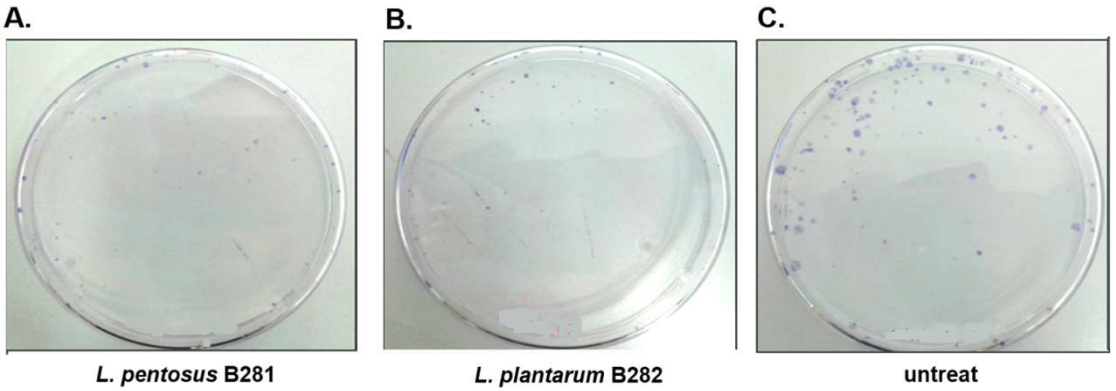
*PLoS One* **2016**, 11(2):e0147960.

# Health promoting properties of probiotic bacteria

**Clonogenic activity:**
The ability of a single cell to reproduce and form a large colony of cells. This is a key characteristic of cells with the potential to form tumors or of cancer stem cells.
**Anti-clonogenic activity:**
The ability of a compound to prevent or significantly reduce the formation of these colonies.
**Mechanism:**
Anti-clonogenic agents can work through various mechanisms, including causing cell cycle arrest, inducing cell senescence, or inhibiting the pathways that allow cells to form colonies.
**Therapeutic potential:**
Compounds with anti-clonogenic activity are of significant interest in cancer therapy because they can target the very cells that drive tumor growth and recurrence, including cancer stem cells.
**Cytotoxicity vs. anti-clonogenicity:**
A substance can kill cells directly (cytotoxic) or prevent them from forming colonies (anti-clonogenic). Some compounds are anti-clonogenic but not cytotoxic, meaning they stop the cells from growing into tumors without necessarily killing them immediately. This distinction is important for developing new cancer drugs.

***Clonogenic High-Throughput assay***

# Health promoting properties of probiotic bacteria




***Clonogenic High-Throughput assay***

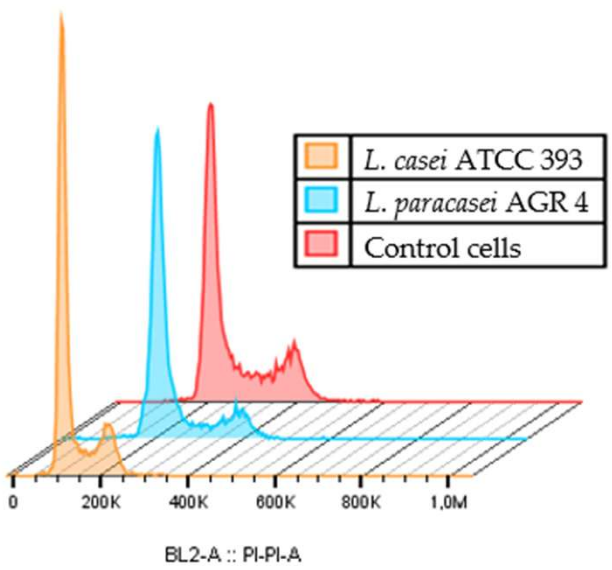
**Genomic and Phylogenetic Analysis of *Lactiplantibacillus plantarum* L125, and Evaluation of Its Anti-Proliferative and Cytotoxic Activity in Cancer Cells**

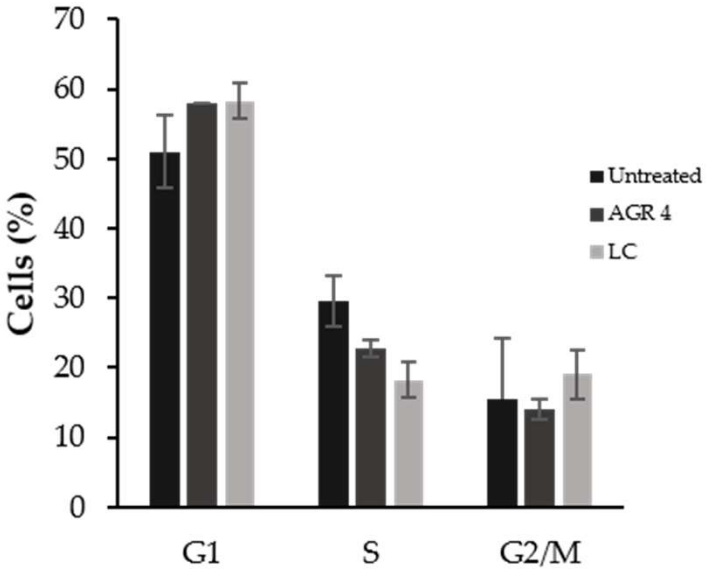
by Konstantinos Tegopoulos [1,†], Odysseas Sotirios Stergiou [1,†], Despoina Eugenia Kiousi [1,†], Margaritis Tsifintaris [1], Ellie Koletsou [1], Aristotelis C. Papageorgiou [1], Anthoula A. Argyri [2], Nikos Chorianopoulos [2], Alex Galanis [1,*] and Petros Kolovos [1,*]

*Biomedicines* **2021**, *9*, 1718

# Health promoting properties of probiotic bacteria

## (Cell cycle arrest)

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*Biomedicines* **2020**, 8, 594.

# Health promoting properties of probiotic bacteria

What anti-migration activity is

- **Inhibiting cell movement:**

It is the ability to stop or slow down the movement of cells, a process crucial for activities like tissue repair, development, and cancer cell invasion.

- **Targeting cellular pathways:**

This activity can work by inhibiting specific molecular pathways that control cell migration, such as the PI3-K/Akt/Rac1 pathway.

- **Blocking key proteins:**

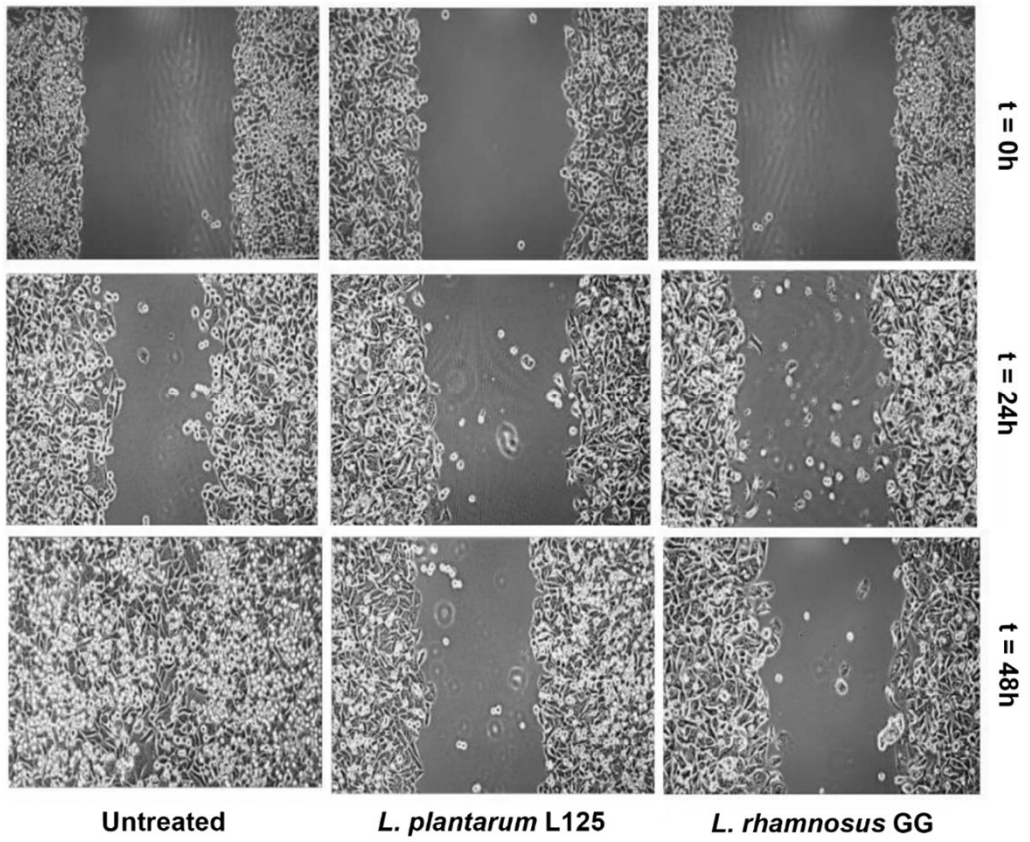
Some agents work by down-regulating proteins essential for cell movement, like matrix-degrading enzymes such as MMP-2 and MMP-9, which are often involved in cancer metastasis.

- **Examples of substances:**

Various natural compounds and drugs have been studied for their anti-migratory properties, including astaxanthin, capsaicin, and certain compounds from plants.

***Wound-healing assay***

# Health promoting properties of probiotic bacteria




***Wound-healing assay***

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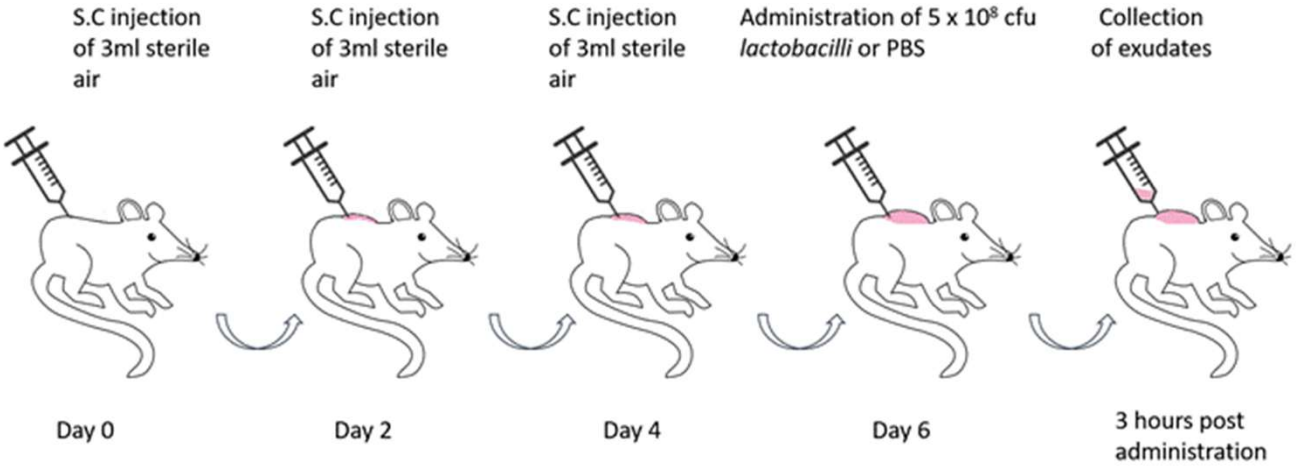
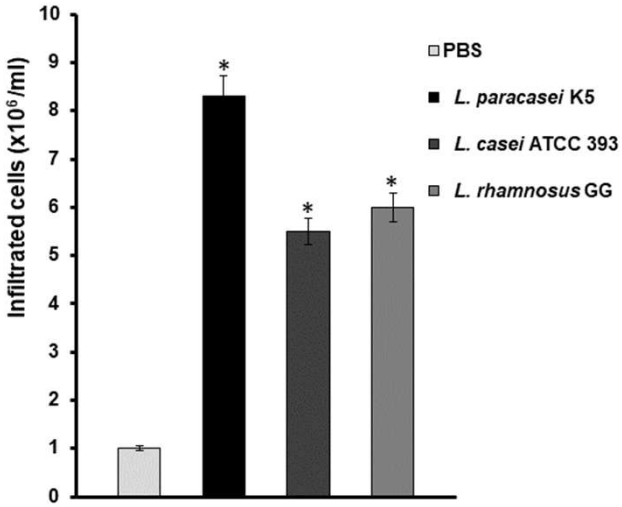
*Biomedicines* **2021**, *9*, 1718

# Health promoting properties of probiotic bacteria

## Immunomodulatory Properties




**Potentially probiotic *Lactobacillus* strains with anti-proliferative activity induce cytokine/chemokine production and neutrophil recruitment in mice**

G. Saxami, A. Karapetsas, P. Chondrou, S. Vasiliadis, E. Lamprianidou, I. Kotsianidis Ypsilantis, S. Botaitis, C. Simopoulos, A. Galanis

*Corresponding author: agalanis@mbg.duth.gr #these authors contributed equally on this work

*Beneficial Microbes* **2018**, 9, 975-983.

***Dorsal air pouch mouse model***

**Assessment of the Immunomodulatory Properties of the Probiotic Strain *Lactobacillus paracasei* K5 In Vitro and In Vivo**

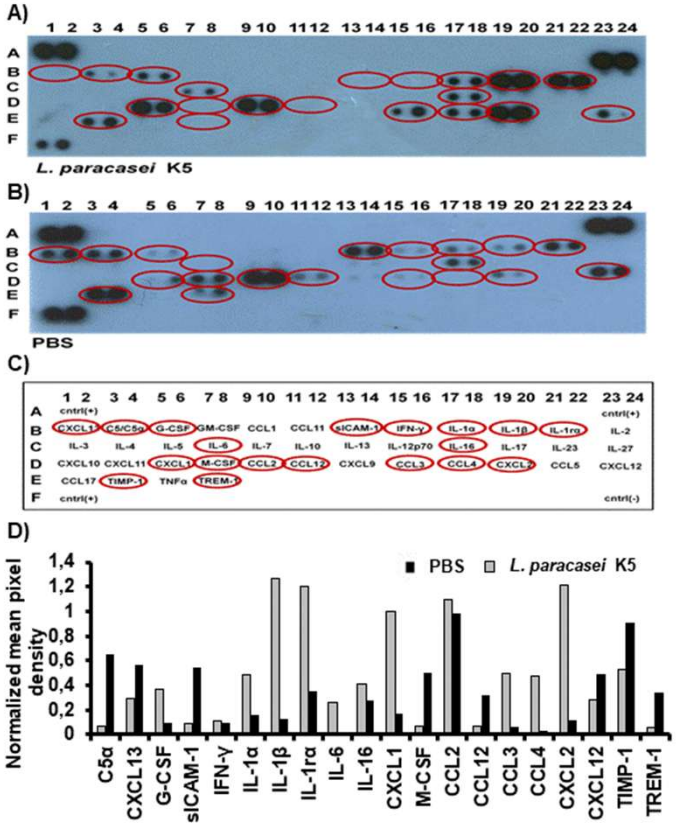
by Pelagia Chondrou, Athanasios Karapetsas, Despoina Eugenia Kiousi, Stavros Vasileiadis, Petros Ypsilantis, Sotiris Botaitis, Athanasios Alexopoulos, Stavros Plessas, Eugenia Bezirtzoglou and Alex Galanis

*Microorganisms* **2020**, 8, 709.

# Health promoting properties of probiotic bacteria

## Immunomodulatory Properties

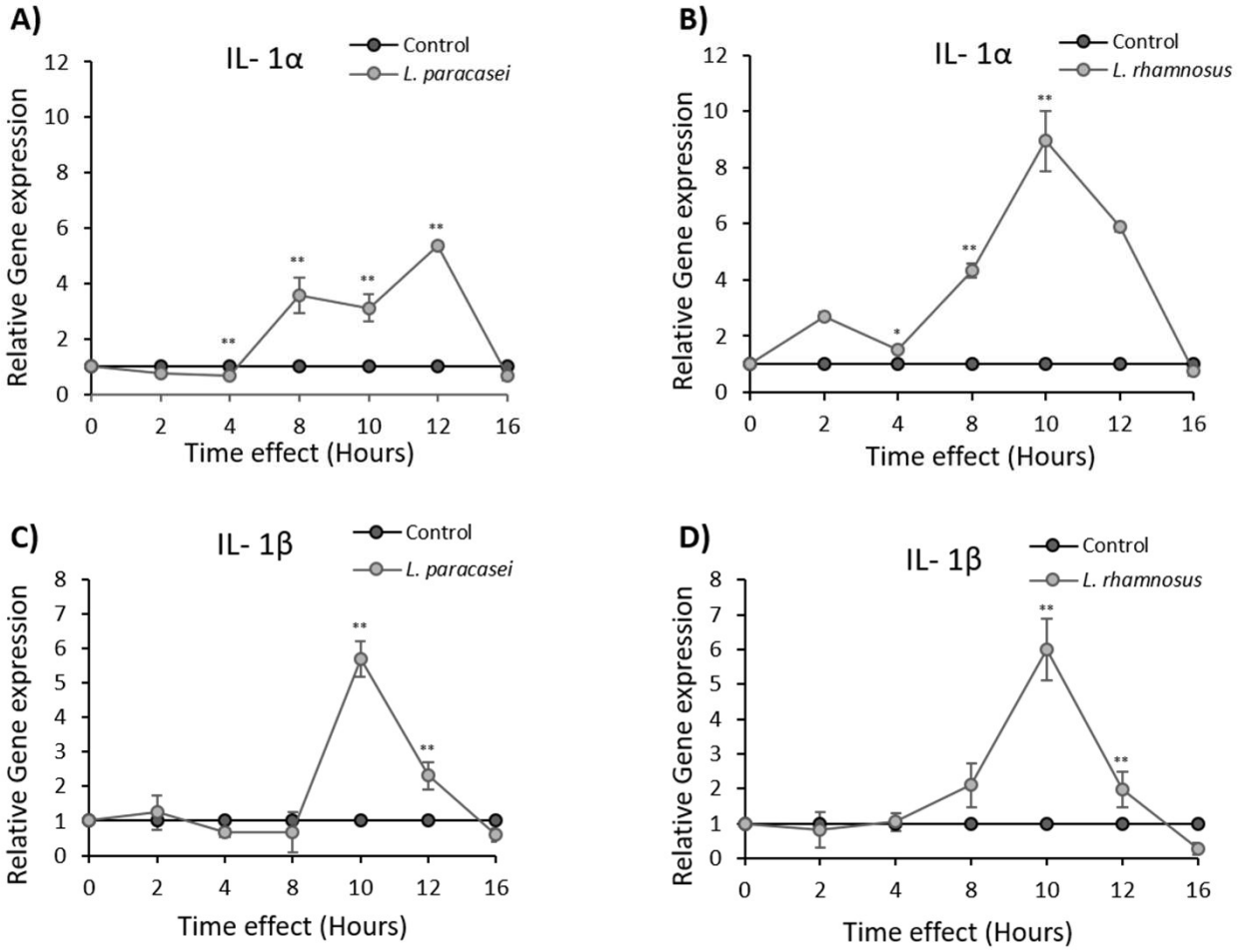
*L. paracasei* or PBS were administered in the air pouches of BALB/c mice, and after 3 h, the exudates were collected, and the supernatants were analyzed for cytokine/chemokine expression, using the Proteome Profiler TM Mouse Antibody Array Panel. The pairs of spots in the upper-left, upper-right, and lower-left corners are positive controls. Data from was quantified as mean pixel density normalized to the density of the positive controls.




| Markers | *L. paracasei* K5 |
|---|---|
| IL-1α | ↑[a] |
| IL-1β | ↑ |
| IL-1rα | ↑ |
| IL-16 | ↑ |
| IL-6 | ↑ |
| C5a | ↓ |
| CCL2 | nd[b] |
| CCL3 | ↑ |
| CCL4 | ↑ |
| CCL12 | ↓[c] |
| IFN-γ | nd |
| sICAM-1 | ↓ |
| CXCL1 | ↑ |
| CXCL2 | ↑ |
| CXCL12 | ↓ |
| CXCL13 | ↓ |
| G-CSF | ↑ |
| M-CSF | ↓ |
| TIMP-1 | ↓ |
| TREM-1 | ↓ |
| [a]upregulation<br>[b]no detection | |
| [c]downregulation | |

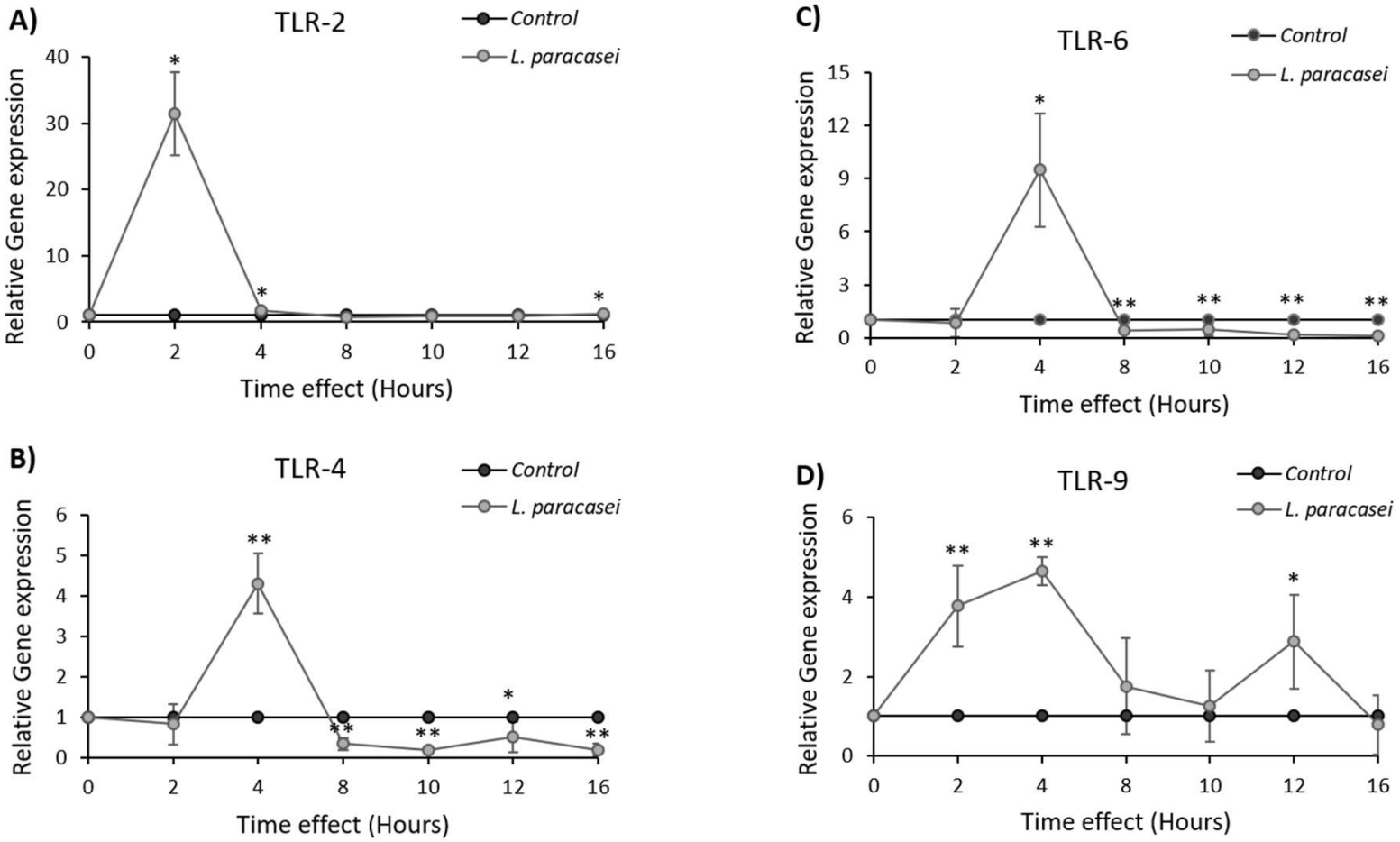
# Health promoting properties of probiotic bacteria

## Immunomodulatory Properties

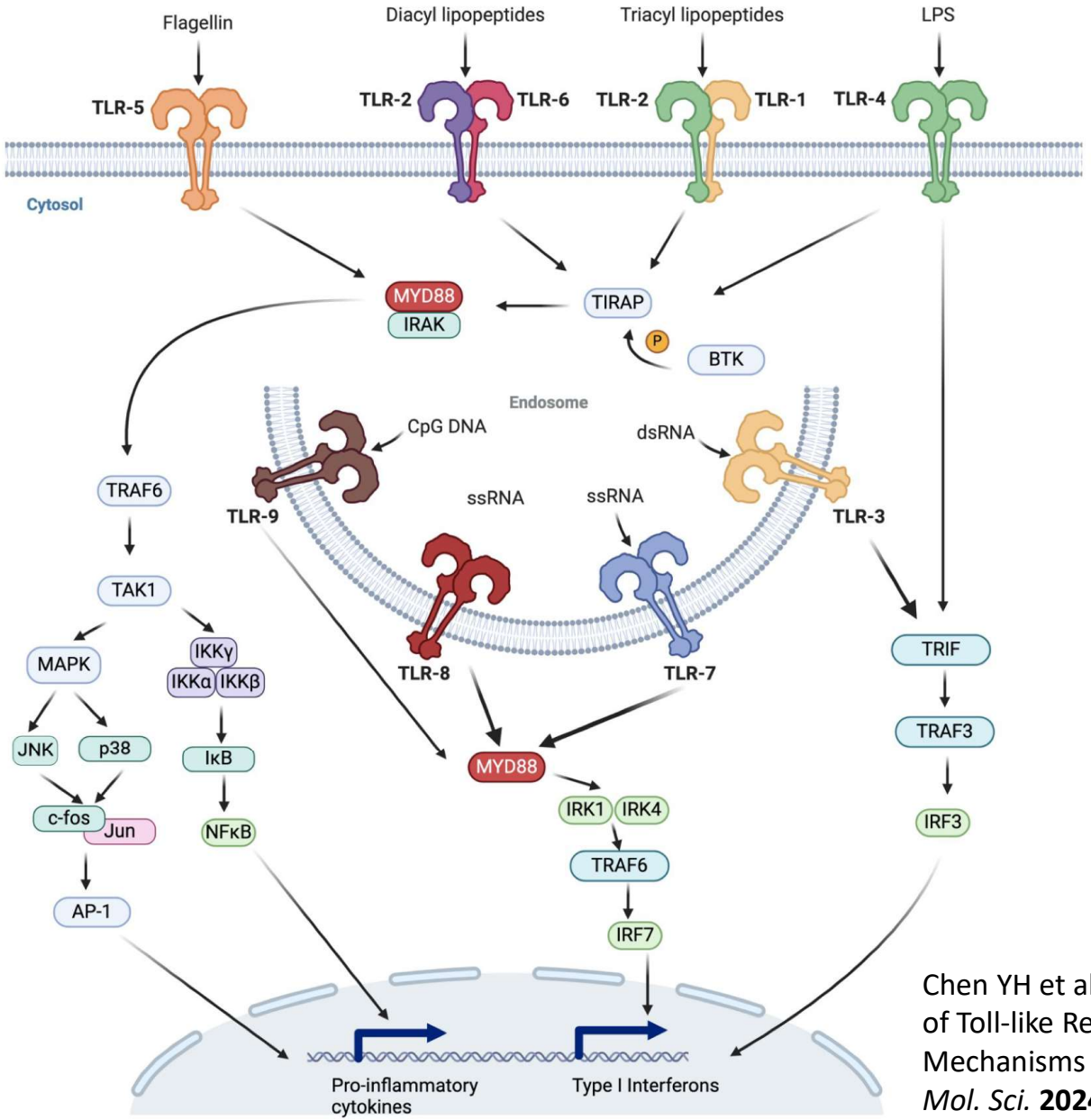


# Health promoting properties of probiotic bacteria

## Immunomodulatory Properties



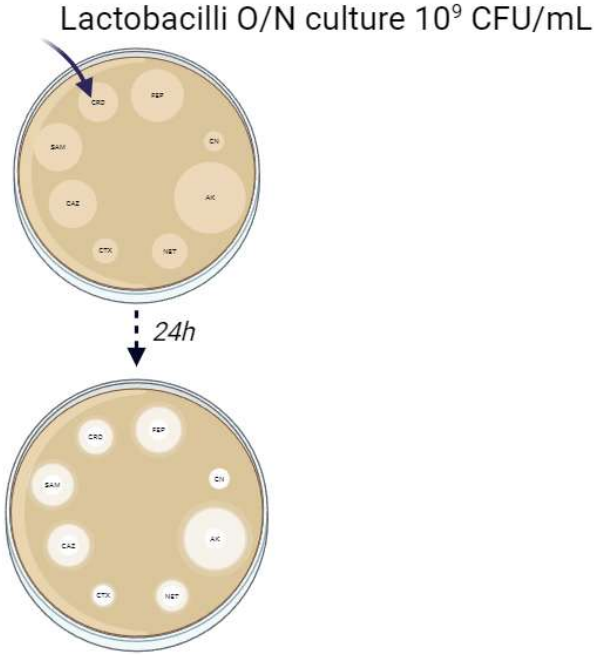
Toll-like receptors (TLRs) are a family of pattern recognition receptors (PRRs) that play a critical role in the innate immune system. They detect molecules shared by pathogens, known as pathogen-associated molecular patterns (PAMPs), as well as host-derived damage-associated molecular patterns (DAMPs) released from damaged or dying cells. The binding of these ligands triggers a signal transduction cascade that stimulates the host's immune response.




Chen YH et al., Unraveling the Complexities of Toll-like Receptors: From Molecular Mechanisms to Clinical Applications. *Int. J. Mol. Sci.* **2024**, 25, 5037.

# Live lactobacilli limit pathogen growth

## Anti-microbial action



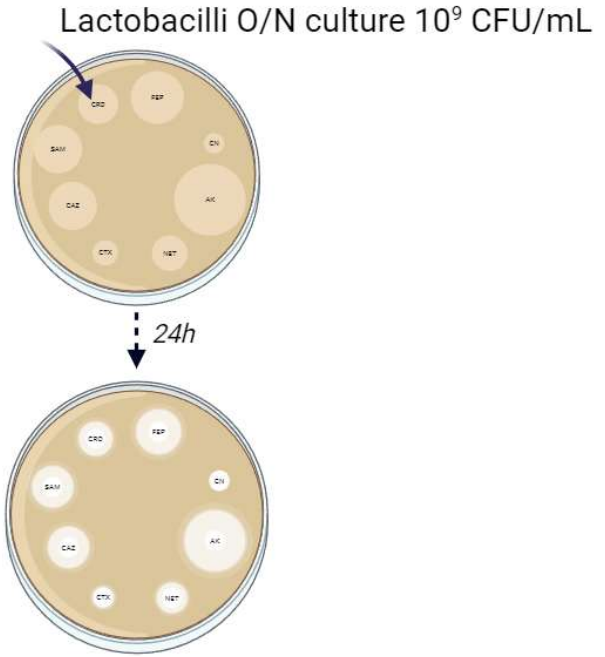

**Agar well diffusion assay**

It is a cost-effective and widely used technique for assessing the antimicrobial activity of various substances, such as plant extracts and microbial products.

- The method provides a qualitative or semi-quantitative assessment of a compound's ability to inhibit microbial growth.
- It is not ideal for all substances; for example, non-polar compounds may not diffuse well, leading to smaller or misleading inhibition zones.
- The size of the zone is influenced by the microbe's growth rate, the diffusion rate of the compound, and the concentration of the substance in the well.

# Live lactobacilli limit pathogen growth

## Anti-microbial action




**How it works**

**Inoculation:** A microbial culture is spread evenly over the surface of an agar plate.

**Well creation:** Sterile wells (4–8 mm in diameter) are punched into the agar using a tool called a borer.

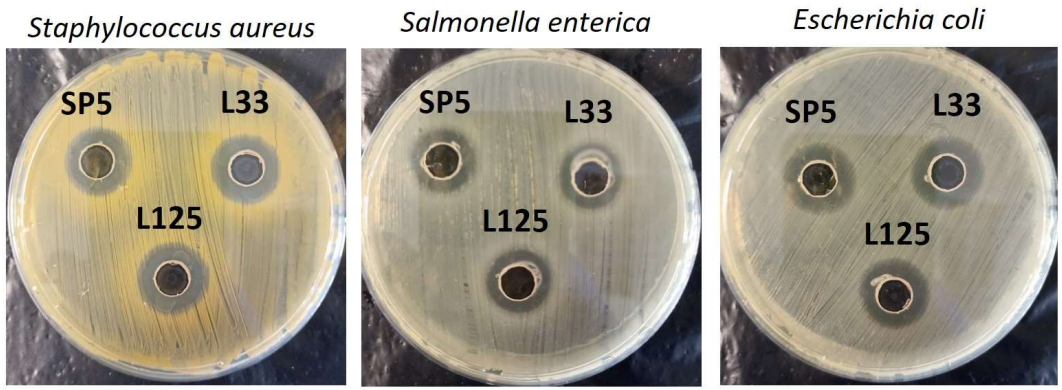
**Sample application:** The antimicrobial substance or extract is placed into the wells.

**Incubation:** The plate is incubated to allow the microorganism to grow and the test substance to diffuse into the agar.

**Measurement:** The clear area around each well, known as the "zone of inhibition," is measured. The size of this zone indicates the effectiveness of the substance.

# Live lactobacilli limit pathogen growth

**A**




**B**

| | ***S. aureus*** (cm) | ***S. enterica*** (cm) | ***E. coli*** (cm) |
|---|---|---|---|
| *Lp. pentosus* L33 | 0.425 ± 0.15 | 0.33 ± 0.16 | 0.46 ± 0.16 |
| *Lp. pentosus* L125 | 0.26 ± 0.03 | 0.22 ± 0.06 | 0.46 ± 0.15 |
| *Lc. paracasei* SP5 | 0.28 ± 0.06 | 0.31 ± 0.11 | 0.56 ± 0.15 |

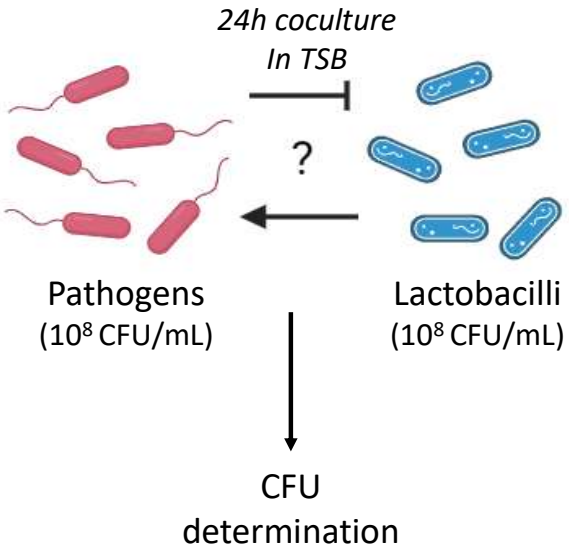
Genetic and phenotypic assessment of the antimicrobial activity of three potential probiotic lactobacilli against human enteropathogenic bacteria

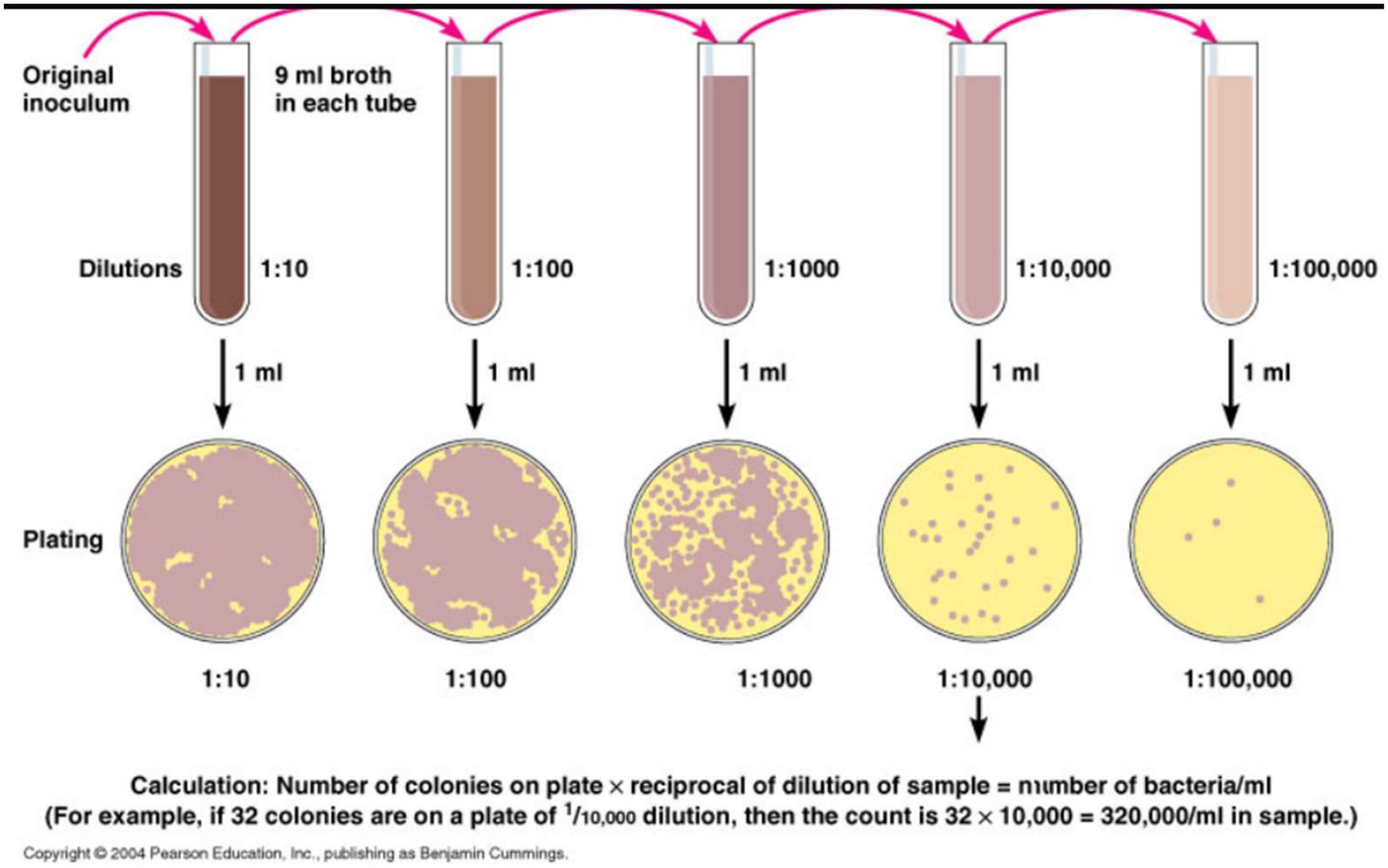
Despoina Eugenia Kiousi[1] Christos Efstathiou[1] Vasilis Tzampazlis[1]
Stavros Plessas[2] Maria Panopoulou[3] Maria Koffa[1] Alex Galanis[1*]

Front. Cell. Infect. Microbiol., 08 February 2023
Sec. Biofilms
Volume 13 - 2023 |
https://doi.org/10.3389/fcimb.2023.1127256

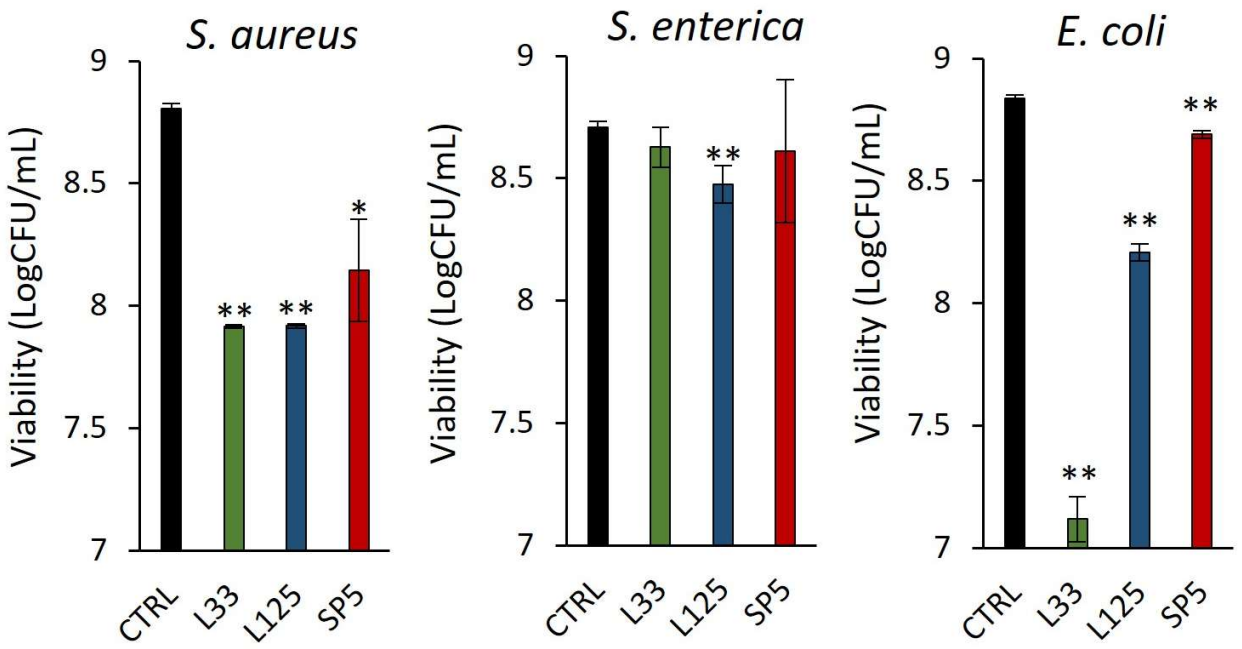
frontiers

# Viable lactobacilli affect pathogen growth in a strain-specific basis



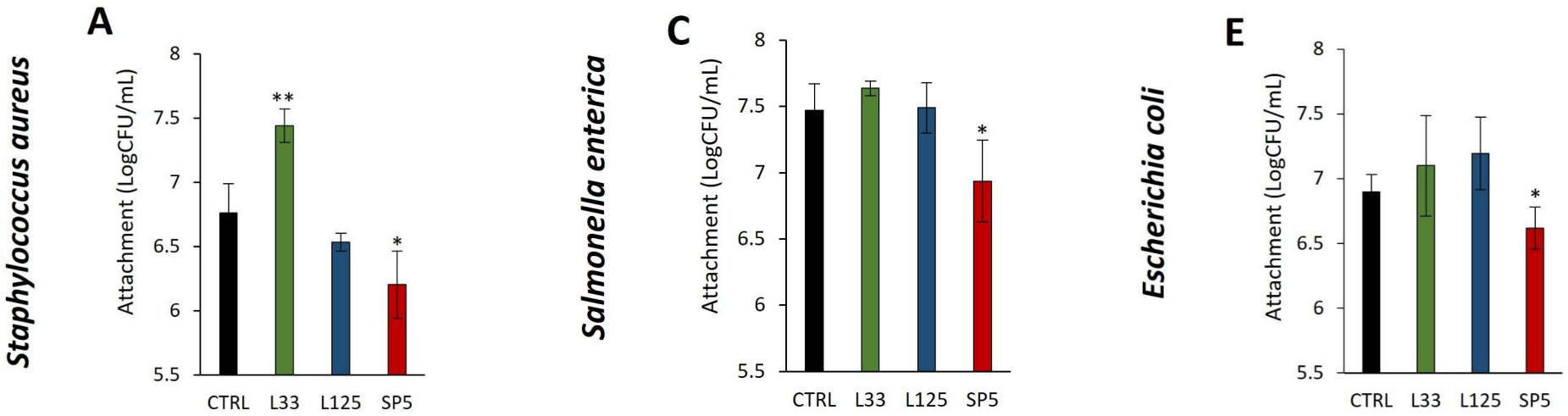



# Viable lactobacilli affect pathogen growth in a strain-specific basis



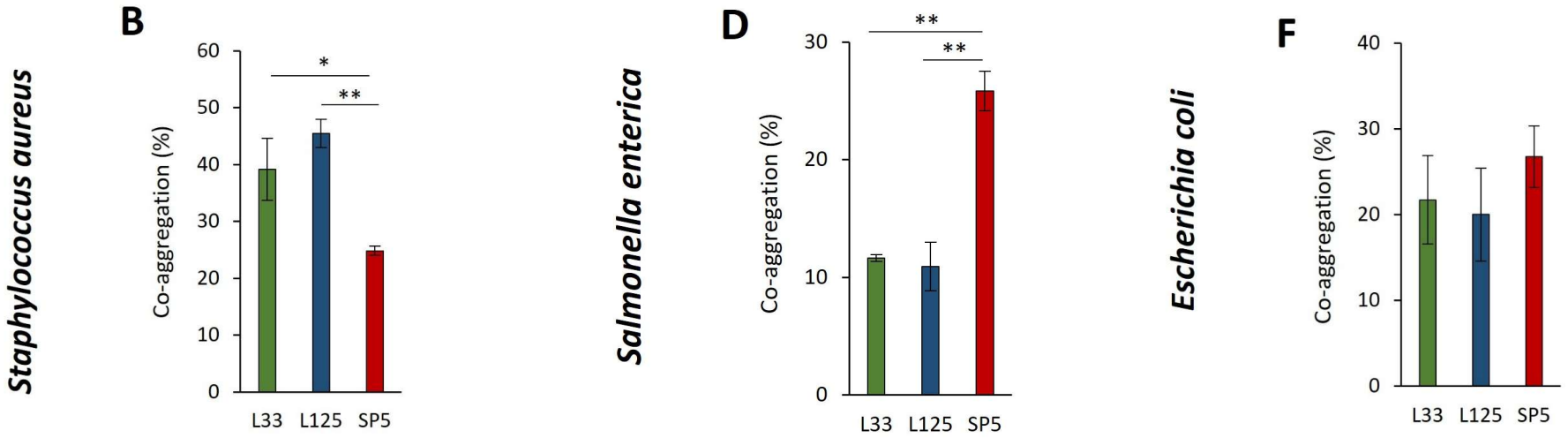

Pathogens ($10^8$ CFU/mL) and lactobacilli ($10^8$ CFU/mL) were co-incubated for 24 h, at 37°C under anaerobic conditions in TSB. The next day, the bacterial suspension was serially diluted in 1× Ringer's solution and spread on agar plates for colony enumeration.

# Competition for adherence and coaggregation ability of lactobacilli



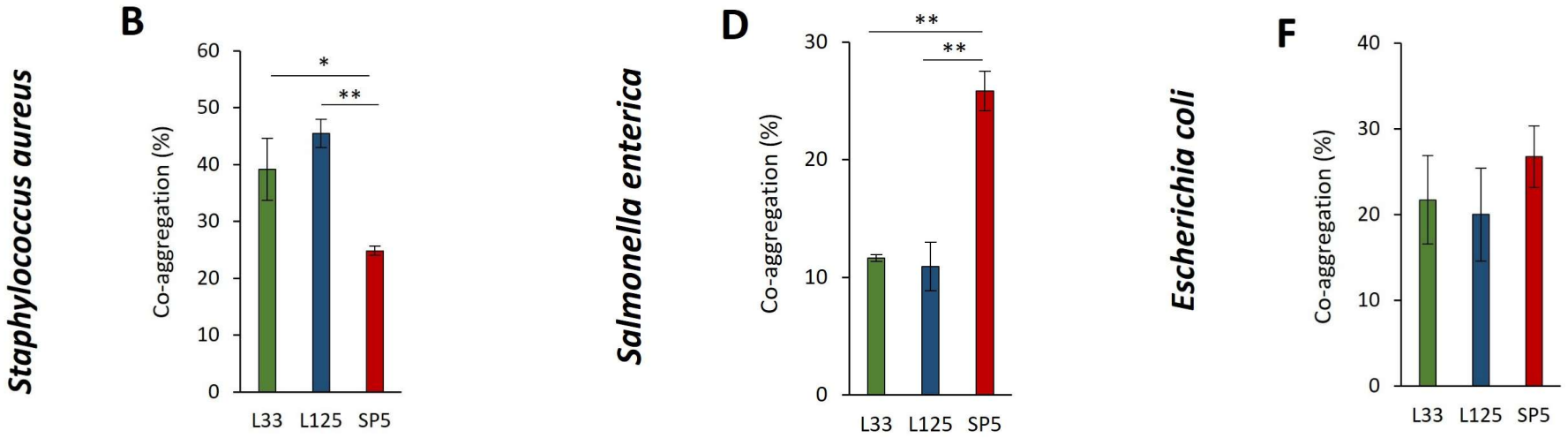

HT-29 cells were seeded in 24-well plates at a density of 4 ×$10^5$ cells per well and were incubated until the formation of a monolayer (100% confluency). Then, cells were treated with lactobacilli and/or pathogens at a concentration of $10^8$ CFU/mL for 4 h. Control samples were incubated with pathogens alone for 4 h. The monolayers were washed twice to remove unattached bacteria, and cells were detached using 1% v/v Trypsin. The suspension was serially diluted in 1× Ringer's solution and plated onto agar plates: TSA plates for *S. aureus* and McConkey agar plates for *S. enterica* and *E. coli* enumeration. Plates were incubated at 37°C, under anaerobic conditions, until the formation of visible colonies. Attached bacteria on epithelial cells are expressed as Log CFU/mL.

# Competition for adherence and coaggregation ability of lactobacilli




**Co-aggregation** is the ability of different microbial strains to stick together, often involving specific cell-to-cell recognition mechanisms. It is measured by observing the degree of aggregation in a mixed culture of two different bacterial strains compared to the strains alone. This property is important in microbiology and is studied to understand biofilm formation and for selecting potential probiotics, which can use co-aggregation to inhibit pathogens.

# Competition for adherence and coaggregation ability of lactobacilli



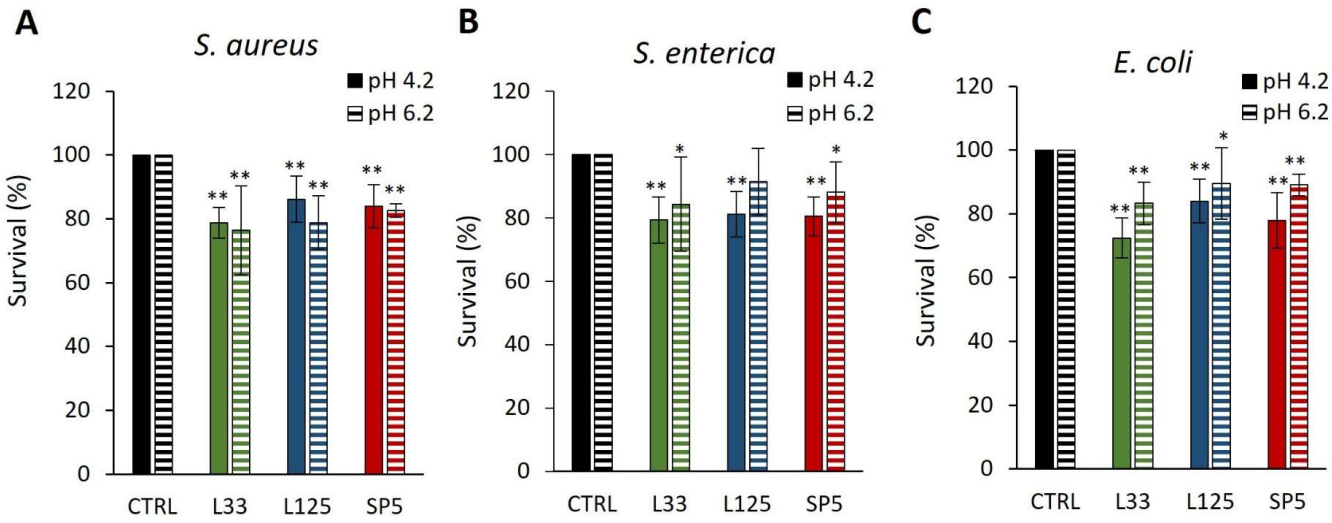

**Why it's important**

**Biofilm formation**: Co-aggregation is a key process in the development of complex microbial communities like biofilms. It allows genetically distinct bacteria to recognize and stick to one another, forming a stable community.

**Probiotic selection**: The co-aggregation ability of probiotic bacteria with pathogens is a desirable trait for selecting candidates.

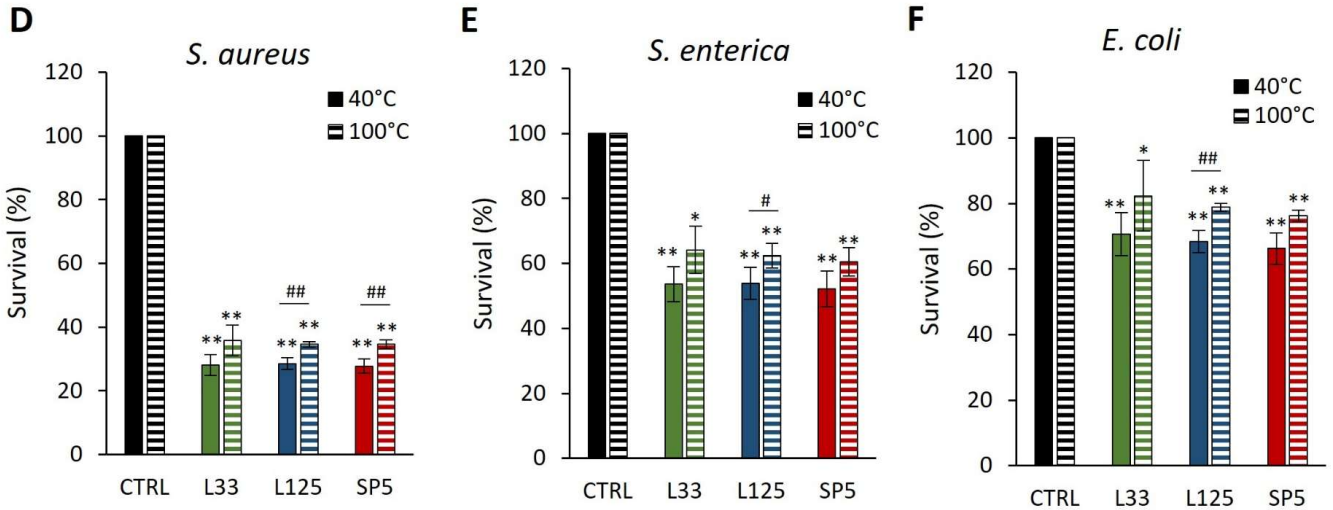
**Pathogen inhibition**: A probiotic's ability to co-aggregate with a pathogen can help prevent the pathogen from attaching to host tissues, as seen with the formation of a barrier in the gastrointestinal tract.

# Antimicrobial capacity of CFCS




$10^8$ CFU/mL of pathogens were added to the CFCS and were left to incubate for 24 h in sterile tubes. MRS was adjusted to the appropriate pH. After the end of the incubation period, samples were vortexed and transferred to a 96-well plate for absorbance reading at 620 nm, using a microplate reader. Results are expressed as the percentage (%) = [(Sample OD620-Media blank OD620)/(Mean control OD620-Media blank OD620)] × 100.

# Antimicrobial capacity of CFCS

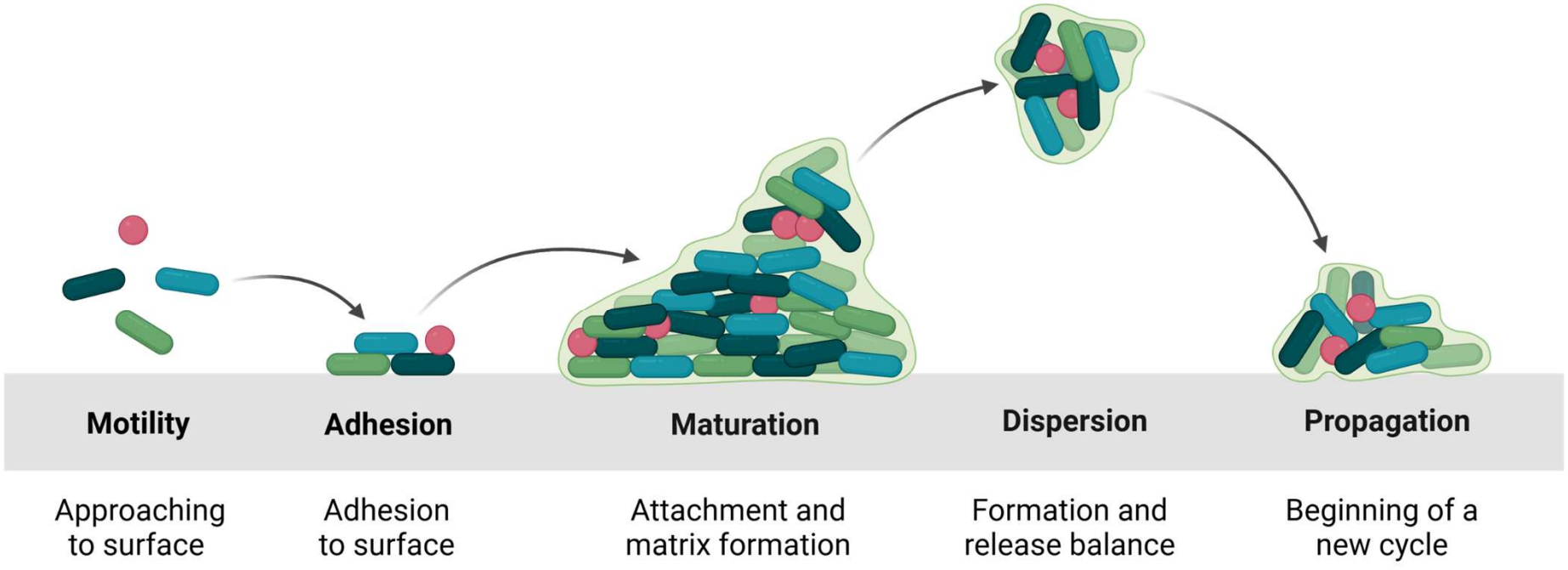



Heat-treated CFCS (pH 4.2) was utilized to determine the stability of the bioactive compounds responsible for the antimicrobial effects at denaturing temperature.

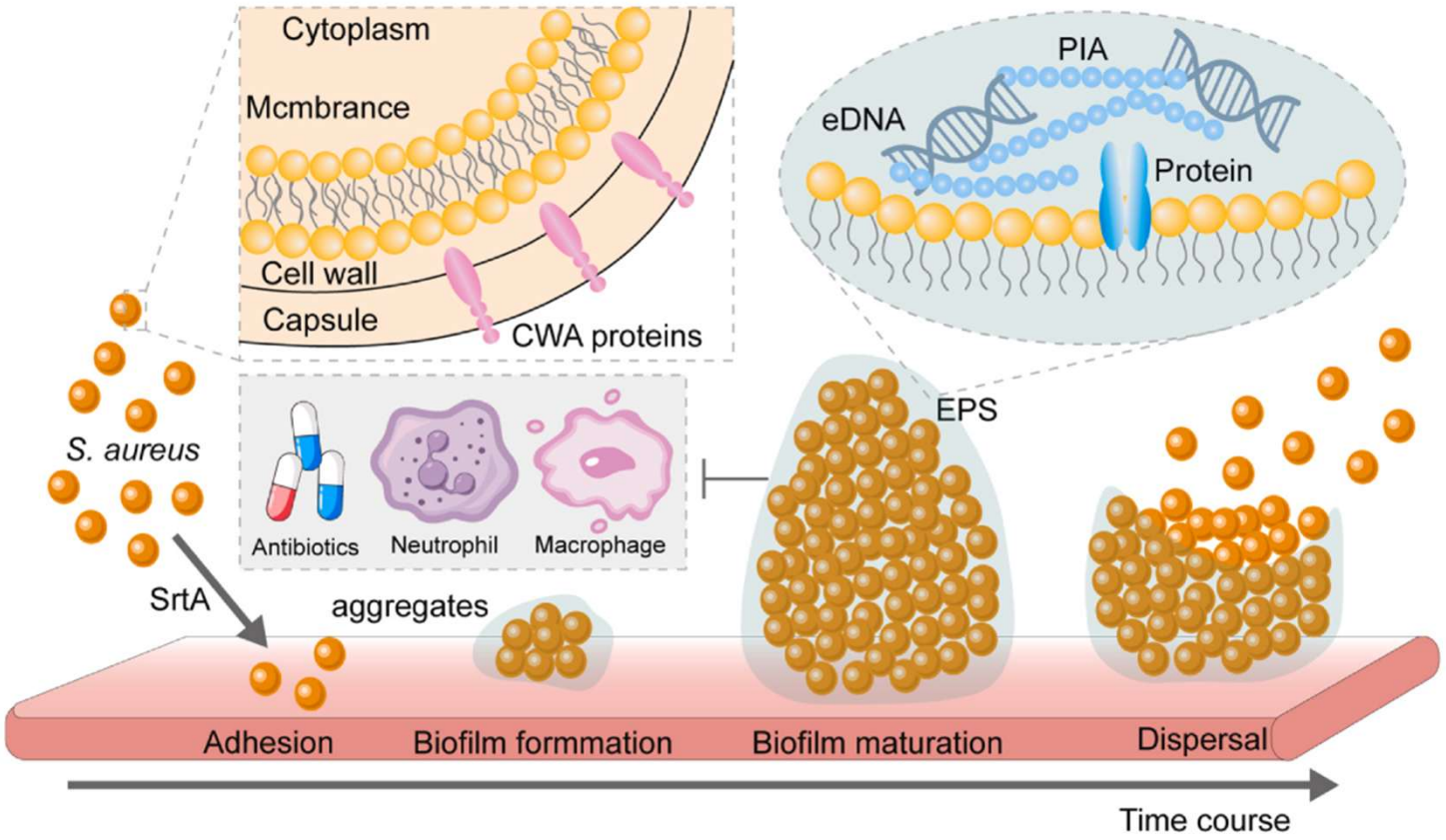
# Pathogen biofilms

**Biofilms**: a complex (poly)microbial community embedded in a matrix comprised by EPS, proteins and DNA

- Increased **antibiotic resistance**
- **Immune evasion**
- **Contamination** of surfaces
- During dispersion they can enter the bloodstream and cause **systemic infections**



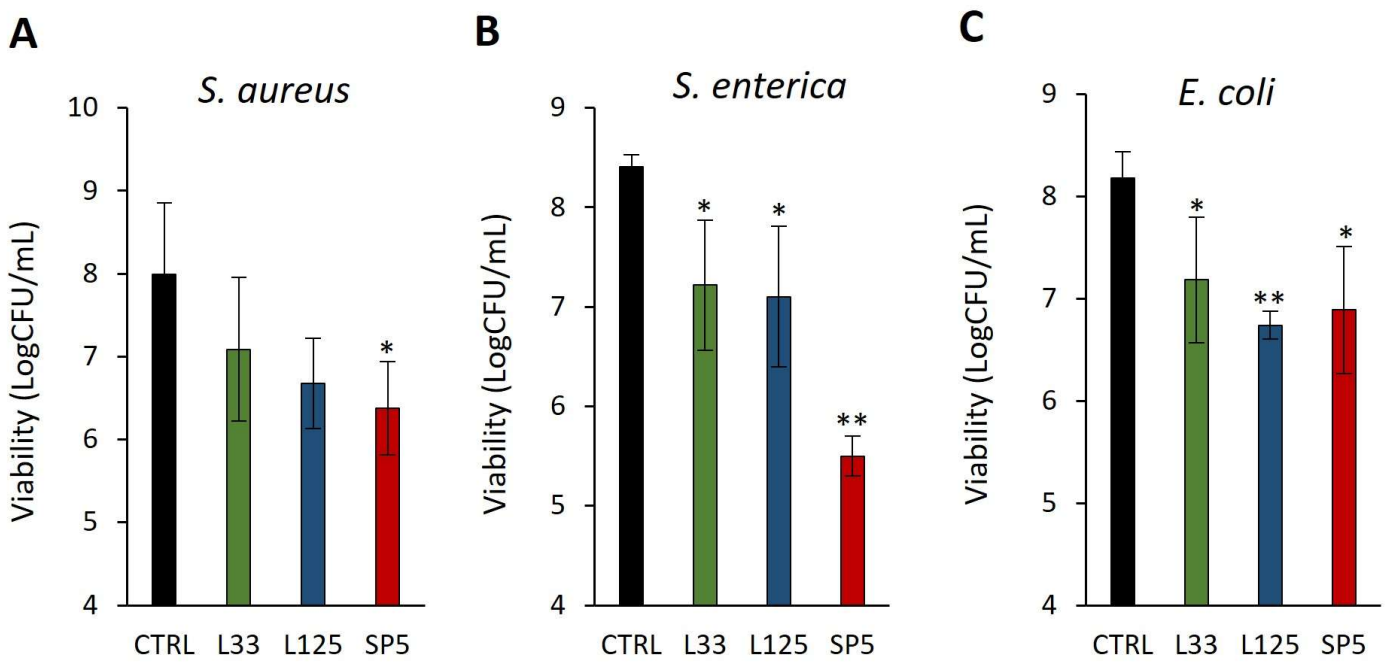
# Pathogen biofilms




***Staphylococcus*** is a leading cause of biofilm-associated infections, which often occur on indwelling medical devices like catheters and prostheses, but can also form in other parts of the body.

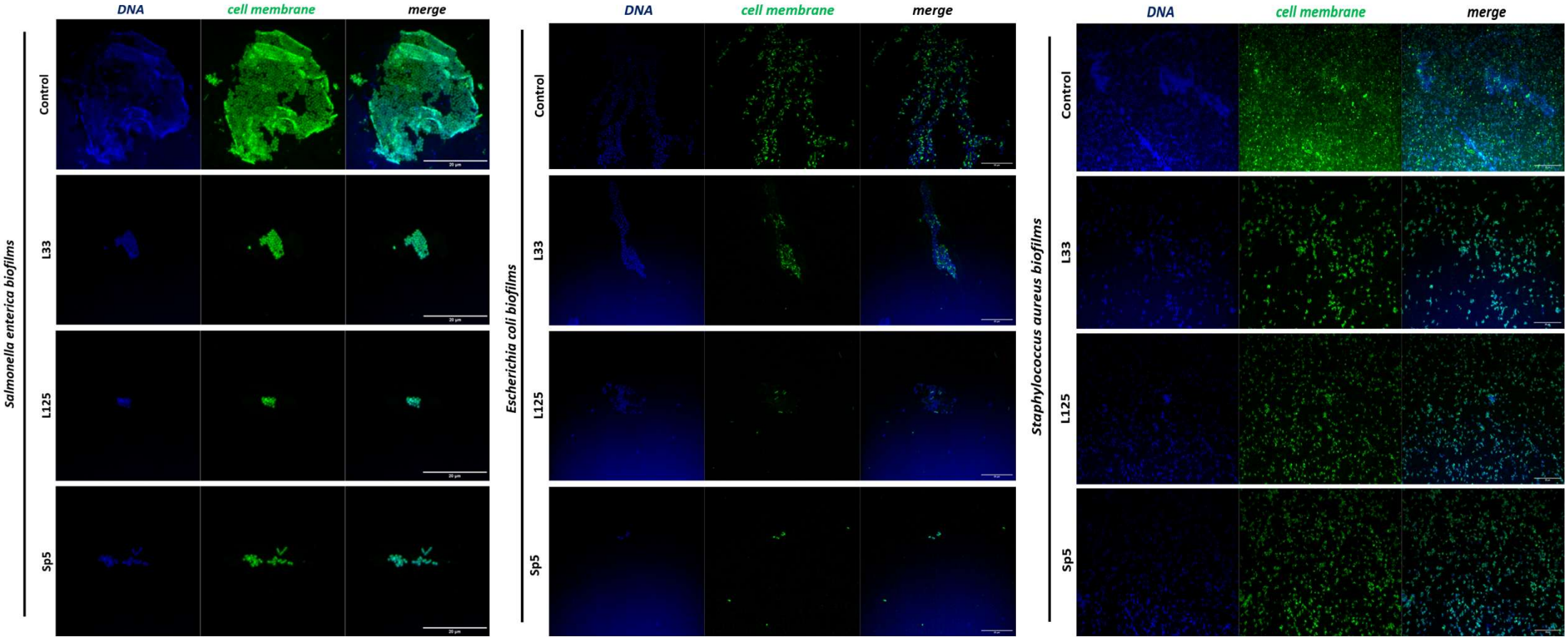
Wang et al., 2025, Animals and Zoonoses, Volume 1, Issue 2, June 2025, Pages 188-202

# Antibiofilm activity of CFCS



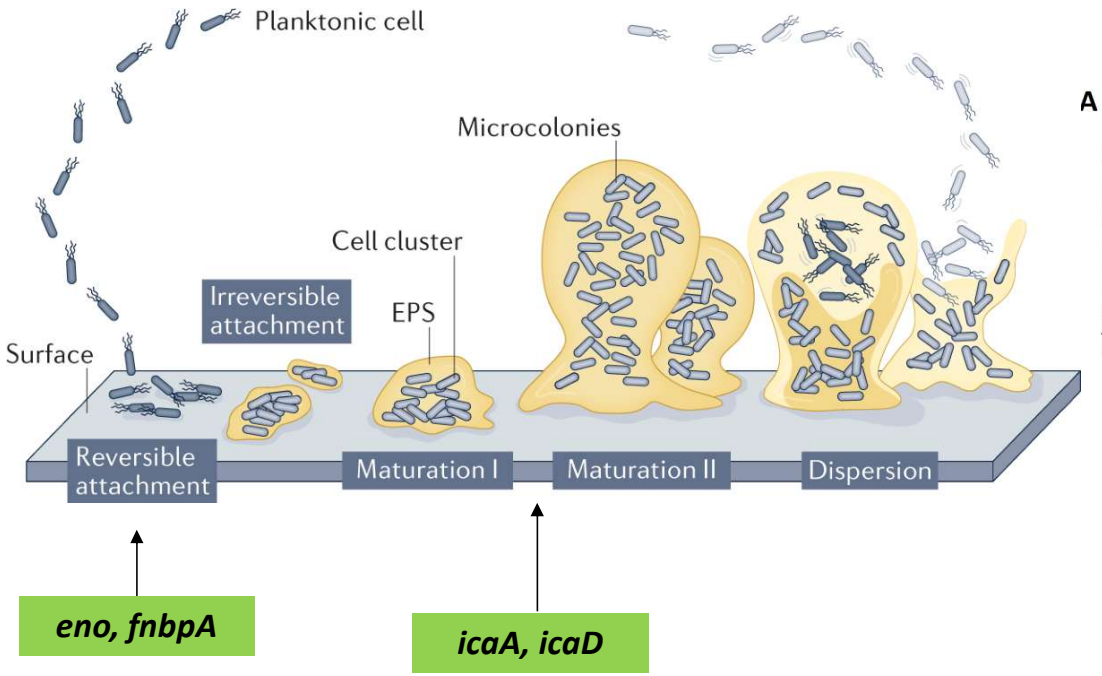

$10^8$ CFU/mL of fresh O/N cultures of pathogens (100 mL) were co-incubated with 100 mL CFCS in a 96-well plate for 24 h. For the estimation of viable cells, biofilms were washed twice post-treatment with PBS, and were mechanically disrupted. The suspension was serially diluted in 1× Ringer's solution and spread on agar plates for colony enumeration. Plates were incubated at 37°C under anaerobic conditions until the formation of visible colonies. Viable bacteria are presented as Log CFU/mL.

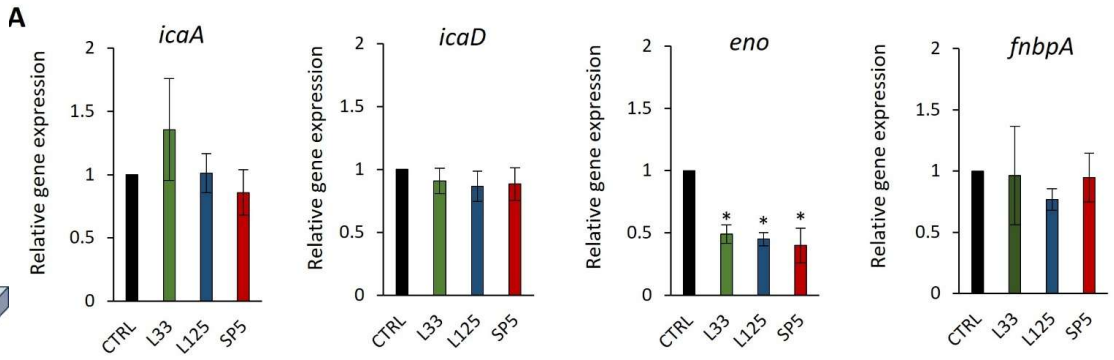
# Antibiofilm activity of CFCS



# Effect of lactobacilli CFCS on the expression levels of biofilm-related genes

***Staphylococcus aureus***

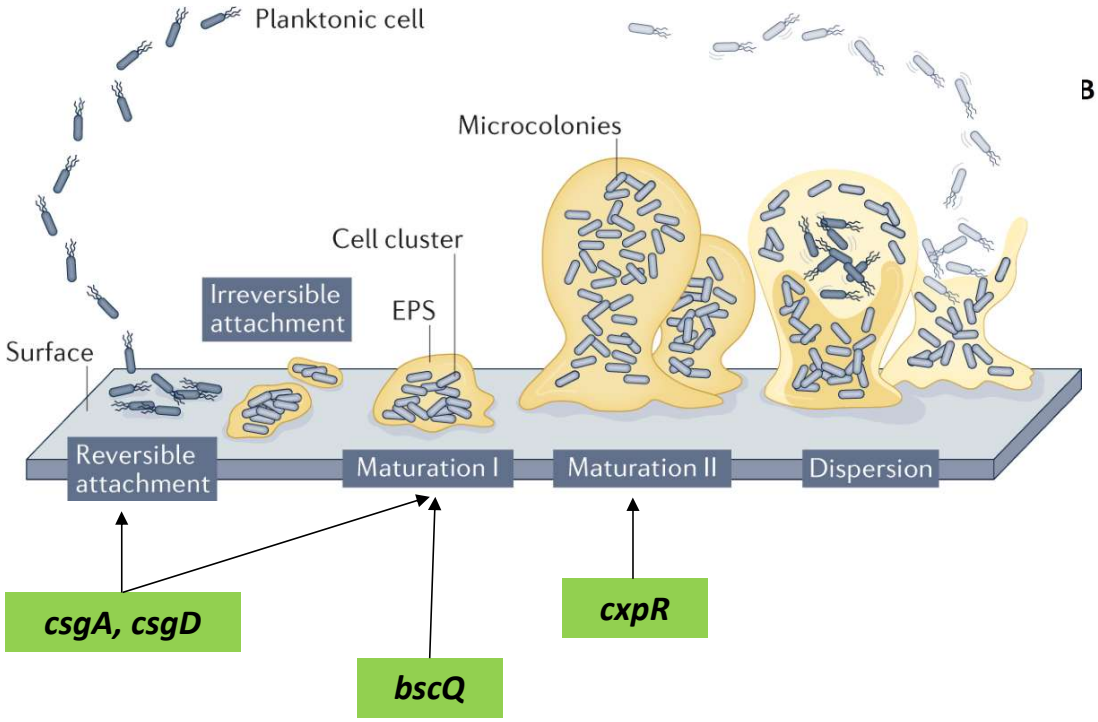


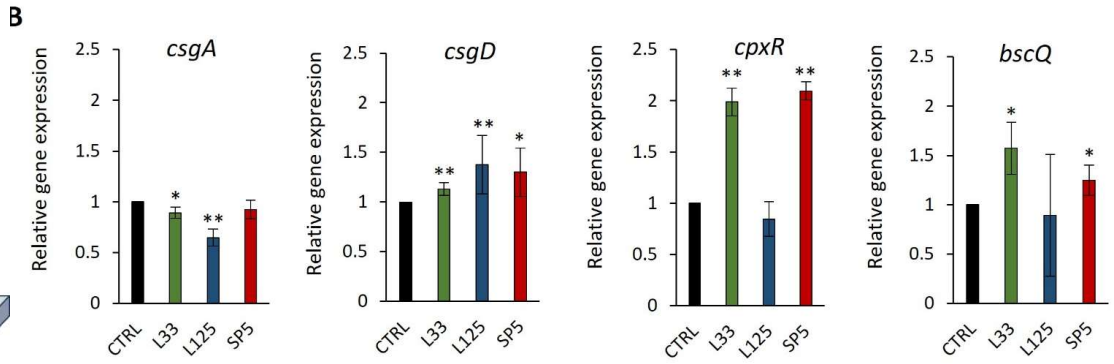




Enolase is an adhesion-related moonlighting protein that mediates attachment on inorganic and organic surfaces, inducing no significant effect on the expression levels of the biofilm formation regulator complex icaA/icaD or the FnbpA adhesin.

# Effect of lactobacilli CFCS on the expression levels of biofilm-related genes

***Salmonella enteritidis***

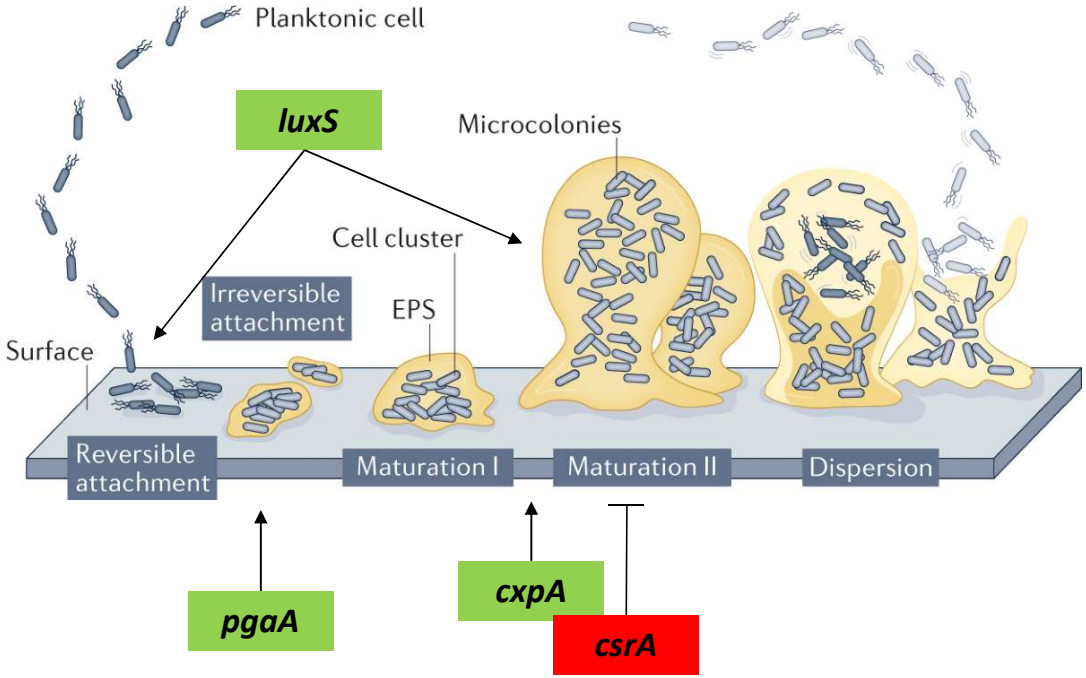


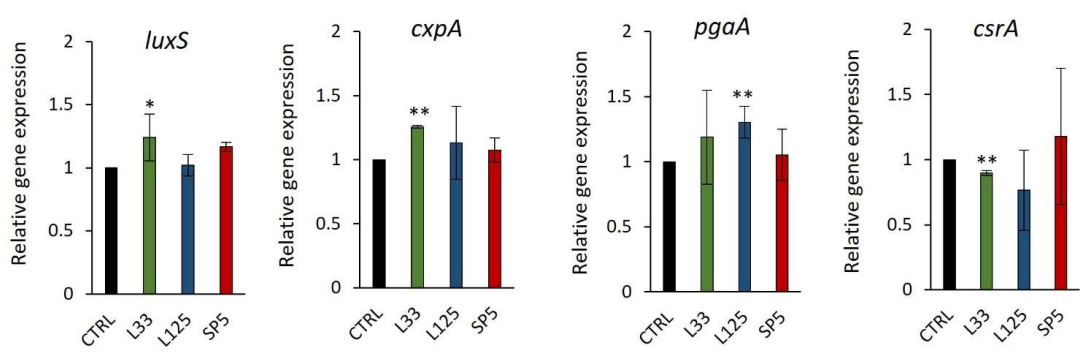




*csgA* is a gene involved in the production of the major Curli protein subunit. *csgD* and *cpxR* code for transcriptional regulators responsible for adhesion on hydrophobic surfaces and biofilm formation. *BscQ* encodes for cellulose synthase.

# Effect of lactobacilli CFCS on the expression levels of biofilm-related genes

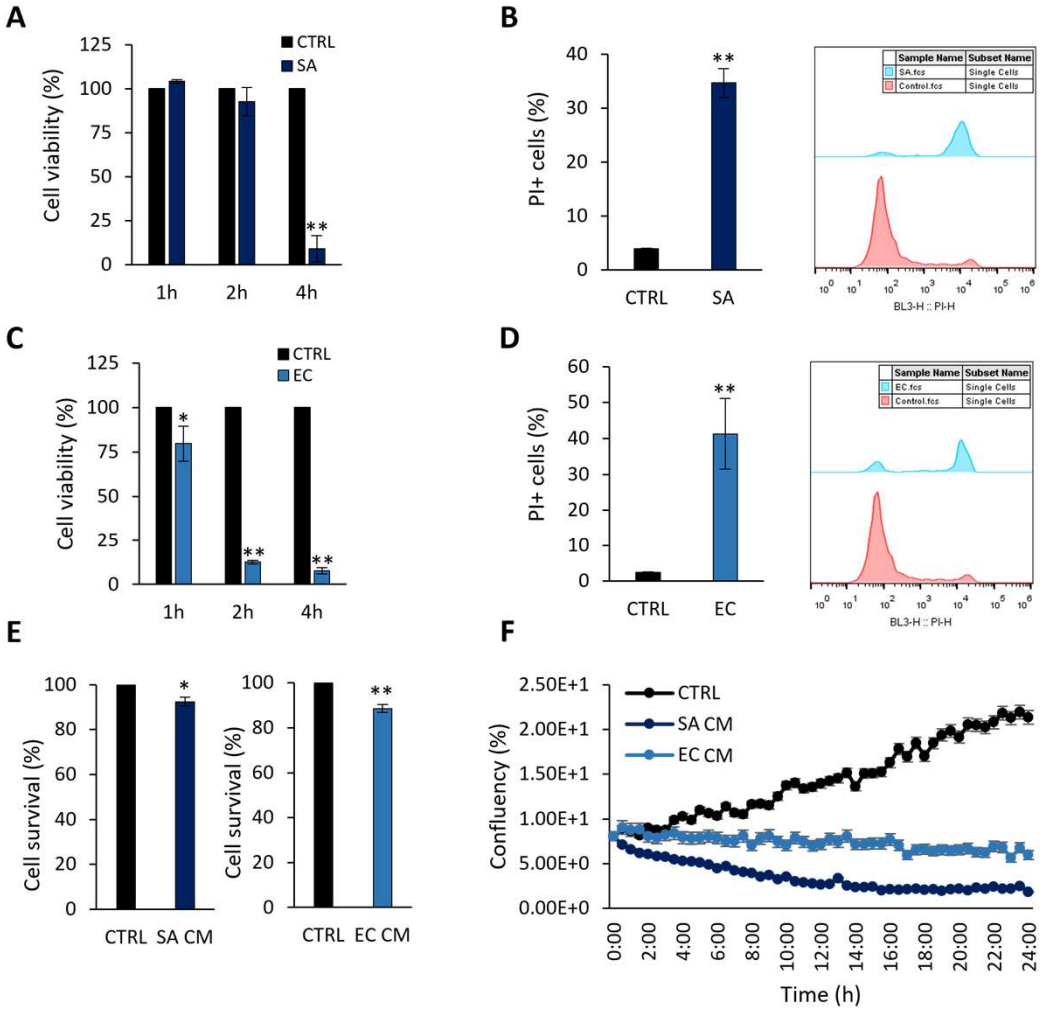
***Escherichia coli***




*luxS* is involved in quorum sensing. *cpxA* is an adhesin-coding gene. c*srA* is a negative biofilm formation regulator and *pgaA is* a gene that stimulates the production of the exopolysaccharide biofilm matrix.

# *Staphylococcus aureus* and *Escherichia coli* induce cell death of HT-29 cells in a time-dependent manner





Lactobacilli-host interactions inhibit *Staphylococcus aureus* and *Escherichia coli*-induced cell death and invasion in a cellular model of infection

Despoina Eugenia Kiousi[1] Maria Panopoulou[2] Aglaia Pappa[1]

Alex Galanis[1*]

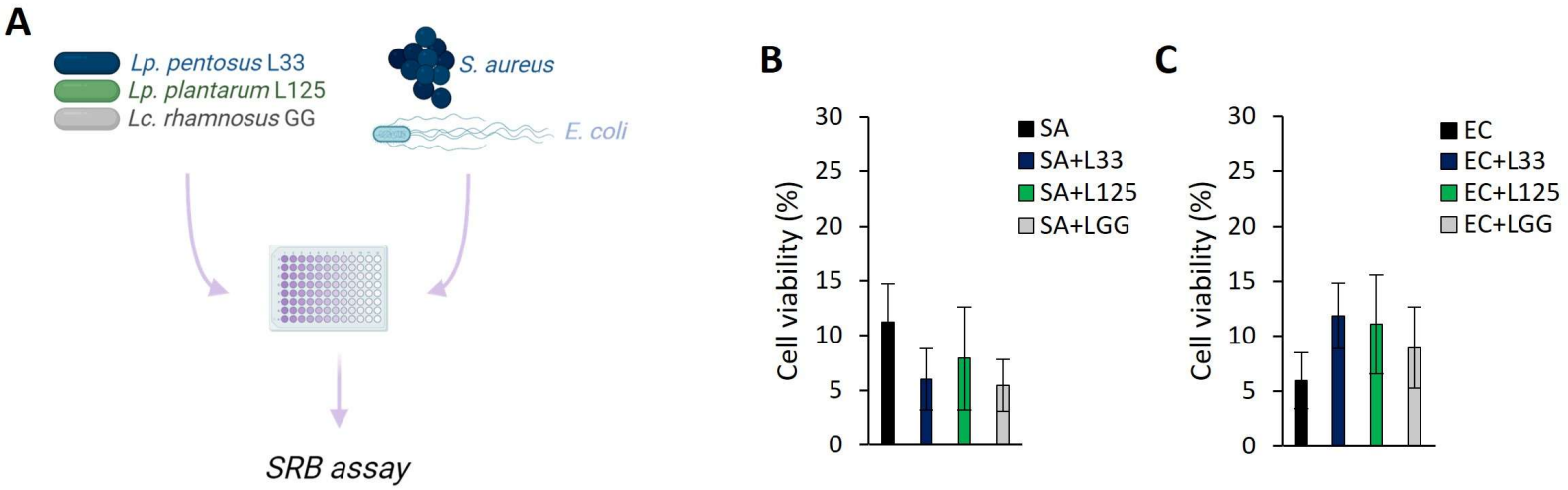
Front. Microbiol., 18 December 2024

Sec. Food Microbiology

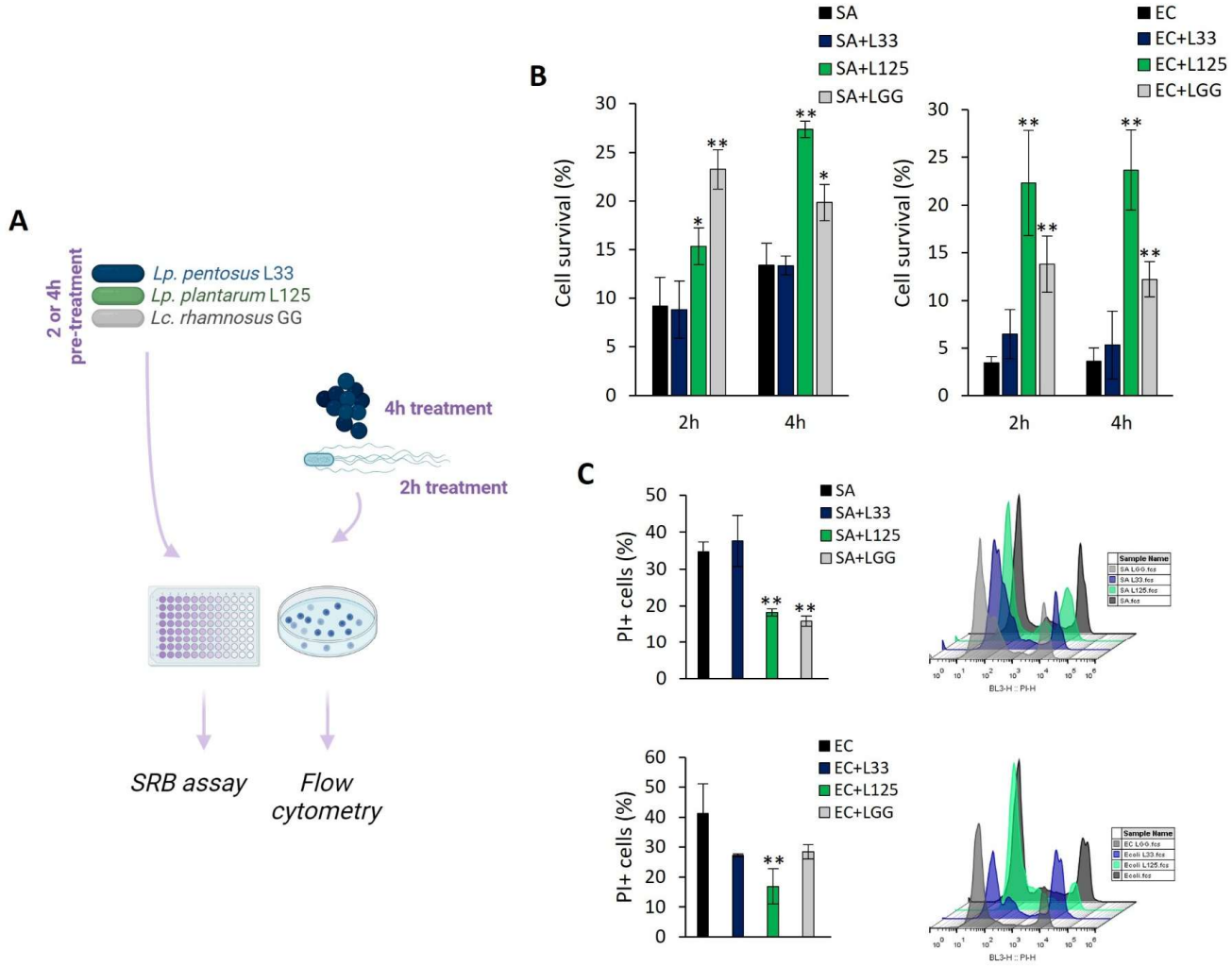
Volume 15 - 2024 |


frontiers

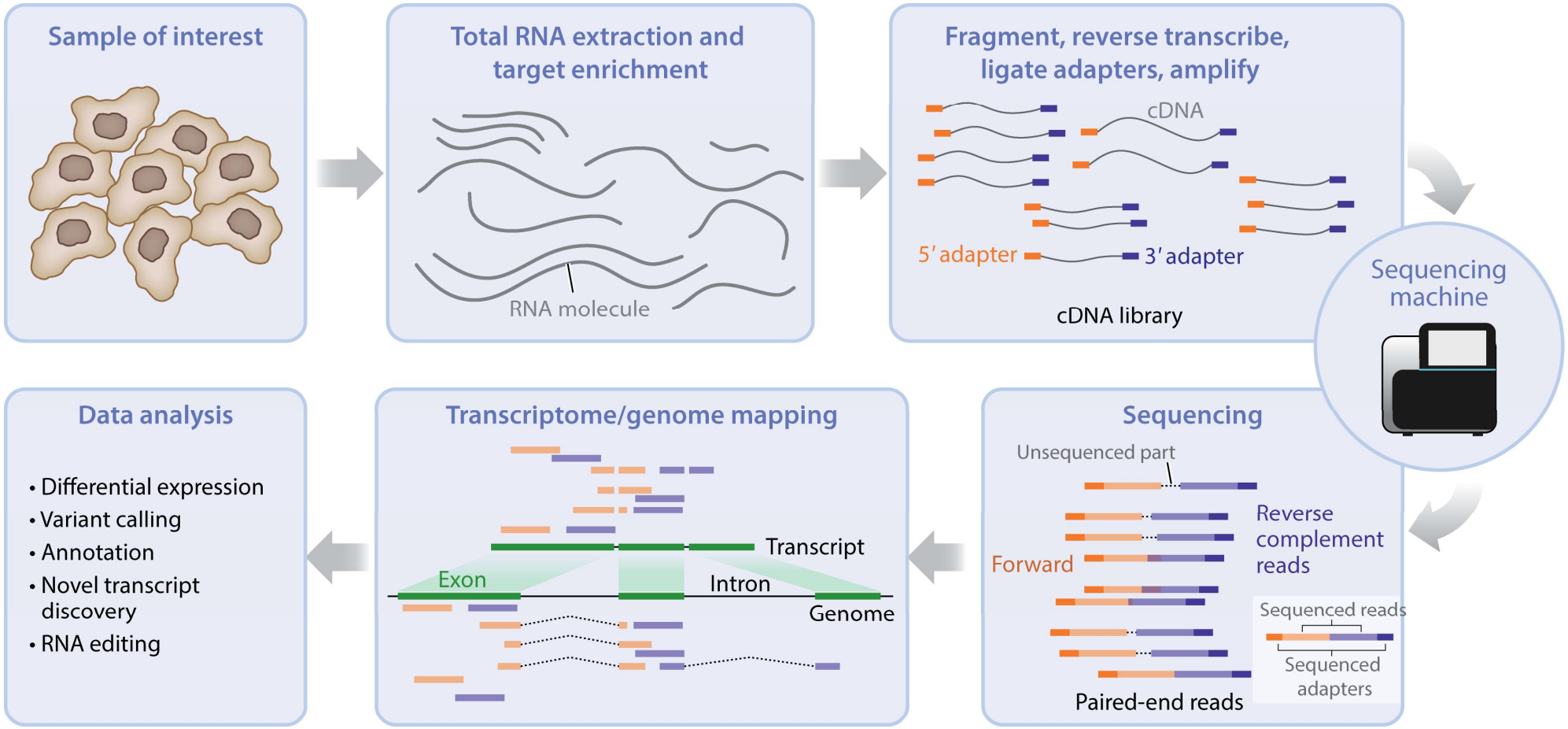
# LAB co-treatment do not protect cells from pathogen-induced cytotoxicity



# L125 pretreatment protects cells from pathogen-induced cytotoxicity



# RNA sequence analysis




Van den Berge K, et al. 2019.
*Annu. Rev. Biomed. Data Sci.* 2:139–73

# RNA sequence analysis

**RNA seq was utilized to analyze the gene expression changes in L125 and HT-29 cells during a 4 h co-incubation period**.

Specifically, for L125, a total of **108 genes** exhibited high expression levels, **2.568 genes** displayed medium expression levels, and **497 genes** showed low expression levels. Additionally, **68 genes** were not expressed in neither of the two independent experiments.

The 108 highly expressed genes clustered into 14 KEGG functional categories, 23 KEGG pathways and 16 clusters of orthologous groups (COGs). The most represented KEGG functional category was “genetic information processing” with “translation” being the most prominent KEGG pathway and COG category.

To identify transcriptional changes in cell-surface exposed proteins, the WGS of L125 was, firstly, re-annotated *in silico*. **Genome mining** was performed to identify proteins potentially involved in the competitive exclusion phenotype, and the strain’s ability to limit pathogen internalization. **77 proteins** containing motifs and domains indicative of cell-wall exposure were identified.

Most predicted surface proteins were found to utilize a Sec/SPI signal recognized by signal peptidase I, and one protein carries a YSIRK processing signal for transport outside the cell. Finally, 16 moonlighting proteins with adhesin function were identified in the L125 genome.

# RNA sequence analysis

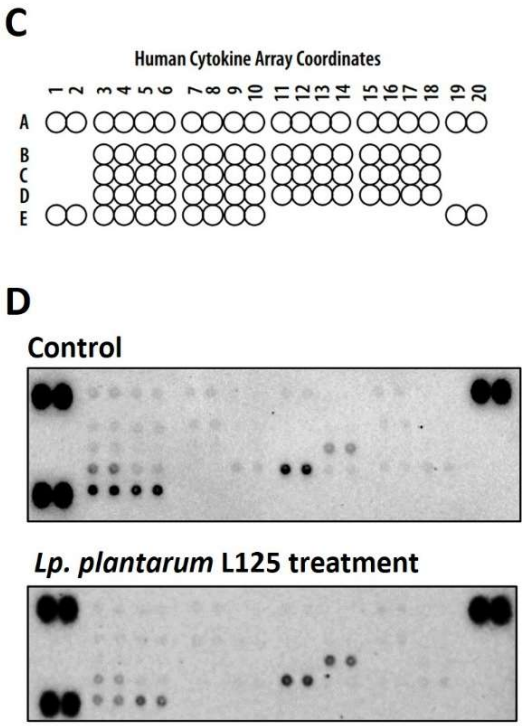
**RNA seq was utilized to explore the capacity of L125 to prime antimicrobial responses in the host cell.**

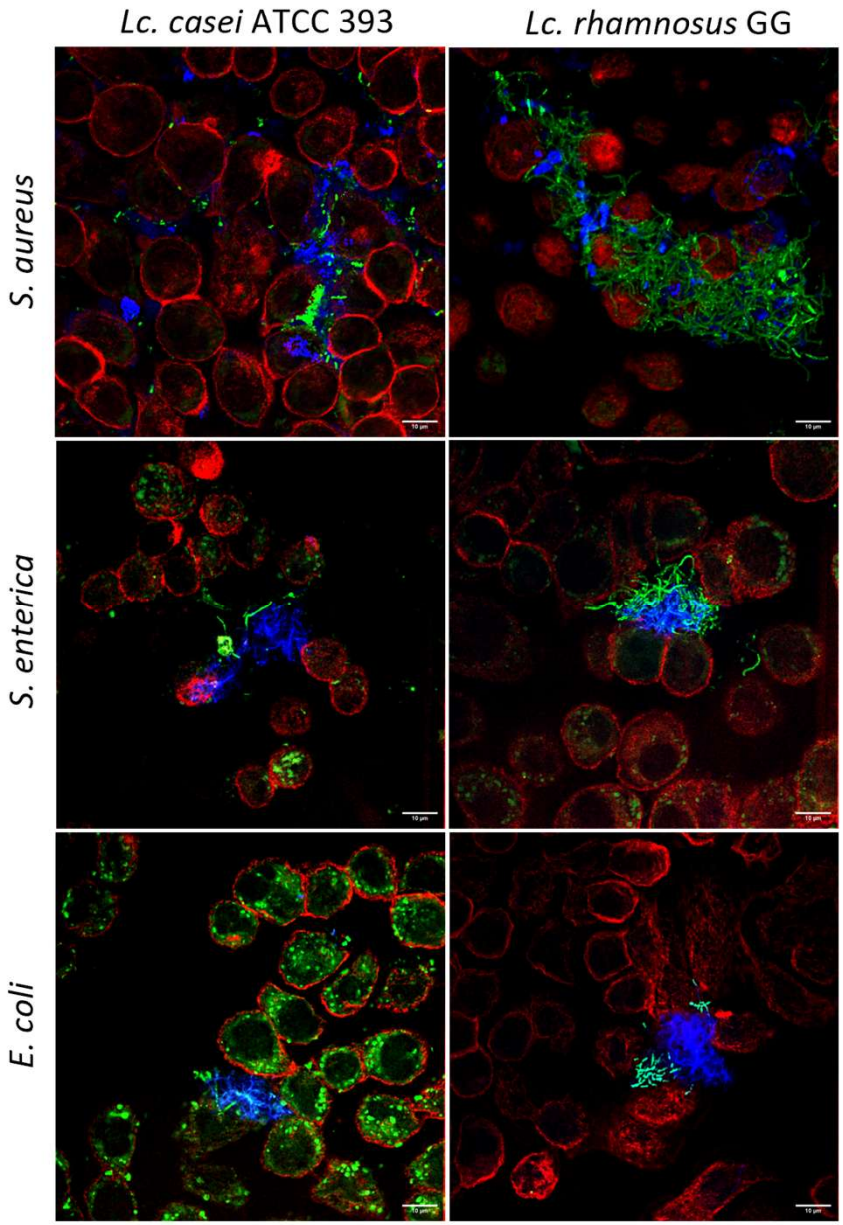
Upregulation of **600 genes** and downregulation of **1,137 genes** in HT-29 cells were recorded, while **26,658 genes** remained unaffected.

L125 **did not significantly modulate immune-related pathways**, including TLR- or NOD-signaling cascades, nor did it affect the production of cytokines and chemokines.

**At the protein level**, L125 was shown to limit the secretion of immunological markers in pooled cell culture supernatants.

**Pathway analysis** revealed that L125 primarily exerted inhibitory effects, negatively impacting bacterial invasion, adherens junction, and endocytosis.






Confocal microscopy visualization of lactobacilli–pathogens–HT-29 interactions. HT-29 cells are stained with SIR-actin (red), lactobacilli with CFCS (green), and pathogens with CellTrace Violet (blue). Scale bar 10 µm.

Article 24 November 2025

*Lacticaseibacillus casei* Combats Biofilm Formation and Exhibits Antibacterial Activity Against Clinical Isolates of *Staphylococcus aureus*, *Salmonella enterica*, and *Escherichia coli*

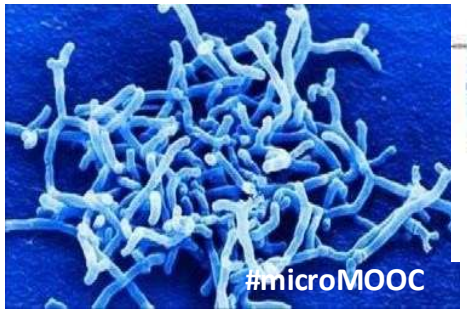
Despoina Eugenia Kiousi[1], Sotiris Kyriakou[2], Christos Efstathiou[1], Stylianos Didaskalou[1], Maria Koffa[1], Aglaia Pappa[1], Maria Panopoulou[3], Mihalis I. Panayiotidis[2,4] and Alex Galanis[1,*]

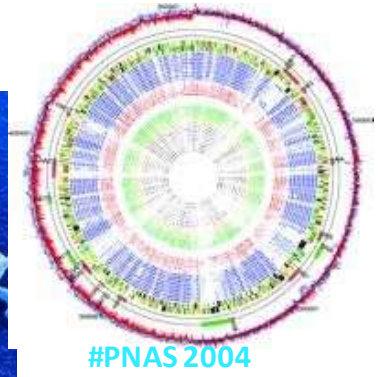
Microorganisms

# PRINCIPLES FOR SCIENTIFIC SUBSTANTIATION (cont.)

***General scientific guidance for stakeholders on health claim applications***

## Characterisation of microorganisms




- **Species identification + strain characterisation/typing** needed, since effects are strain specific unless the contrary is demonstrated
- New **molecular tools** (multilocus sequence typing, optical mapping, whole-genome sequencing, etc.). Open list to others.
- **Several methods** often needed in combination

# Experimental workflow

**1. Genome Assembly**

was performed using SPAdes (version 3.15.1).

Visualization via ARTEMIS tool

**2. Genome Annotation**

was executed using Prokka (version 1.14.5) and PGAP.

**3. Comparative genomics**

- Average nucleotide identity (ANI) analysis

(Pyani version 0.2.10)

- Approximately-maximum-likelihood

phylogenetic tree (FastTree 2.1)

**4. Genes involved in technological and functional characteristics**

investigated using EggNOG, BlastKOALA

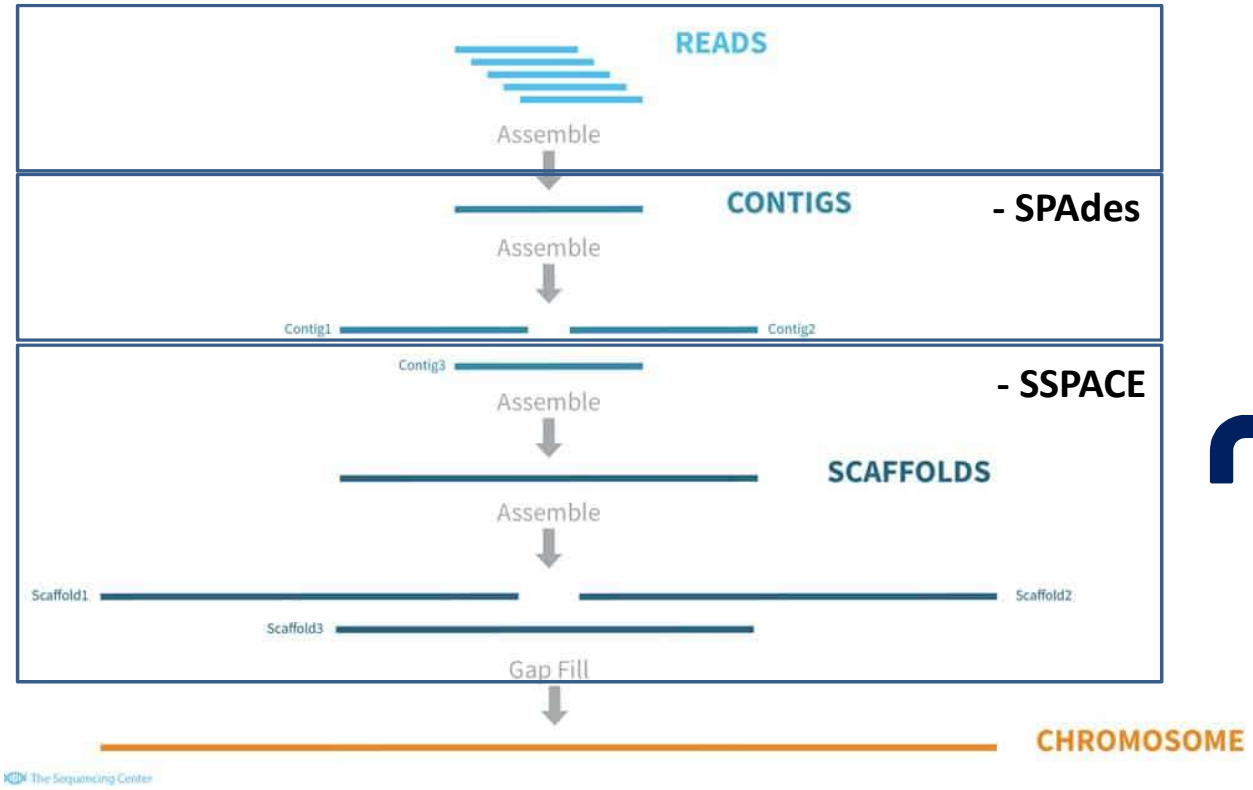
**5. Safety assessment**

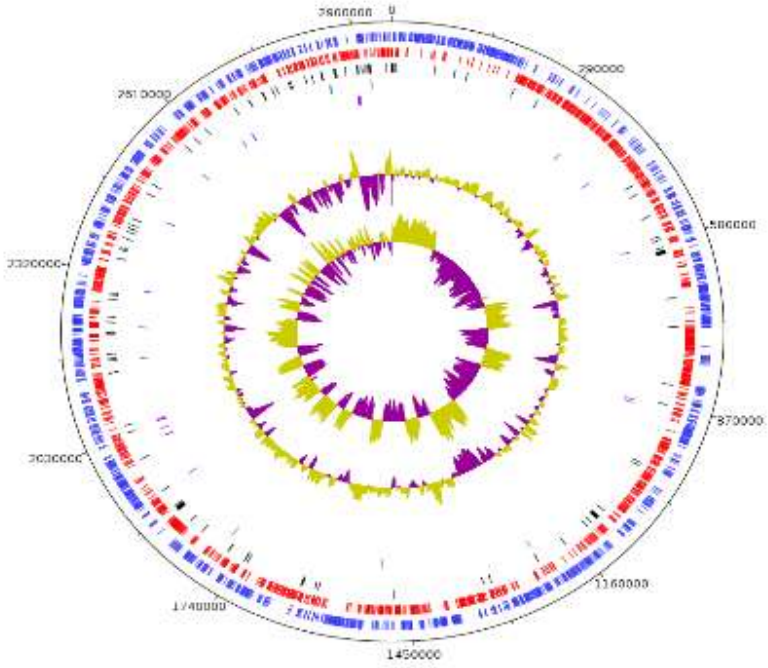
- presence of plasmids (PlasmidFinder)
- antibiotic resistance (ResFinder 4.1)
- virulence genes (VirulenceFinder 2.0)
- pathogenicity (PathogenFinder 1.1)

# Genome Assembly



*De novo* assembly



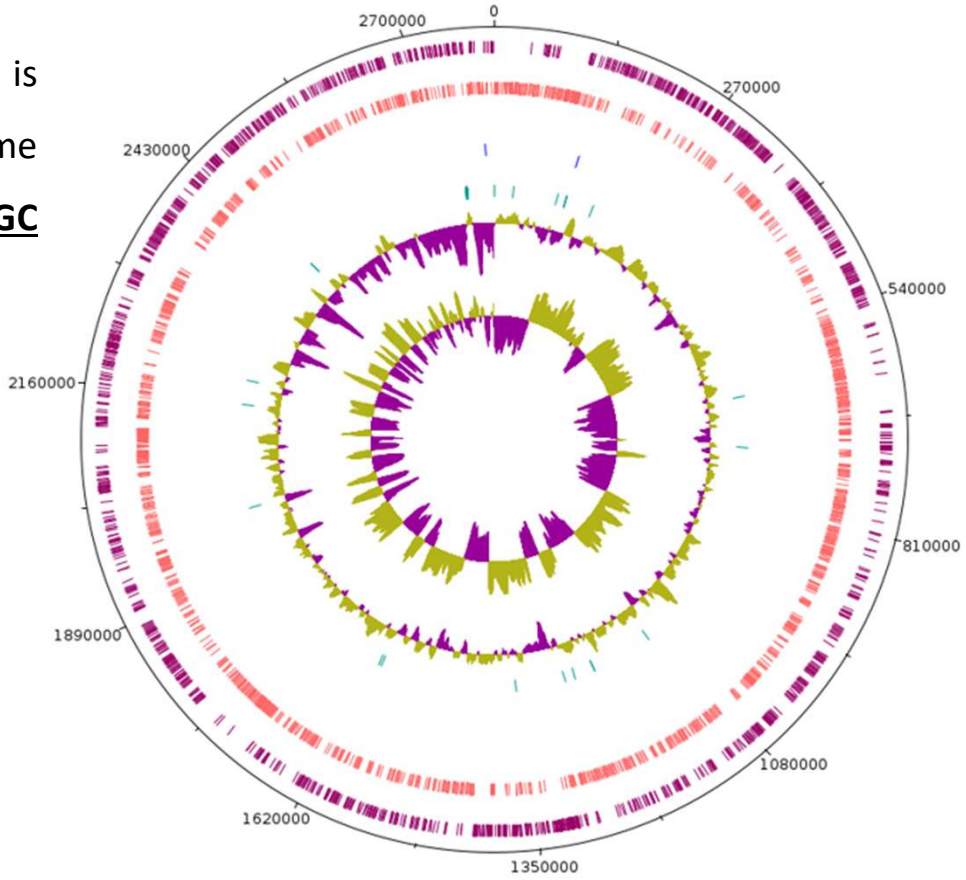



# Genome feature analysis for SRX10

The genome of *Lc. paracasei* SRX10 is comprised of a circular chromosome with a total **length of 2.81 Mb** and a **GC content 46.4%**

Members of the emended *Lactobacillus* genus exhibit genomes that vary in size, ranging from 1.27 to 4.91 Mb (Duar et al., 2017)

*Lc. paracasei* strains possess a median genome length of 3.01 Mb and a median GC content of 46.3%




Circular chromosome map of *Lb. paracasei* SRX10

| Genome characteristics | Value |
|---|---|
| Length | 2,813,407 bp |
| GC content | 46.40 % |
| Total genes | 2,764 |
| CDSs | 2,711 |
| rRNAs | 6 |
| tRNAs | 44 |
| ncRNAs | 3 |
| Pseudogenes | 106 |
| Contamination (%) | 1.89 |

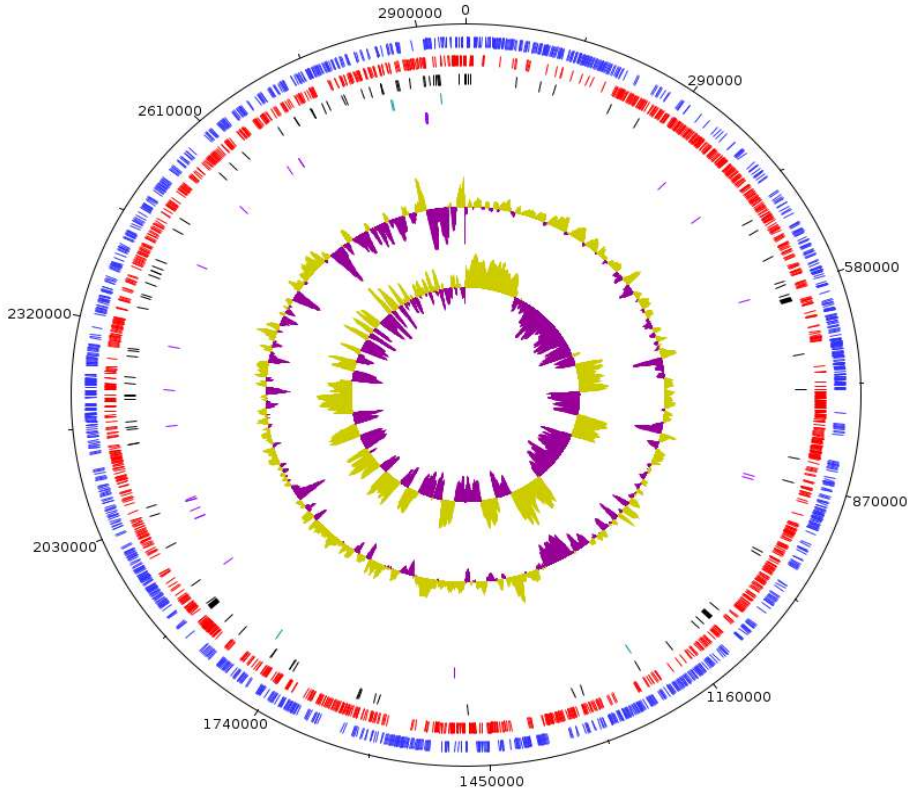
› Microorganisms. 2024 Jan 2;12(1):93. doi: 10.3390/microorganisms12010093.

**Dissecting the Genetic Basis of the Technological, Functional, and Safety Characteristics of *Lacticaseibacillus paracasei* SRX10**

Christina S Kamarinou [1,2], Despoina E Kiousi [1], Panagiotis Repanas [1], Anthoula A Argyri [2], Nikos G Chorianopoulos [3], Alex Galanis [1]

# Genome feature analysis for SP5

| Genome Characteristics | Value |
|---|---|
| **Length** | **2,958,982 bp** |
| **GC content** | **46.3 %** |
| Total genes | 2,920 |
| **CDSs** | **2,870** |
| rRNAs | 5 |
| tRNAs | 42 |
| ncRNAs | 3 |
| Pseudogenes | 105 |
| No. of CRISPR Arrays | 0 |
| Cas proteins | 0 |
| IS elements | 122 |
| Phages | |
| *Intact* | 1 |
| *Incomplete* | 2 |
| *Questionable* | 2 |
| Antibiotic resistance genes | |
| *Perfect hits* | 0 |
| *Strict hits* | 0 |
| *Loose hits* | 202 |
| Virulence genes | 0 |
| Probability of being a human pathogen | 0.099 |
| Plasmids | 0 |



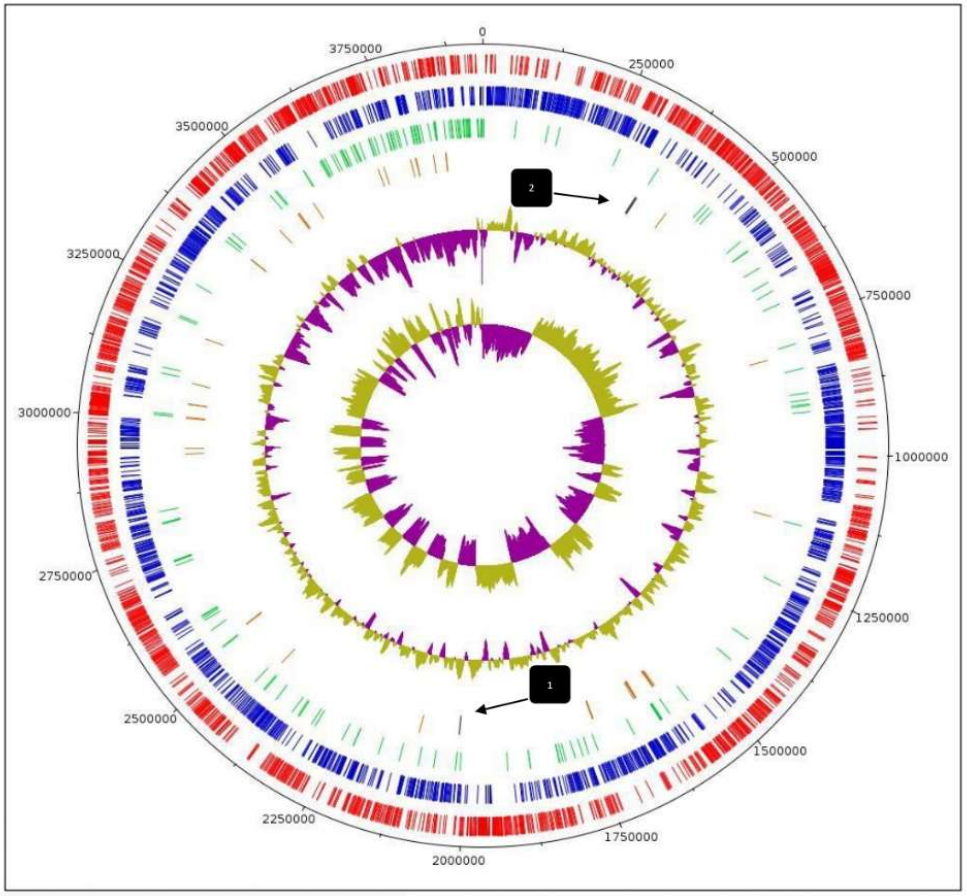

***Lc. paracasei* SP5 genome metrics are typical of the *Lc. paracasei* species**

Genomic Insight Into *Lacticaseibacillus paracasei* SP5, Reveals Genes and Gene Clusters of Probiotic Interest and Biotechnological Potential

Despoina Eugenia Kiousi[1], Christos Efstathiou[1], Konstantinos Tegopoulos[1], Ioanna Mantzourani[2], Athanasios Alexopoulos[2], Stavros Plessas[2*], Petros Kolovos[1], Maria Koffa[1] and Alex Galanis[1*]

Frontiers Micro 2022;13:922689.

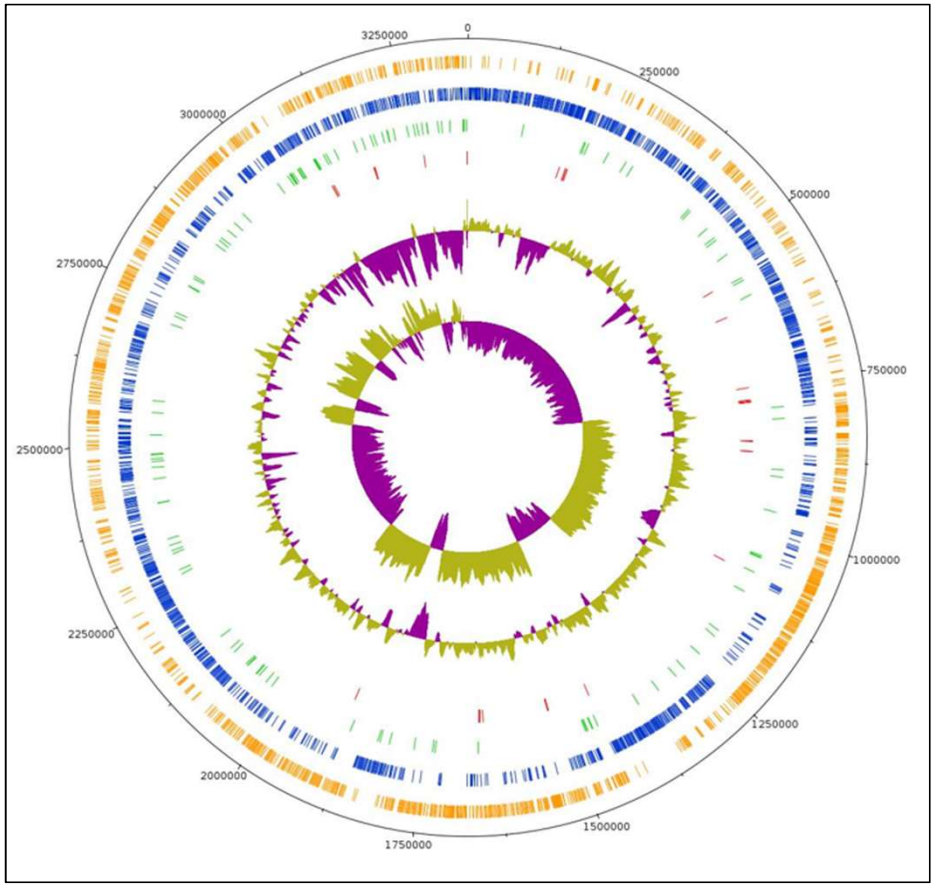
# Characterisation of novel probiotic strains (Whole-genome sequencing)




Whole-Genome Sequencing, Phylogenetic and Genomic Analysis of *Lactiplantibacillus pentosus* L33, a Potential Probiotic Strain Isolated From Fermented Sausages

Odysseas Sotirios Stergiou[1†], Konstantinos Tegopoulos[1†], Despoina Eugenia Kiousi[1], Margaritis Tsifintaris[1], Aristotelis C. Papageorgiou[1], Chrysoula C. Tassou[2], Nikos Chorianopoulos[2], Petros Kolovos[1*] and Alex Galanis[1*]

Frontiers Micro 2021;12:746659.



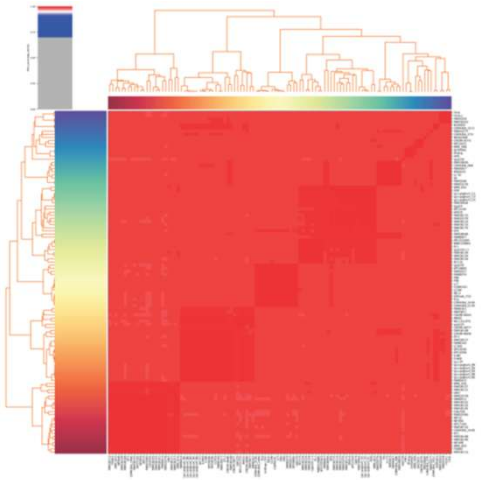

**Genomic and Phylogenetic Analysis of *Lactiplantibacillus plantarum* L125, and Evaluation of Its Anti-Proliferative and Cytotoxic Activity in Cancer Cells**

by Konstantinos Tegopoulos [1,†], Odysseas Sotirios Stergiou [1,†], Despoina Eugenia Kiousi [1,†], Margaritis Tsifintaris [1], Ellie Koletsou [1], Aristotelis C. Papageorgiou [1], Anthoula A. Argyri [2], Nikos Chorianopoulos [2], Alex Galanis [1,*] and Petros Kolovos [1,*]

Biomedicines 2021;9(11):1718.

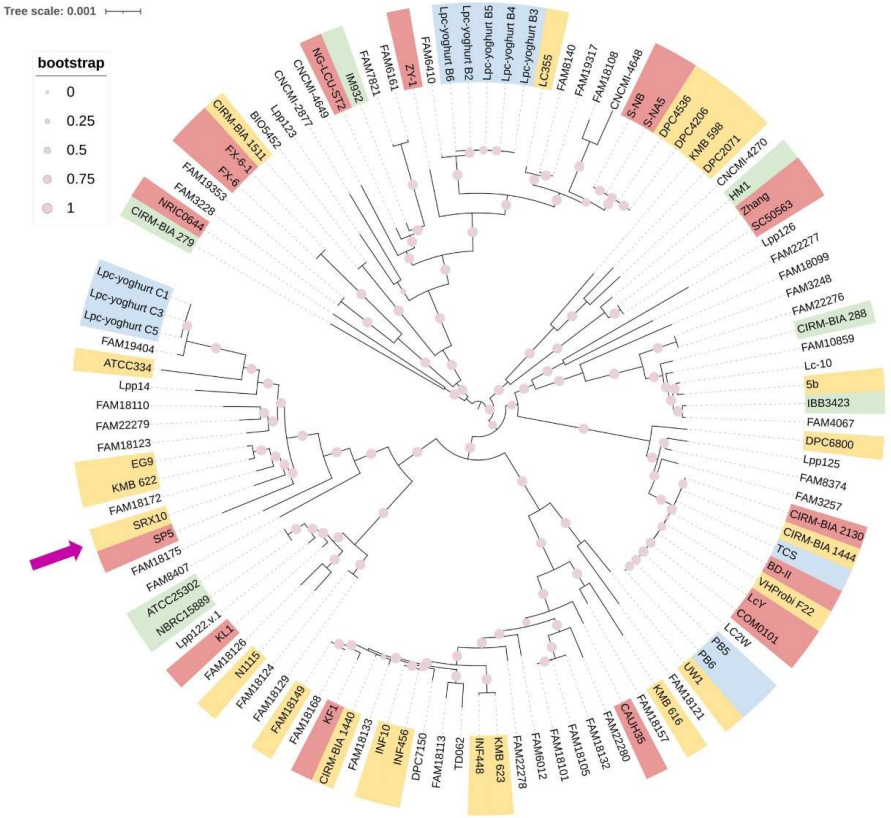
# Phylogenomic and Pangenome analysis for SRX10

| Strain | ANI (%) with SRX10 |
|---|---|
| SRX10 | 100 |
| KMB_622 | 99.67 |
| EG9 | 99.62 |
| FAM18172 | 99.58 |
| FAM18123 | 99.51 |
| FAM22279 | 99.50 |
| SP5 | 99.43 |
| FAM18110 | 99.40 |
| Lpp14 | 99.34 |
| Lpc-yoghurt_C5 | 99.34 |
| Lpc-yoghurt_C1 | 99.31 |
| Lpc-yoghurt_C3 | 99.31 |
| FAM19404 | 99.26 |
| FAM18175 | 99.26 |
| ATCC334 | 99.23 |
| FAM8407 | 99.21 |



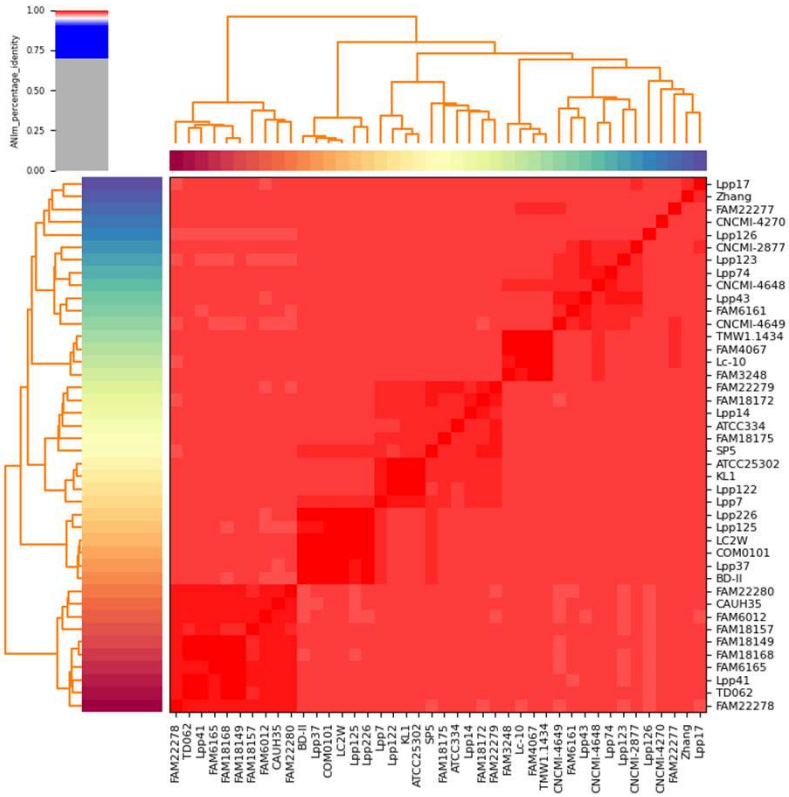
ANI of *Lc. paracasei* SRX10 derived from fermented milk products (A) ANI heatmap of all dairy isolates (B) ANI of *Lc. paracasei* SRX10 with dairy isolates

Approximately-maximum-likelihood phylogenetic tree of *Lc. paracasei* strains isolated from dairy products (pink – fermented milk products, yellow– cheese, blue- yoghurt, green- raw milk products)



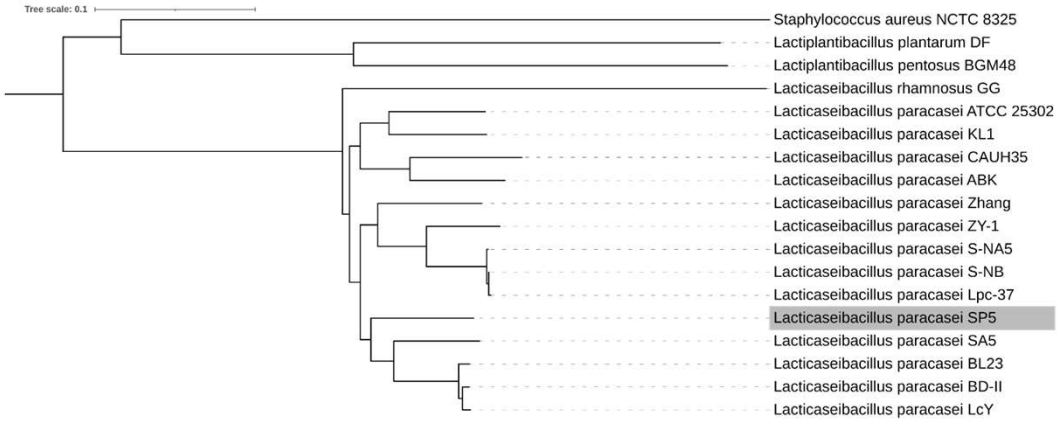

Average nucleotide identity (ANI) analysis using the python module Pyani, showed that the strain possessed **unique genomic sequences**, and further confirmed its **classification in the *Lc. paracasei* species**

# Phylogenomic and Pangenome analysis for SP5




| | SP5 |
|---|---|
| SP5 | 1 |
| FAM18172 | 0.994177 |
| FAM22279 | 0.993258 |
| Lpp14 | 0.991895 |
| ATCC334 | 0.991474 |
| FAM18175 | 0.990251 |
| ATCC25302 | 0.990191 |
| KL1 | 0.990157 |
| LC2W | 0.990041 |
| Lpp7 | 0.990039 |
| COM0101 | 0.989907 |
| Lpp37 | 0.989872 |
| BD-II | 0.989491 |
| Lpp226 | 0.989313 |
| Lpp125 | 0.988772 |
| Lpp122 | 0.988018 |
| CNCMI-4270 | 0.987437 |
| FAM3248 | 0.987297 |
| FAM4067 | 0.98708 |
| FAM22277 | 0.986993 |
| TMW1.1434 | 0.986689 |
| CNCMI-4648 | 0.986671 |
| CNCMI-2877 | 0.986671 |
| Zhang | 0.986314 |
| FAM6161 | 0.986299 |
| Lpp17 | 0.986282 |
| Lpp123 | 0.986054 |
| Lpp43 | 0.986051 |
| Lpp74 | 0.985998 |
| Lc-10 | 0.985682 |
| TD062 | 0.98547 |
| FAM18157 | 0.985405 |
| Lpp126 | 0.985383 |
| FAM6165 | 0.985242 |
| Lpp41 | 0.985202 |
| FAM18149 | 0.985153 |
| FAM18168 | 0.98509 |
| CNCMI-4649 | 0.985044 |
| FAM22280 | 0.985032 |
| CAUH35 | 0.985001 |
| FAM6012 | 0.984923 |
| FAM22278 | 0.984349 |

**Average Nucleotide Identity (ANI)** for species identification/**strain characterization** through whole-genome similarity analysis




**ANI species cut off > 96%**

***Lc. paracasei* SP5 is a unique strain that belongs to the *Lc. paracasei* species**

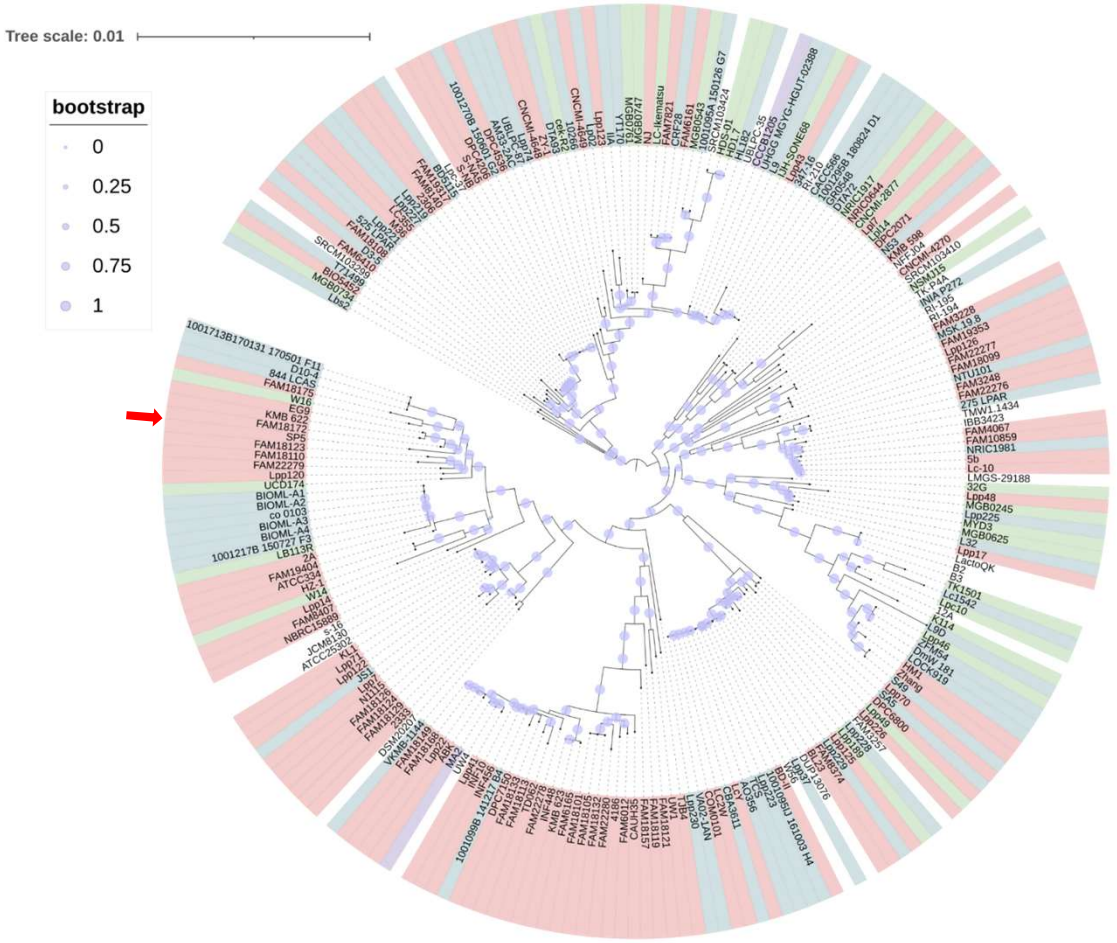
Genomic Insight Into *Lacticaseibacillus paracasei* SP5, Reveals Genes and Gene Clusters of Probiotic Interest and Biotechnological Potential

Despoina Eugenia Kiousi[1], Christos Efstathiou[1], Konstantinos Tegopoulos[1], Ioanna Mantzourani[2], Athanasios Alexopoulos[2], Stavros Plessas[2]*, Petros Kolovos[1], Maria Koffa[1] and Alex Galanis[1]*

Frontiers Micro 2022;13:922689.

# Phylogenomic analysis for SP5

- *Lc. paracasei* strains do not cluster based on isolation source
- *Lc. paracasei* SP5 clusters with dairy strains – however it exhibits close phylogenetic relationship with human- and vegetable-derived strains

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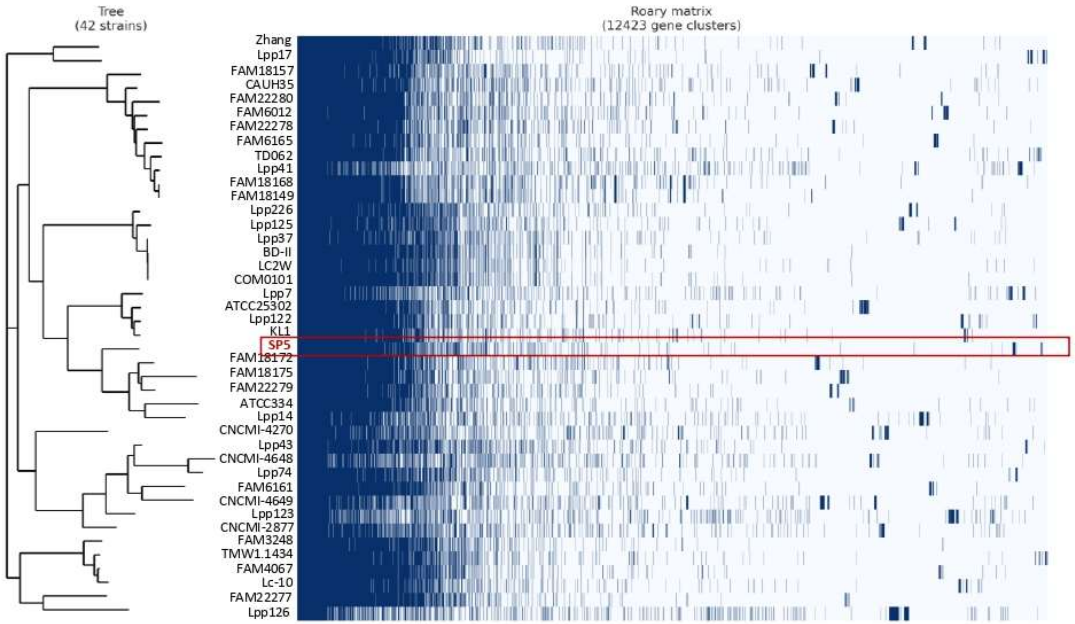
Frontiers Micro 2022;13:922689.

# Pangenome analysis for SP5

**Pangenome Analysis** to define functional diversity and evolution, niche adaptation and host interactions




| Class | SP5 | Pangenome | Unique |
|---|---|---|---|
| C, Energy production and conversion | 111 (4.81%) | 299 (3.17%) | 1 (2.3%) |
| D, Cell cycle control and mitosis | 38 (1.62%) | 134 (1.42%) | 0 (0%) |
| E, Amino Acid metabolism and transport | 184 (7.83%) | 539 (5.71%) | 1 (2.38%) |
| F, Nucleotide metabolism and transport | 111 (4.72%) | 237 (2.51%) | 0 (0%) |
| G, Carbohydrate metabolism and transport | 231 (9.83%) | 879 (9.32%) | 7 (16.67%) |
| H, Coenzyme metabolism | 66 (2.81%) | 164 (1.74%) | 2 (4.76%) |
| I, Lipid metabolism | 57 (2.43%) | 134 (1.42%) | 0 (0%) |
| J, Translation | 166 (7.06%) | 306 (3.24%) | 0 (0%) |
| K, Transcription | 211 (8.98%) | 632 (6.70%) | 6 (14.29%) |
| L, Replication and repair | 157 (6.68%) | 1521 (16.11%) | 5 (11.90%) |
| M, Cell wall/membrane/envelop biogenesis | 125 (5.32%) | 598 (6.34%) | 4 (9.52%) |
| N, Cell motility | 8 (0.34%) | 29 (0.31%) | 2 (4.76%) |
| O, Post-translational modification, protein turnover, chaperone functions | 52 (2.21%) | 139 (1.47%) | 0 (0%) |
| P, Inorganic ion transport and metabolism | 132 (5.62%) | 450 (4.77%) | 1 (2.38%) |
| Q, Secondary Structure | 26 (1.11%) | 72 (0.76%) | 1 (2.38%) |
| T, Signal Transduction | 52 (2.21%) | 155 (1.64%) | 1 (2.38%) |
| U, Intracellular trafficking and secretion | 45 (1.91%) | 144 (1.53%) | 2 (4.76%) |
| V, Defense mechanisms | 74 (3.15%) | 365 (3.87%) | 1 (2.38%) |
| S, Function Unknown | 502 (21.36%) | 1614 (17.11%) | 8 (19.05%) |
| No category, General function prediction only | 269 (11.45%) | 1019 (10.81%) | 5 (11.9%) |
| Total | 2350 (100%) | 9430 (100%) | 42 (100%) |

***Lc. paracasei* SP5 unique genes are involved in carbohydrate metabolism and DNA transposition**


Genomic Insight Into *Lacticaseibacillus paracasei* SP5, Reveals Genes and Gene Clusters of Probiotic Interest and Biotechnological Potential

Frontiers Micro 2022;13:922689.

# Genome-Wide Analysis for SRX10

## Detection of genes associated with

### Technological characteristics

1. **Stress tolerance responses** (cold shock, heat shock proteins and genes responsible for the resistance to osmotic shock)
2. **Metabolic pathways** and genes associated with flavor development

- *Carbohydrate metabolism (catabolism of lactose)*
- *Lipid metabolism (lipid degradation, fatty acid biosynthesis (e.g., fabD, fabH, fabZ)*
- *Amino acid metabolism (proteolytic enzymes (e.g., pepN, pepX, and pepC)*

3. **EPS production** (e.g., epsA, epsB, and epsC) were annotated in the genome of the strain

### Functional characteristics

1. **Resistance to Gastrointestinal conditions** (a full cluster for the production of F0F1 ATP synthetase atpABCDEFGH)

2. **Adhesion capacity** (moonlighting proteins)

3. **Antimicrobial compounds**

4. **Vitamin synthesis** (Vitamin B1)

# Genome-Wide Analysis for SP5

**Table 3.** Annotation of genes coded by *Lc. paracasei* SP5 that are implicated in stress response and host-microbe interactions.

| Locus Tag | Gene Function | Gene | E-value |
|---|---|---|---|
| ***Gastrointestinal tract survival & stress response*** | | | |
| SP5_000699 | Penicillin-binding protein | *pbpX* | 0.0 |
| SP5_001309 | Penicillin-binding protein | *mrdA* | 0.0 |
| SP5_000894 | Penicillin-binding protein 1A | *ponA* | 0.0 |
| SP5_001372 | Penicillin-binding protein 2A | *pbp2A* | 0.0 |
| ***Acid tolerance*** | | | |
| SP5_002673 | Sodium proton antiporter | *yvgP* | 0.0 |
| SP5_002530 | ATP synthase subunit alpha | *atpA* | 0.0 |
| SP5_002526 | ATP synthase subunit a | *atpB* | 1.23e-162 |
| SP5_002533 | ATP synthase epsilon chain | *atpC* | 1.88e-91 |
| SP5_002532 | ATP synthase subunit beta | *atpD* | 0.0 |
| SP5_002527 | ATP synthase subunit c | *atpE* | 2.57e-37 |
| SP5_002528 | ATP synthase subunit b | *atpF* | 3.59e-80 |
| SP5_002531 | ATP synthase gamma chain | *atpG* | 1.92e-211 |
| SP5_002529 | ATP synthase subunit delta | *atpH* | 3.93e-116 |
| SP5_000635 | Decarboxylase | *yphJ* | 1.07e-72 |
| ***Bile salt tolerance*** | | | |
| SP5_002916 | Linear amide C-N hydrolase, choloylglycine hydrolase family | - | 2.38e-252 |
| ***Extreme temperature tolerance*** | | | |
| SP5_001004 | 'Cold-shock' DNA-binding domain protein | *cspA* | 3.08e-43 |
| SP5_000711 | Cold shock protein | *cspB* | 4.62e-48 |
| SP5_002482 | Cold shock protein | *cspC* | 6.22e-43 |
| SP5_001854 | Heat shock 40 kDa protein | *dnaJ* | 9.45e-261 |
| SP5_001853 | Heat shock 70 kDa protein | *dnaK* | 0.0 |
| SP5_001695 | Member of the small heat shock protein (HSP20) family | *hsp* | 1.8e-99 |
| SP5_000918 | Member of the small heat shock protein (HSP20) family | *hsp1* | 1.05e-111 |
| SP5_001851 | Negative regulator of class I heat shock genes (grpE- dnaK-dnaJ and groELS operons) | *hrcA* | 1.6e-246 |
| SP5_002404 | Recovery of the cell from heat-induced damage, in cooperation with DnaK, DnaJ and GrpE | *clpC* | 0.0 |
| SP5_001504 | Molecular chaperone | *GroEL* | 0.0 |
| SP5_001503 | Co-chaperonin | *GroES* | 1.7e-59 |
| SP5_000774 | Molecular chaperone | *clpB* | 0.0 |
| ***Cell wall formation*** | | | |
| SP5_000209 | Cell wall formation | *murA* | 9.48e-300 |
| SP5_002250 | Glycosyltransferase like family 2 | *epsIIG* | 3.79e-89 |
| SP5_002585 | Glycosyltransferase like family 2 | *epsG* | 4.18e-151 |
| SP5_002316 | Glycosyl transferases group 1 | *tagE3* | 0.0 |
| SP5_002317 | Glycosyl transferases group 1 | *tagE2* | 0.0 |
| SP5_002642 | Glycosyl transferase family 8 | *arbx* | 1.17e-211 |
| SP5_001889 | Glycosyl transferase family 2 | *ykcC* | 8.07e-233 |
| SP5_000560 | D-alanine--D-alanyl carrier protein ligase | *dltA* | 0.0 |
| SP5_000558 | D-alanyl carrier protein | *dltC* | 6.97e-49 |
| SP5_000557 | Involved in the D-alanylation of LTA | *dltD* | 8.29e-312 |
| SP5_000561 | D-Ala-teichoic acid biosynthesis protein | *dltX* | 1.04e-27 |
| ***Adhesion capacity*** | | | |
| *Putative adhesins* | | | |
| SP5_000853 | Fibronectin-binding protein A (Fibronectin binding domain A) | *FbpA* | 0.0 |
| SP5_002267 | Putative adhesin | *yvlB* | 0.0 |
| SP5_001633 | Internalin J (MucBP domain) | *inlJ* | 5.78e-287 |
| SP5_002881 | LPxTG domain protein (PillinD1 domain, SpaA domain, Ig-like fold) | - | 1.25e-236 |
| SP5_002694 | Hydrolase, Collagen-binding protein | *mapA* | 0.0 |
| SP5_000963 | NlpC P60 family protein (SlpA domain) | *p75* | 7.12e-202 |
| SP5_002397 | CHAP domain protein (SibA CHAP domains) | *p40* | 4.92e-201 |
| *Moonlighting proteins* | | | |
| SP5_002227 | Glycosyl hydrolase (LysM domain) | - | 8.06e-232 |
| SP5_000794 | Leucine-rich repeat (LRR) protein (LysM domain) | - | 1.68e-104 |
| SP5_001575 | Hydrolase (LysM domain) | - | 1.64e-184 |
| SP5_000723 | Phosphoglycerate mutase | *pgm6* | 4.69e-159 |
| SP5_002706 | Triosephosphate isomerase | *tpiA* | 2.81e-180 |
| SP5_000756 | Elongation factor Tu | *tuf* | 2.74e-285 |
| *Supporting functions* | | | |
| SP5_000470 | Sortase family protein | *srtA* | 4.31e-166 |
| SP5_002372 | Sortase family protein | *srtA* | 2.1e-143 |
| SP5_002167 | Sortase family protein | *srtB* | 5.2e-188 |
| SP5_002880 | Sortase family protein | - | 3.16e-258 |
| ***Biofilm formation*** | | | |
| SP5_002245 | Capsular polysaccharide biosynthesis protein | *epsB* | 2.52e-169 |
| SP5_000584 | S-ribosylhomocysteine lyase | *luxS* | 1.69e-112 |
| SP5_000661 | Transcriptional regulatory protein DesR | *desR* | 8.42e-135 |
| SP5_001469 | Catabolite control protein A | *ccpA* | 3.03e-232 |
| SP5_000097 | Cell envelope-like function transcriptional attenuator common domain protein | *brpA* | 4.84e-256 |

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Front. Micro 2022;13:922689

# In silico Safety Assessment for SRX10

*Genome stability*

Three prophage regions were predicted in the genome of *Lc. paracasei* SRX10. **No plasmids or mobile genetic elements** were detected, while a total of 98 insertion elements were identified in the genome of the strain. Finally SRX10 **lacks functional CRISPR arrays** and does not code for Cas proteins.

*Virulence and antibiotic resistance*

SRX10 does not possess **any virulence factors** that could negatively affect the host. **No acquired antibiotic resistance genes** were identified by RGI and ResFinder.

BLAST analysis confirmed that SRX10 **does not harbor genes encoding** the enzymes responsible for the production **of biogenic amines or genes involved in hemolysis.**

**5. Safety assessment**

- presence of plasmids (PlasmidFinder)
- antibiotic resistance (ResFinder 4.1)
- virulence genes (VirulenceFinder 2.0)
- pathogenicity (PathogenFinder 1.1)

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