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Μοριακή Βιοτεχνολογία και Διατροφή

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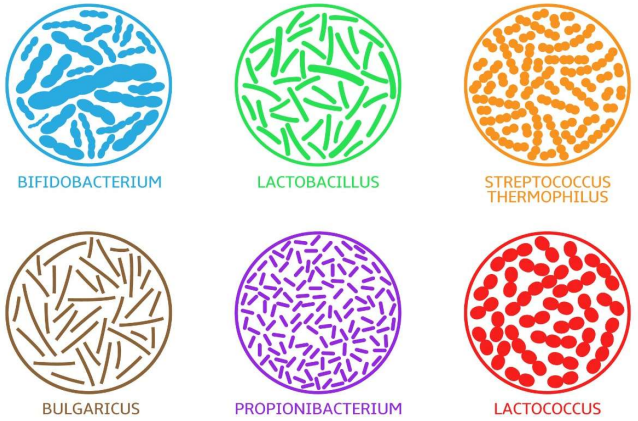
Pro (latin) + Biotic (greek) = For Life

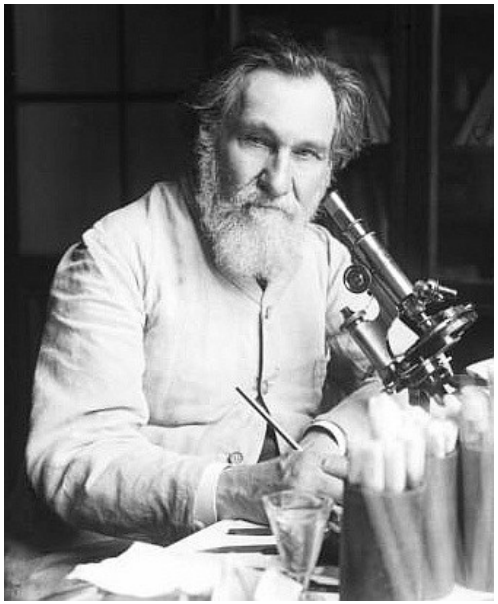
The term was first used in 1965, by **Lilly and Stillwell**, to describe substances secreted by one organism which stimulate the growth of another.

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).

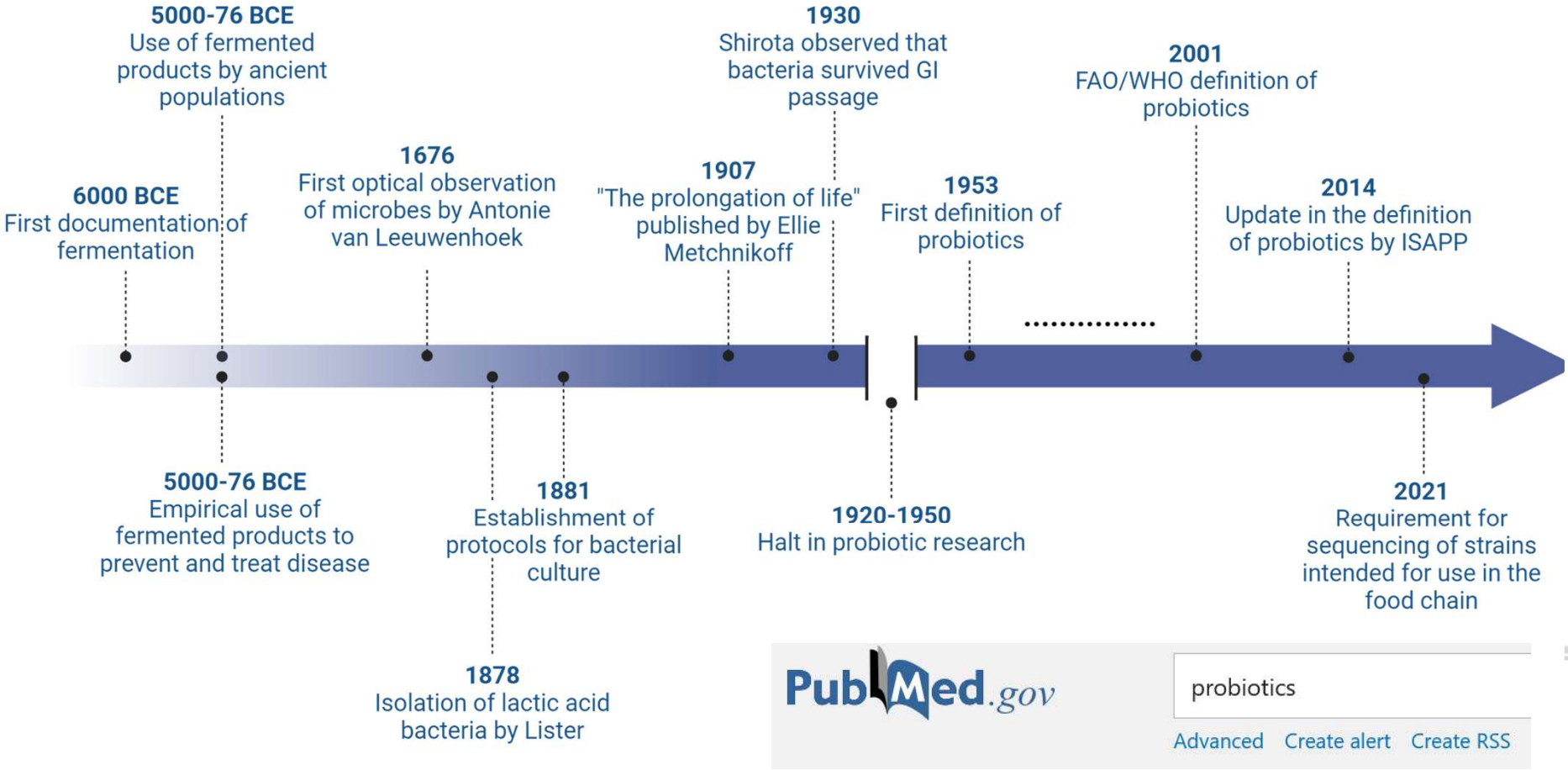
In 1989, **R. Füller** defined probiotics as *"a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance".*

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

Digestive health
Irritable Bowel Syndrome (IBS): Crohn's disease and ulcerative colitis, antibiotics-associated diarrhea, lactose intolerance

Oral health
Periodontitis, tooth decay, halitosis - chronic bad breath

Skin health
Neurogenic Skin Inflammation, Acne Rosacea, and Acne Vulgaris, Psoriasis and Atopic Dermatitis, Aging Skin

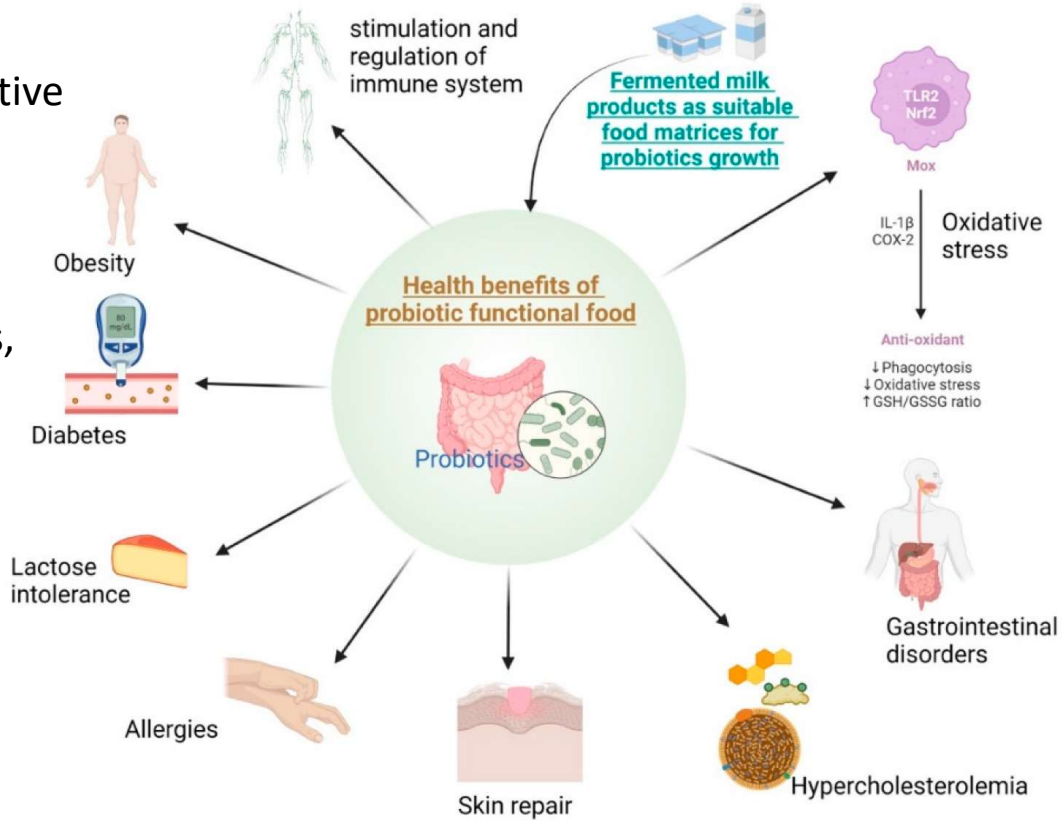
Allergic disorders
Atopic dermatitis - eczema, allergic rhinitis

Stress related diseases
Anxiety and depression Alzheimer's disease

Neurodegenerative and Demyelinating Diseases
Alzheimer's disease, Multiple Sclerosis

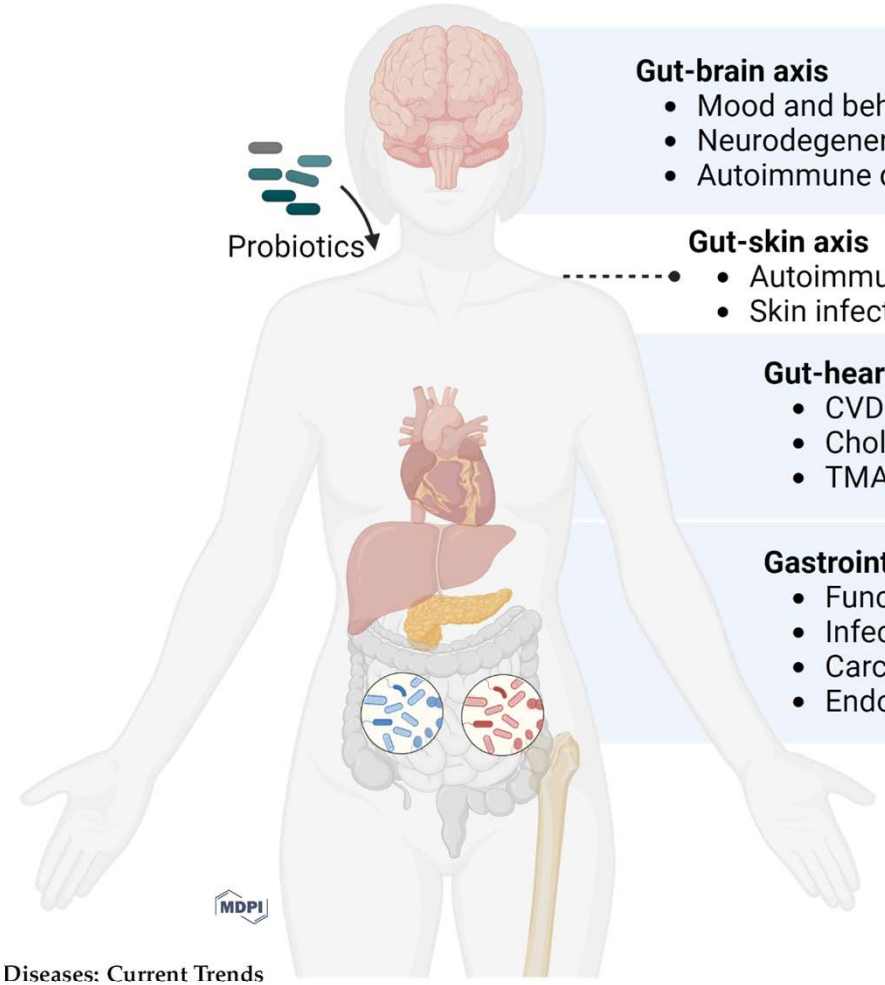
Bone diseases
Primary Osteoporosis, Rheumatoid arthritis

Cancer
Prevention of the early stages of colon cancer development



Kaur, H. et al., *Fermentation* **2022**, 8, 425.

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

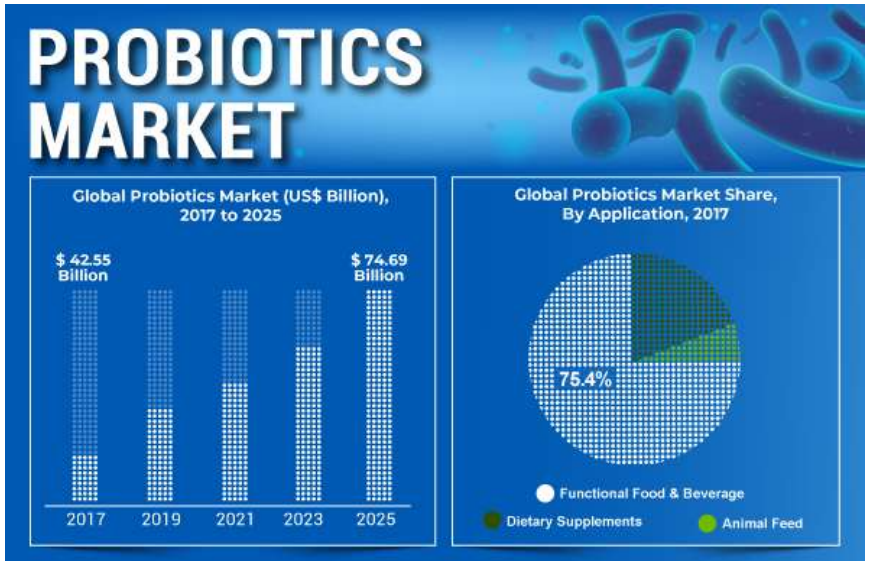
Despoina E. Kiousi [1], Athanasios Karapetsas [1,†], Kyriaki Karolidou [1], Mihalis I. Panayiotidis [2], Aglaia Pappa [1] and Alex Galanis [1,*]

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

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

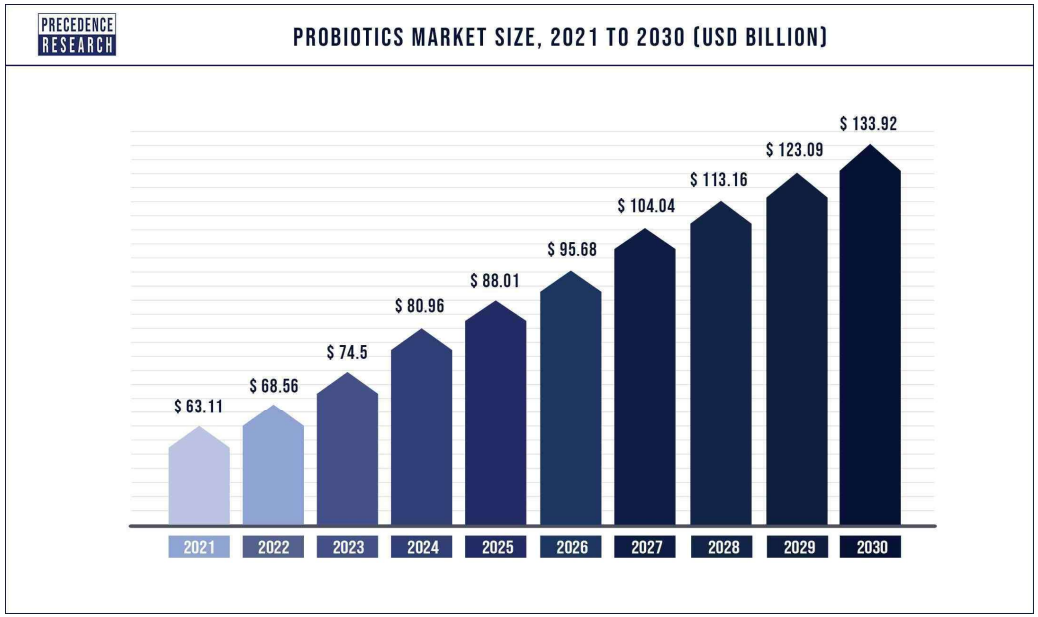
Probiotic health benefits and applications

The product was developed in 1935 by scientist Dr. Minoru Shirota who founded the company in 1955. Yakult has expanded globally, offering a range of products that contribute to the health and happiness of people around the world. In 1994 the first products were produced in the Netherlands for the European market. Yakult sells over 40 million bottles globally every day, with approximately 31.6 million bottles sold daily in international markets and about 9 million bottles sold daily in Japan. As of October 2025 Yakult has a market cap of $4.57 Billion USD.



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

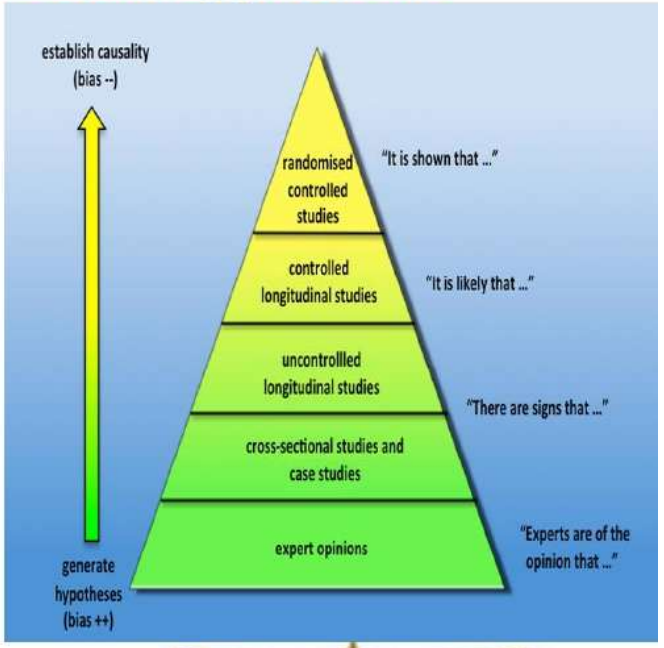
Parma, 18 January 2019

efsa

PRINCIPLES FOR SCIENTIFIC SUBSTANTIATION (cont.)

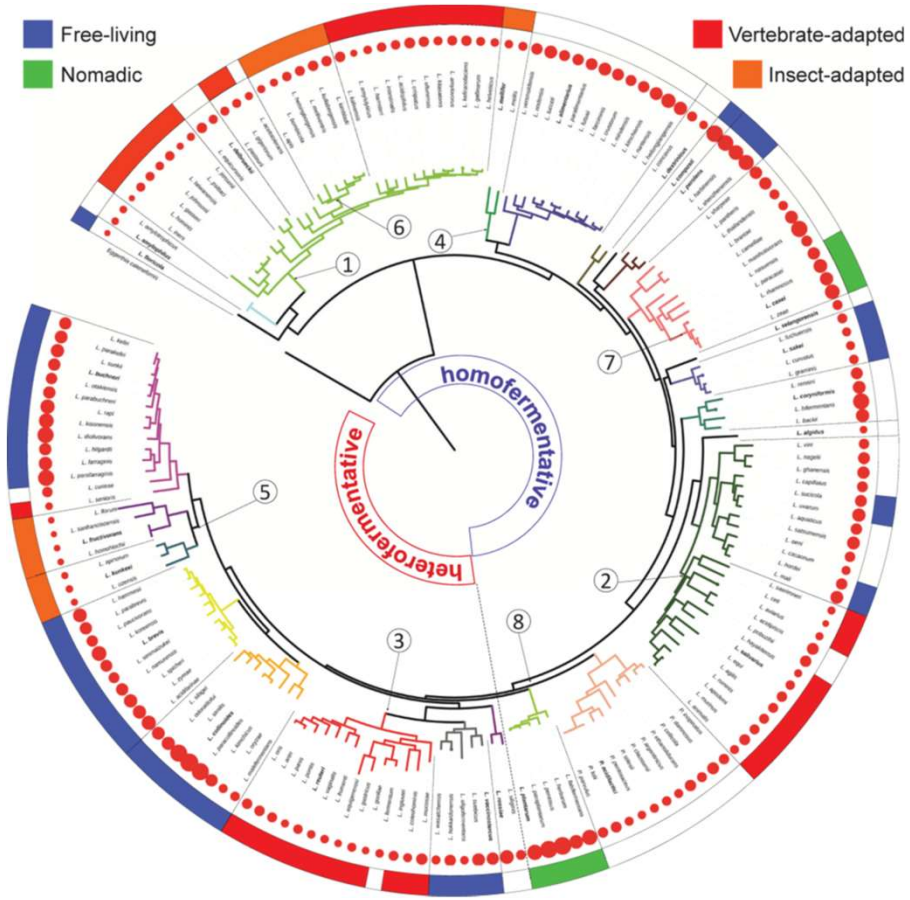
General scientific guidance for stakeholders on health claim applications

- **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



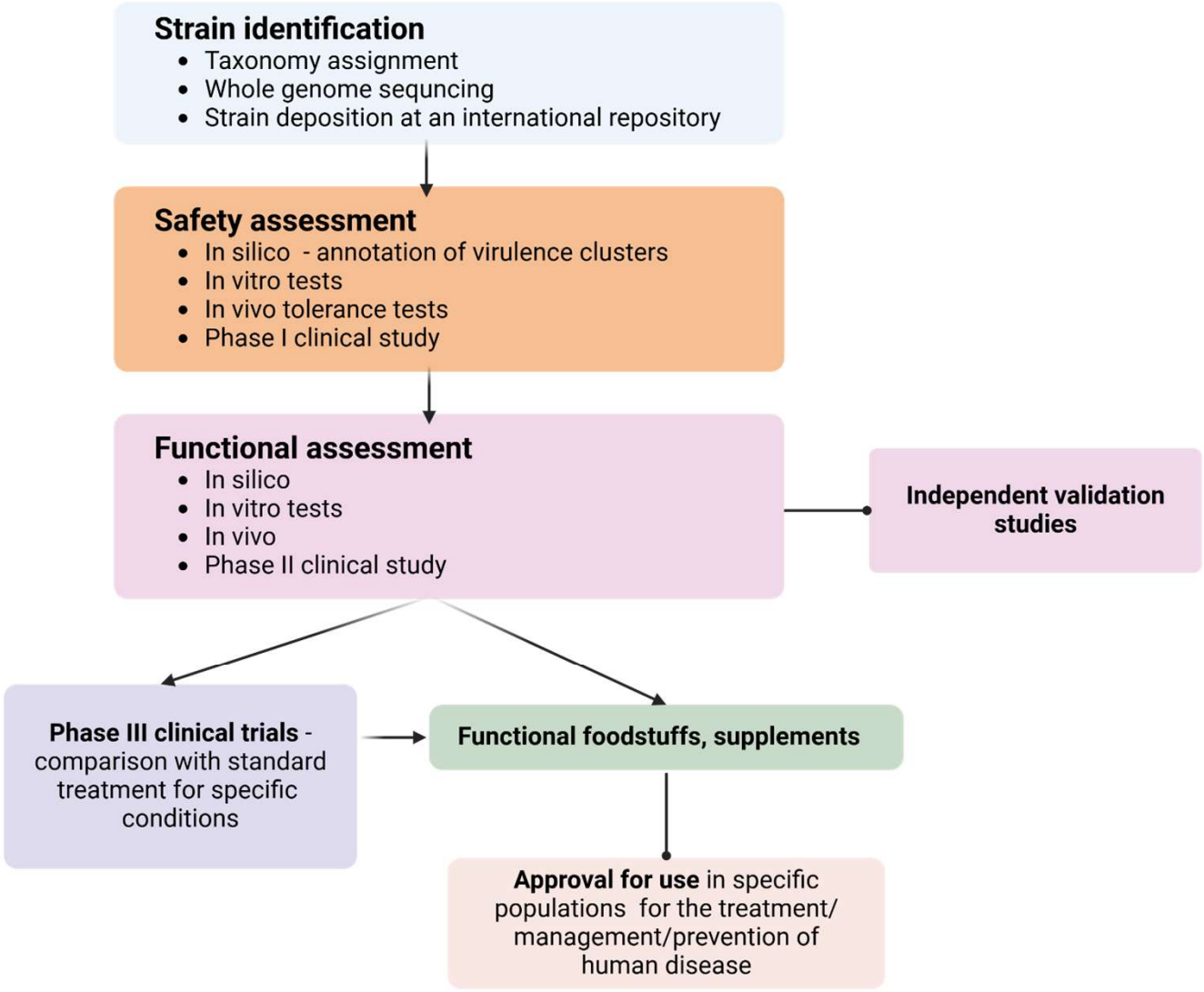
Lactobacilli

- Gram-positive
- Non-motile
- Non-spore forming
- Oxygen tolerant or anaerobic
- Optimal temperature: 2-53°C
- Nutrient-dense environments
- ~2-4 Mb



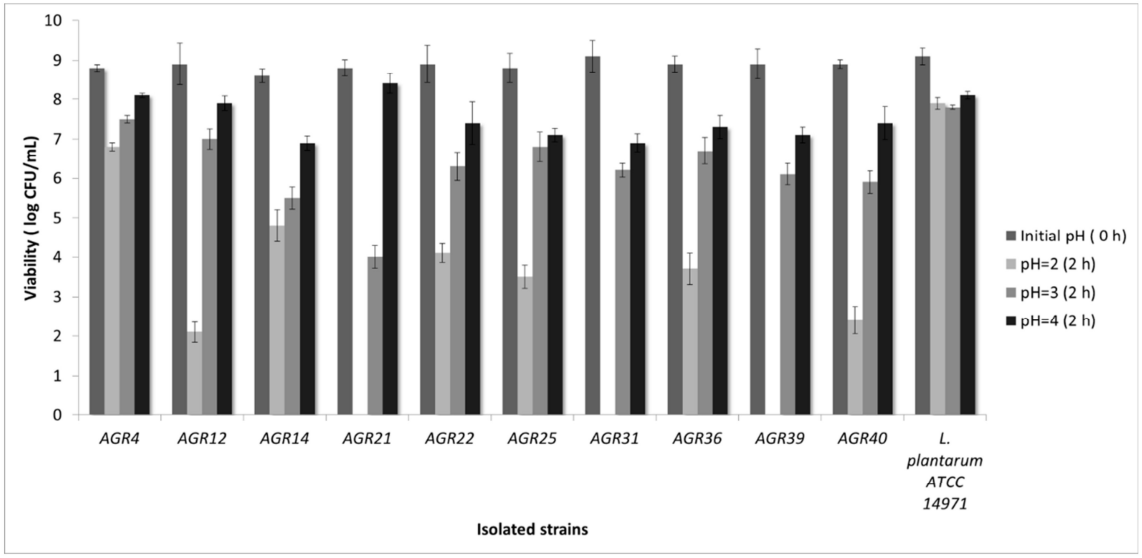
Zheng J. et al., *Int J Syst Evol Microbiol* **2020**, 70, 2782-2858.

Criteria for probiotic selection

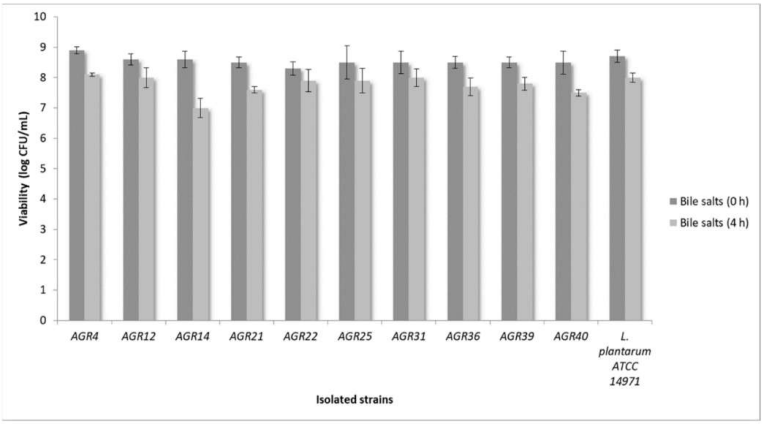


In vitro tests simulating the human gastrointestinal tract

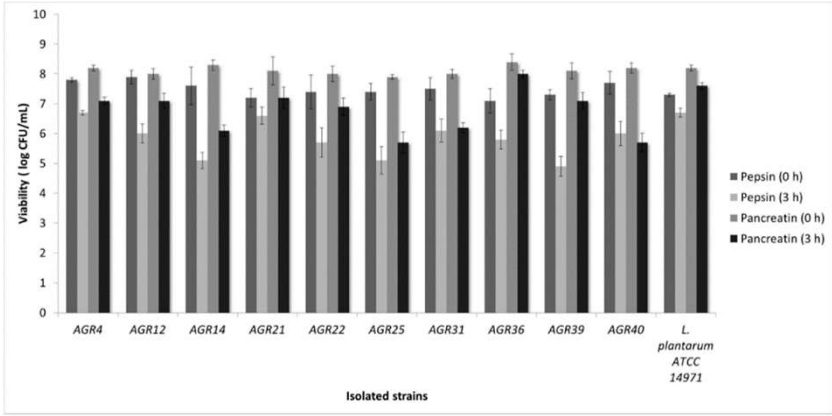
Resistance to low pH



Tolerance to bile salts



Resistance to pepsin and pancreatin

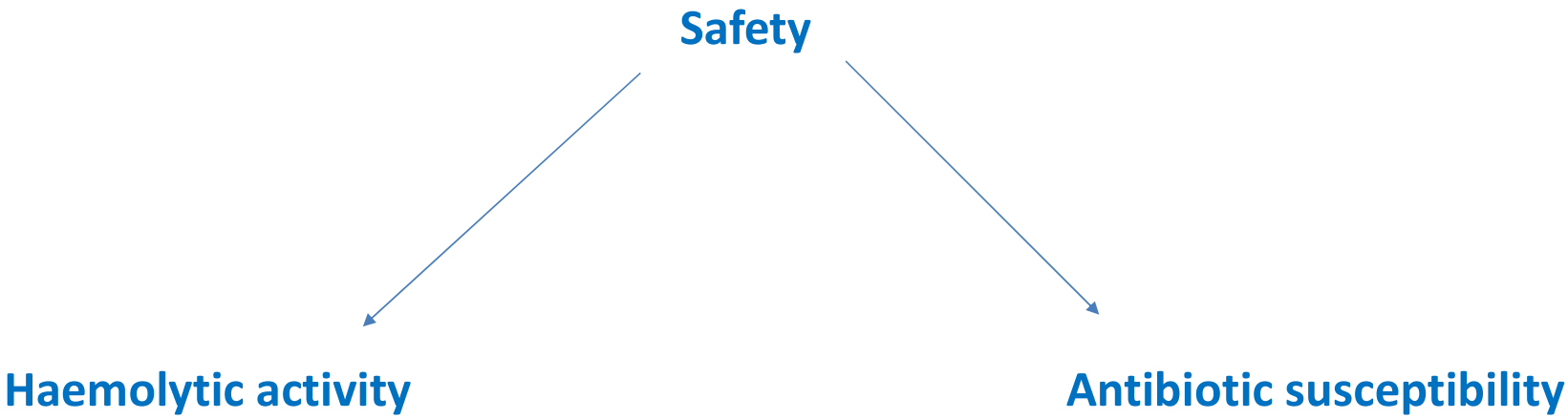


Isolation of a *Lactobacillus paracasei* Strain with Probiotic Attributes from Kefir Grains

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

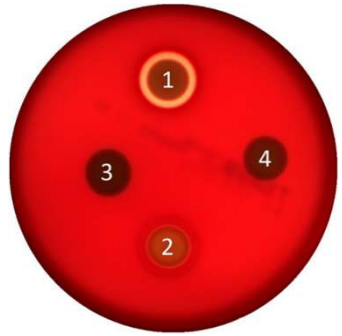


In vitro tests simulating the human gastrointestinal tract

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

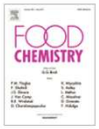
Antibiotic susceptibility expressed as minimum inhibitory concentration (MIC)

Tests included the following antibiotics:

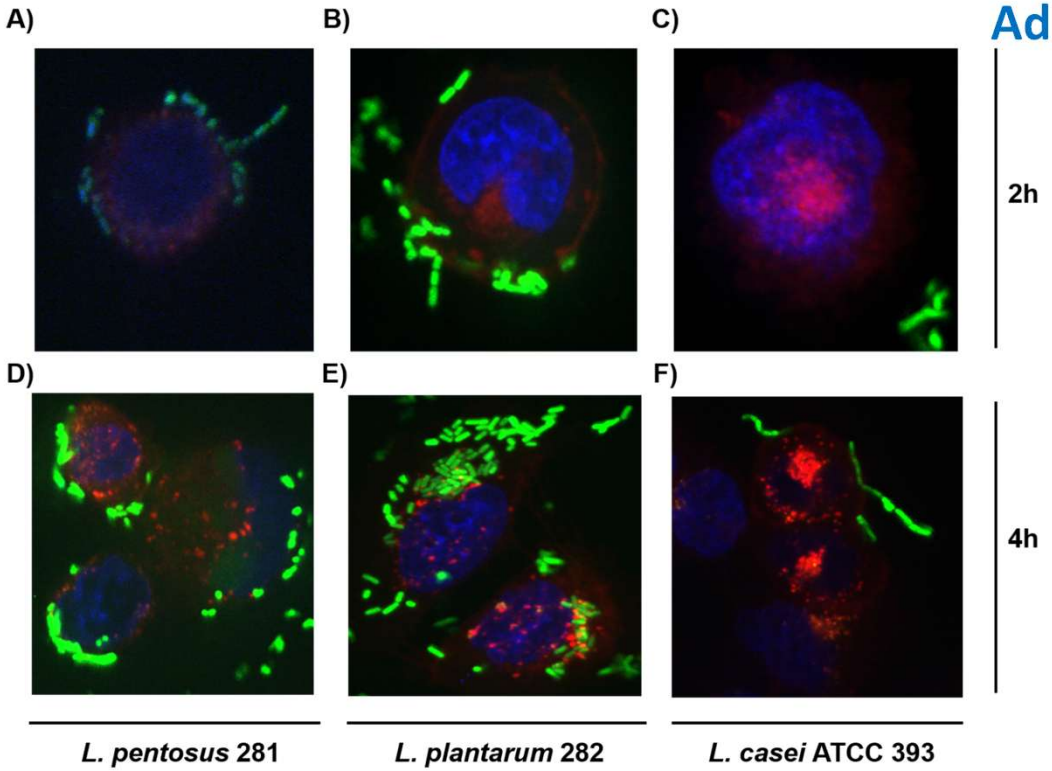
amoxycillin (256–0.015 g/mL),
amoxycillin + clavulanic acid (256–0.015 g/mL),
ampicillin (256–0.015 g/mL),
clindamycin (256–0.015 g/mL),
erythromycin (256–0.015 g/mL),
gentamycin (1024–0.06 g/mL),
metronidazole (256–0.015 g/mL),
tetracycline (256–0.015 g/mL),
tigecycline (256–0.015 g/mL)
vancomycin (256–0.015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

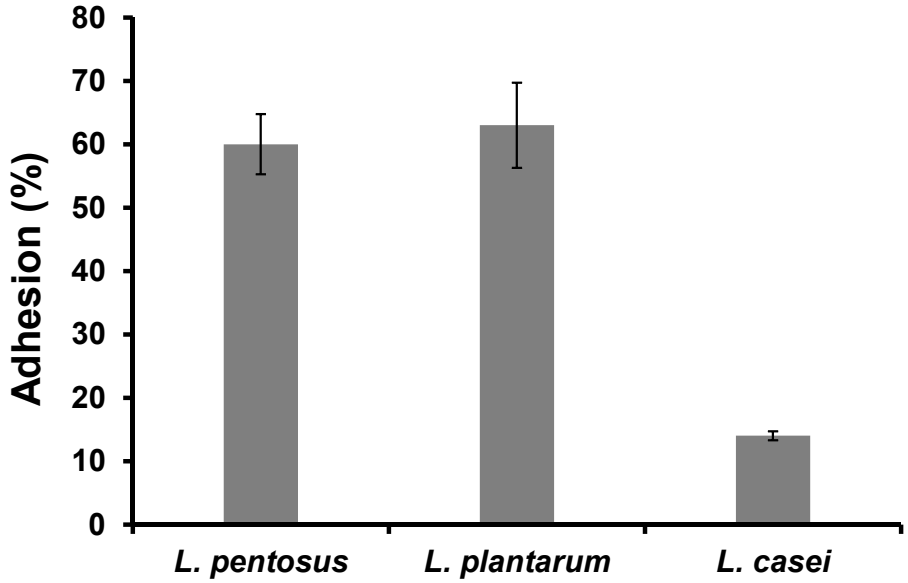
Food Chemistry
Volume 226, 1 July 2017, Pages 102-108



In vitro tests simulating the human gastrointestinal tract



Adherence capacity



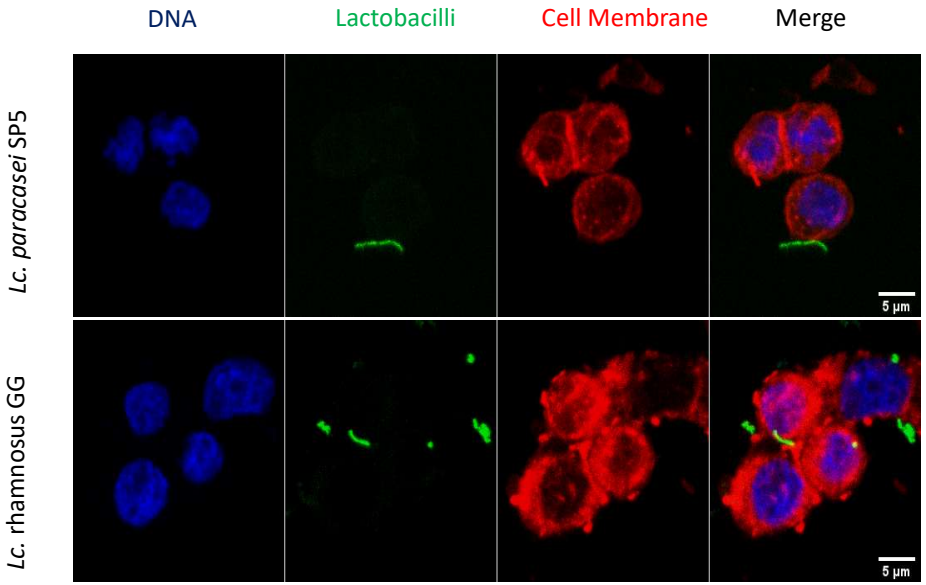
Two potential probiotic lactobacillus strains isolated from olive microbiota exhibit adhesion and anti-proliferative effects in cancer cell lines

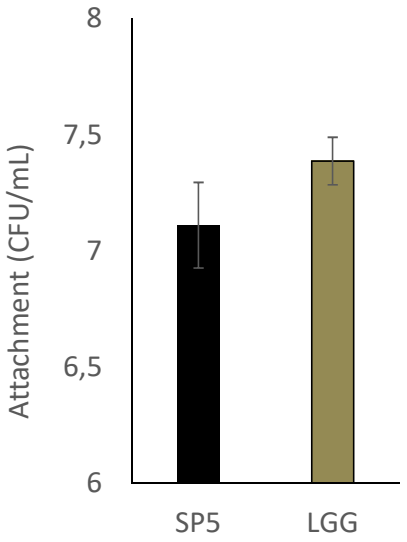
Georgia Saxami [a], *Athanasios Karapetsas* [a], *Eleftheria Lamprianidou* [b], *Ioannis Kotsianidis* [b], *Aikaterini Chlichlia* [a], *Chrysoula Tassou* [c], *Vassilis Zoumpourlis* [d], *Alex Galanis* [a,*]

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

In vitro tests simulating the human gastrointestinal tract

Adherence capacity





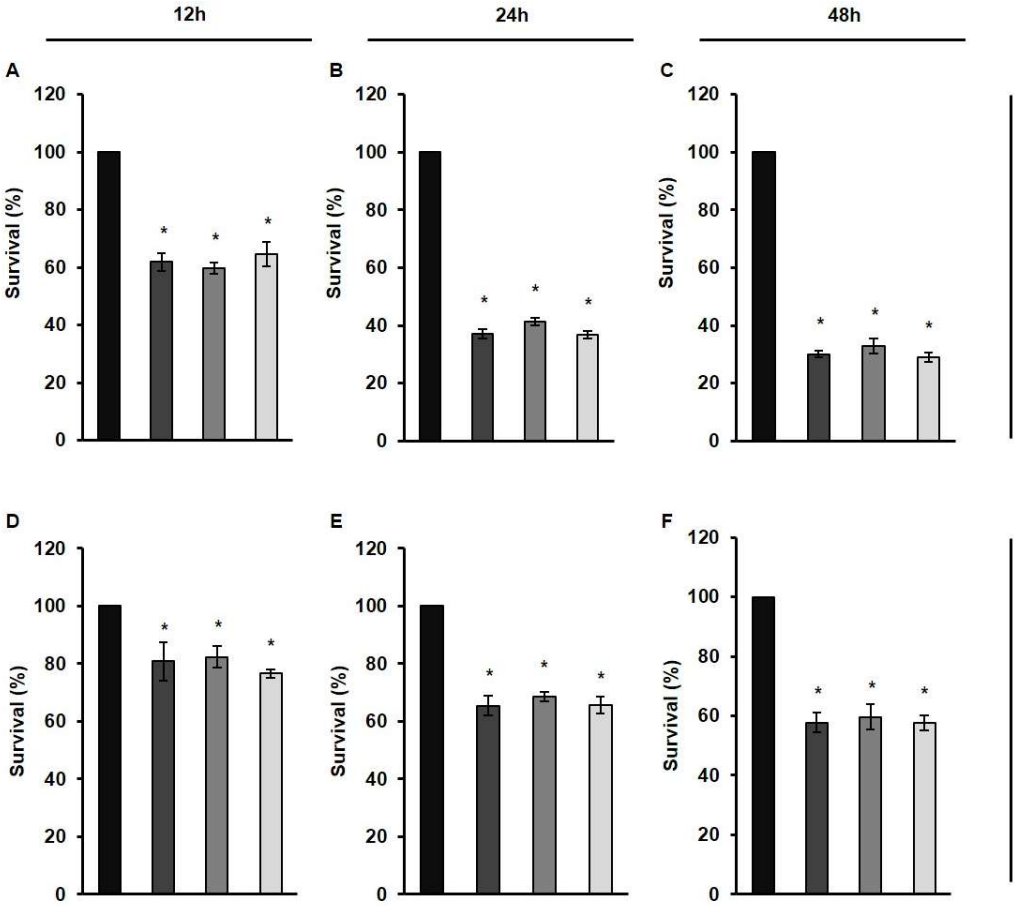
Genomic Insight Into *Lacticaseibacillus paracasei* SP5, Reveals Genes and Gene Clusters of Probiotic Interest and Biotechnological Potential

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*]

Front. Microbiol., 16 June 2022
Sec. Food Microbiology
https://doi.org/10.3389/fmicb.2022.922689

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

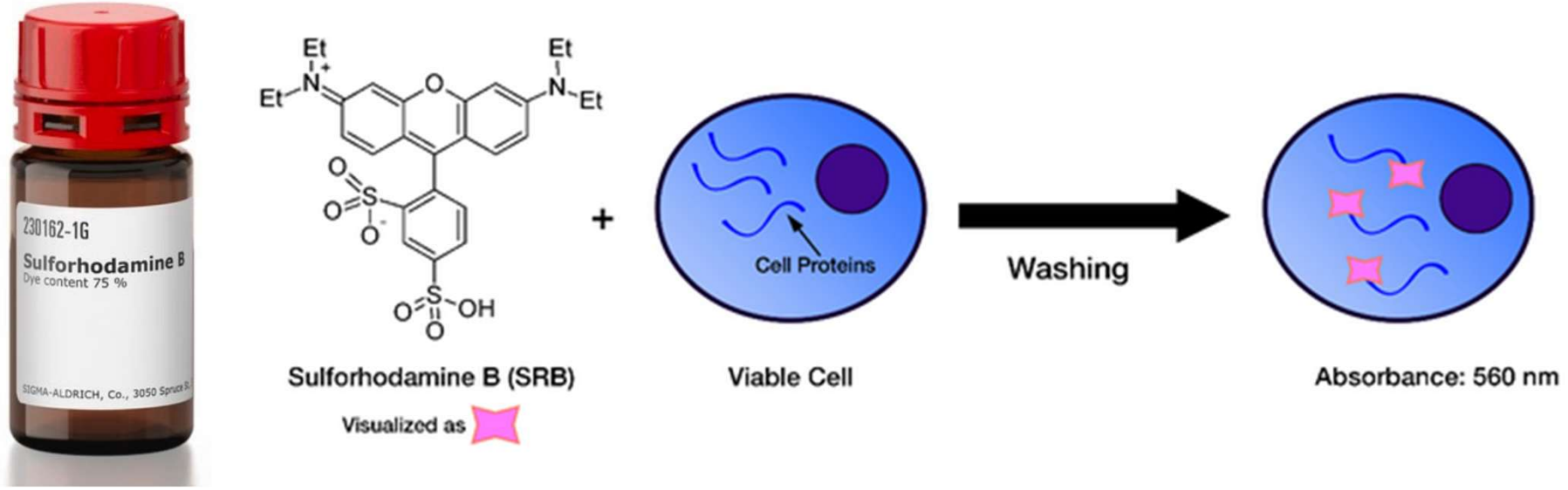
P. Chondrou, A. Karapetsas, D.E. Kiousi, D. Tsela, A. Tiptiri-Kourpeti, I. Anestopoulos Kotsianidis, E. Bezirtzoglou, A. Pappa, A. Galanis
*Corresponding author: agalanis@mbg.duth.gr

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

Health promoting properties of probiotic bacteria

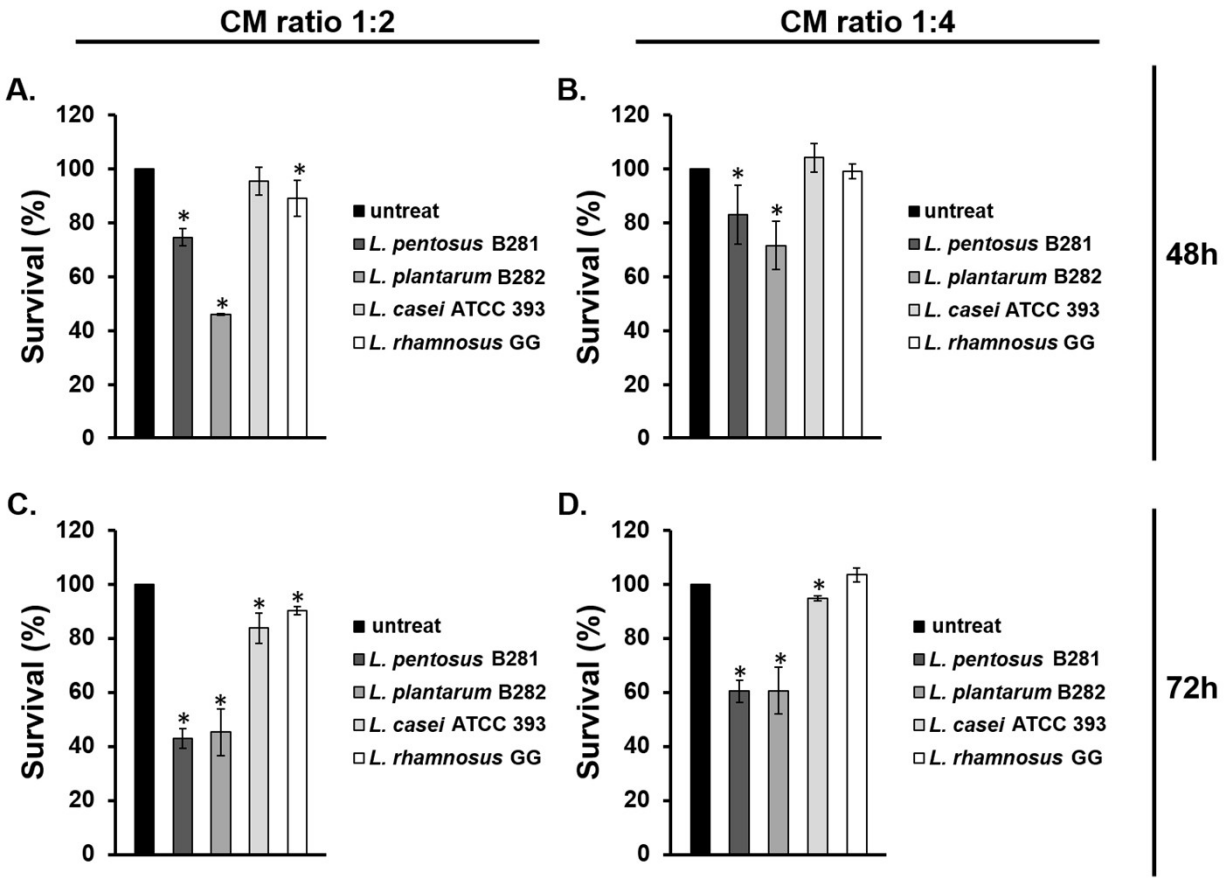
The **Sulforhodamine B (SRB)** colorimetric assay is a method for measuring cell viability, proliferation, and protein content by staining cellular proteins with SRB dye, which is then solubilized and measured using a spectrophotometer.

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 (Cell Free Supernatant)



Sulforhodamine B colorimetric assay

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

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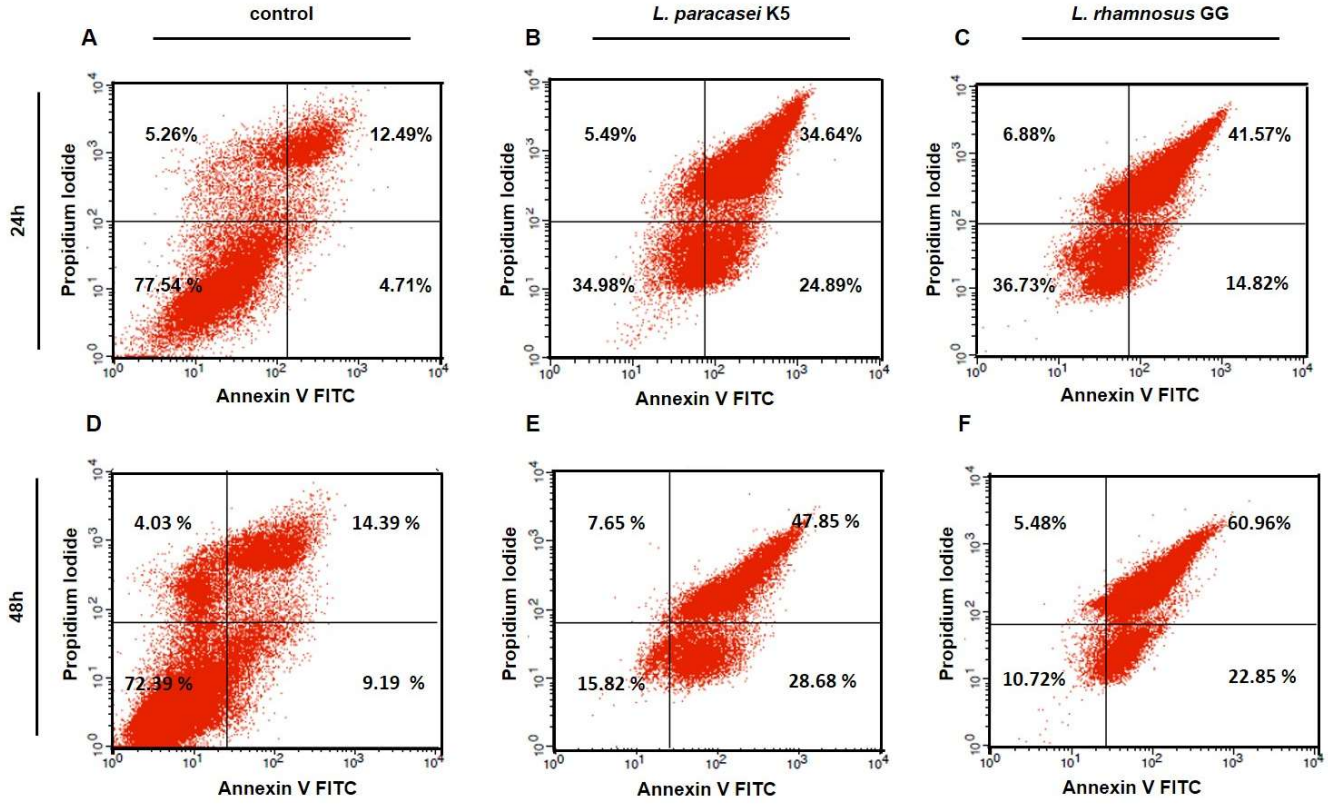
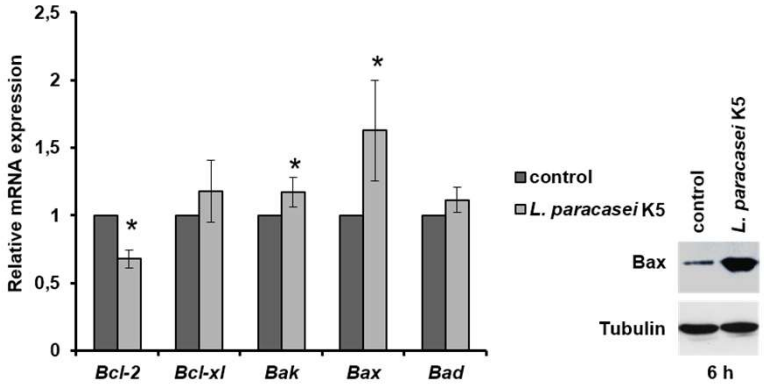
Journal of Functional Foods **2016** 24, 461–471

Health promoting properties of probiotic bacteria

Anti-proliferative activity (Induction of apoptosis)





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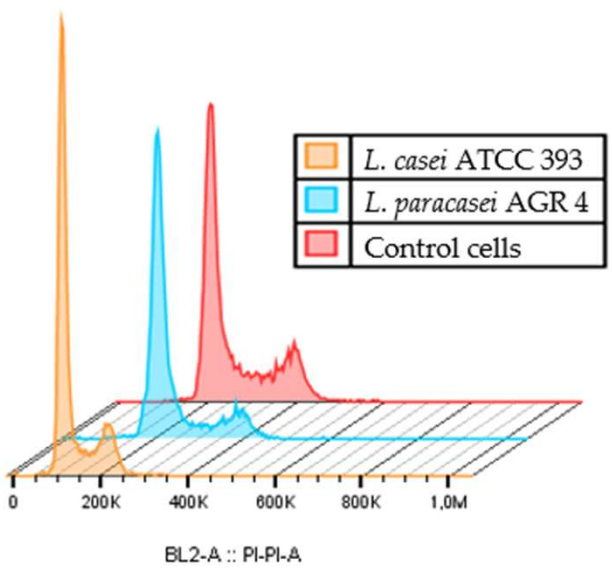
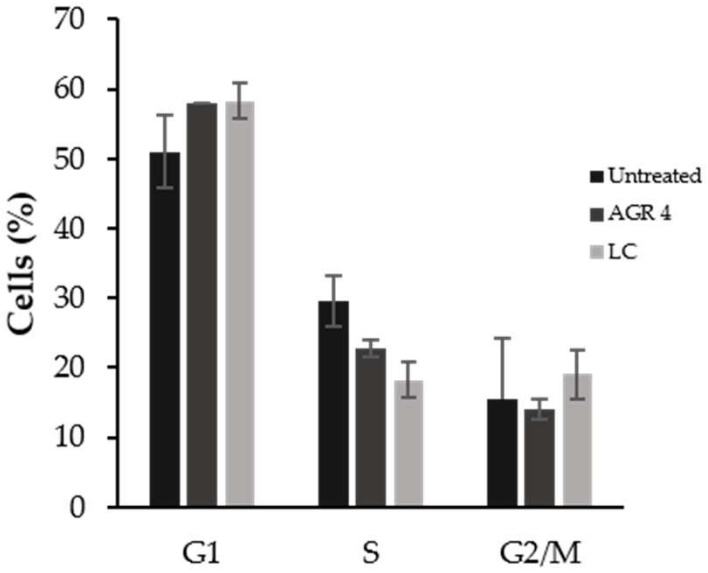
*Corresponding author: agalanis@mbg.duth.gr

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

Health promoting properties of probiotic bacteria

Anti-proliferative activity (Cell cycle arrest)





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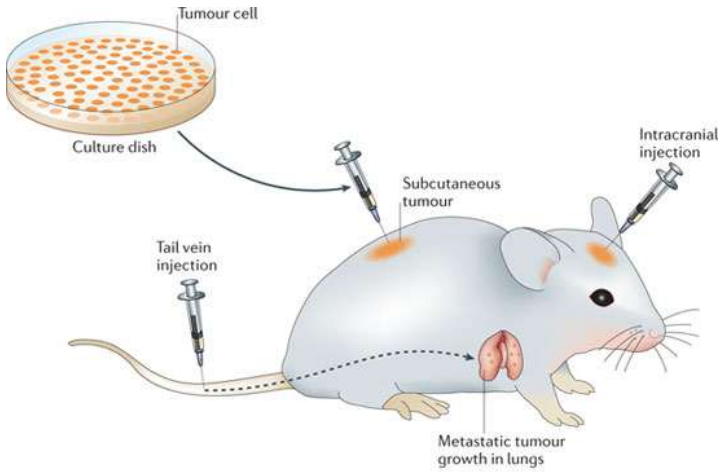
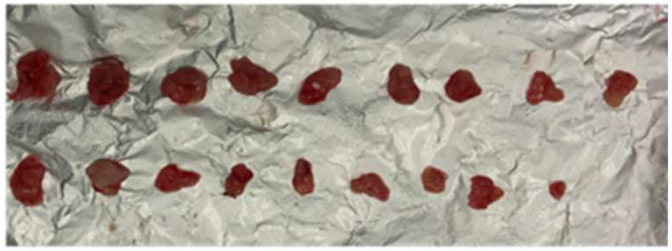
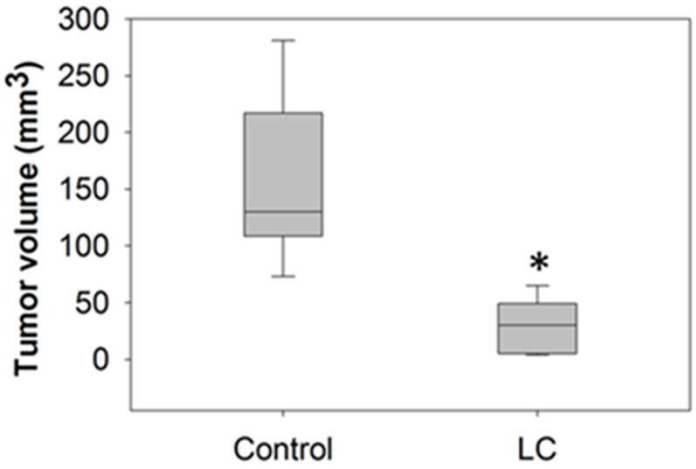
Biomedicines **2020**, 8, 594.

Health promoting properties of probiotic bacteria

Anti-proliferative activity (in vivo)







murine colon carcinoma model

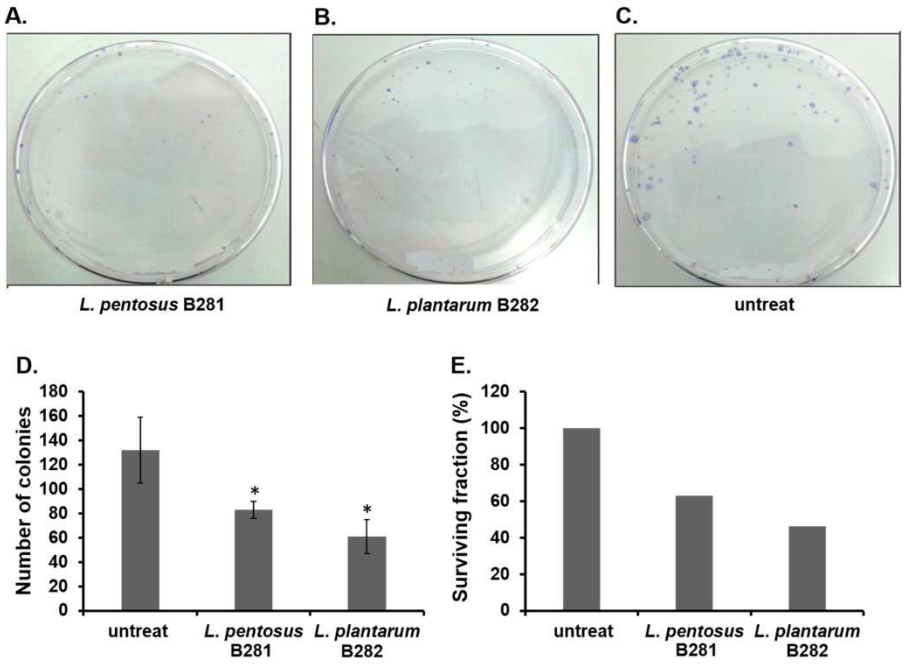
Lactobacillus casei Exerts Anti-Proliferative Effects Accompanied by Apoptotic Cell Death and Up-Regulation of TRAIL in Colon Carcinoma Cells

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

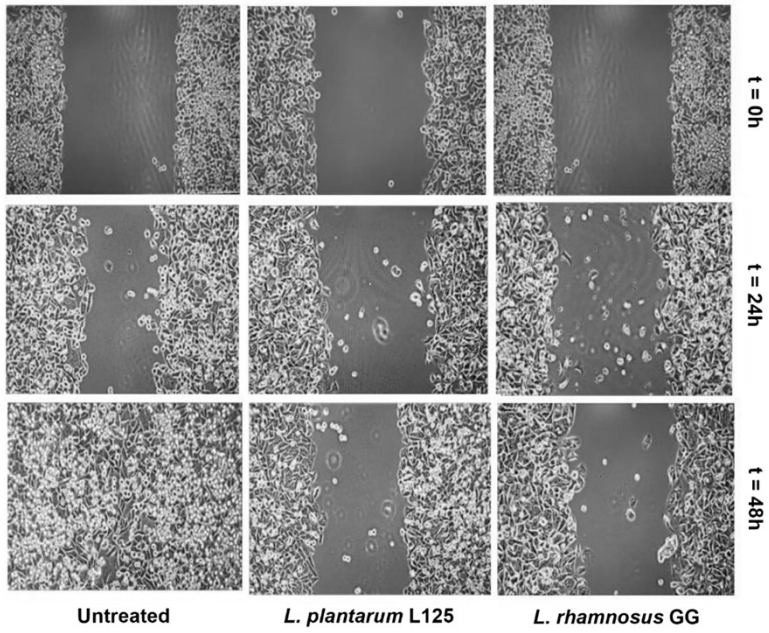
PLoS One **2016**, 11(2):e0147960.

Health promoting properties of probiotic bacteria

Anti-clonogenic activity



Anti-migration activity



Clonogenic High-Throughput assay

Wound-healing 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

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

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.

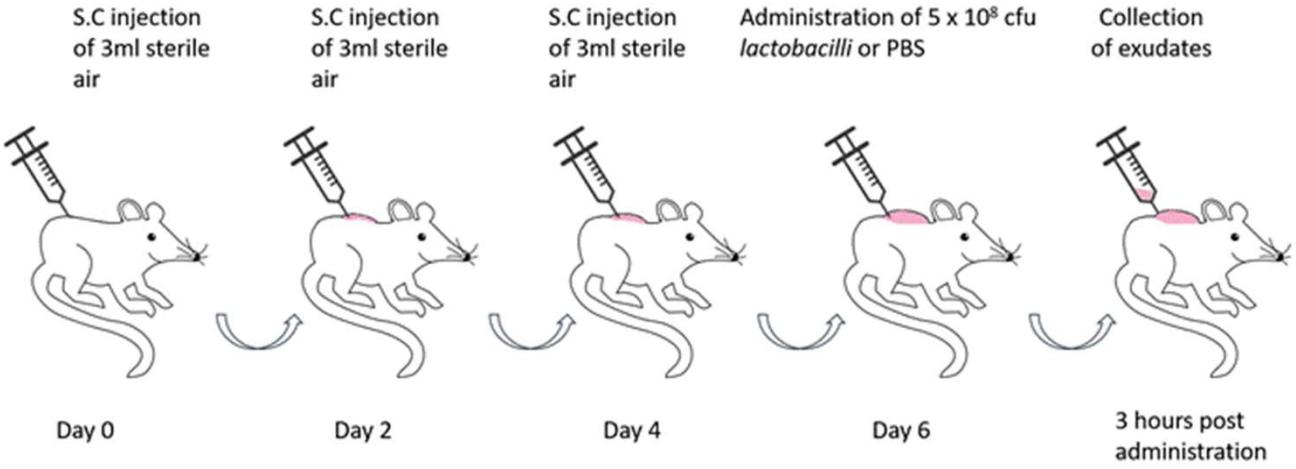
- **Examples of substances:**

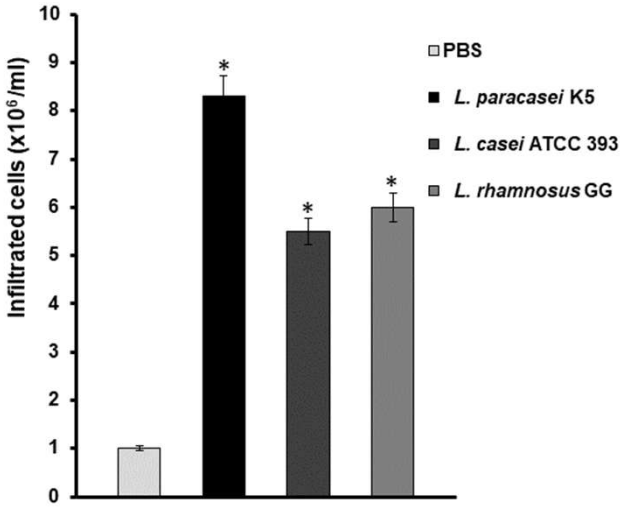
Various natural compounds and drugs have been studied for their anti-migratory properties, including astaxanthin, capsaicin, and certain compounds from plants.

Wound-healing assay

Health promoting properties of probiotic bacteria

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

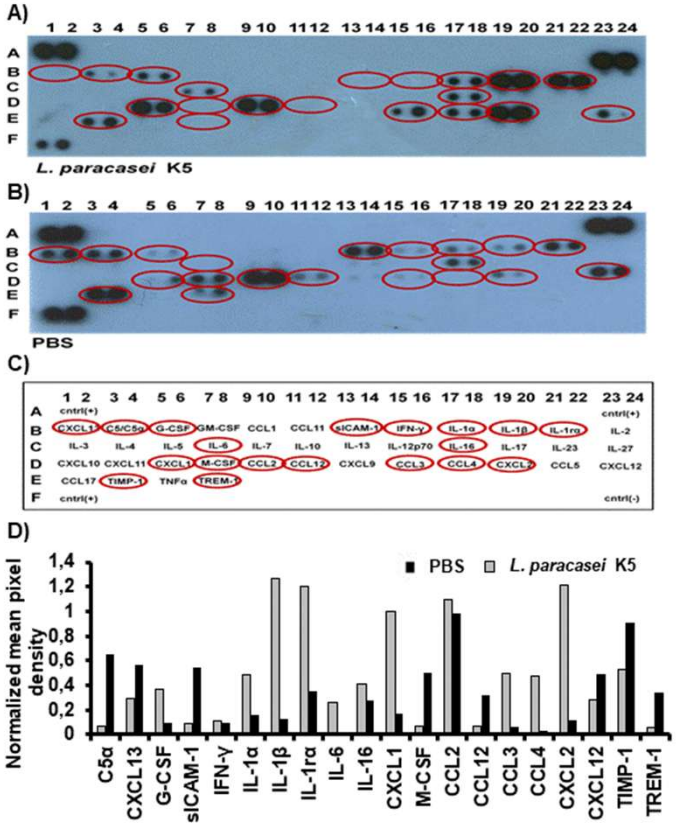
by Pelagia Chondrou, Athanasios Karapetsas, Despoina Eugenia Kiousi, Stavros Vasileiadis, Petros Ypsilantis, Sotiris Botaitis, Athanasios Alexopoulos, Stavros Plessas, Eugenia Bezirtzoglou and Alex Galanis

Microorganisms **2020**, 8, 709.

Health promoting properties of probiotic bacteria

Immunomodulatory Properties

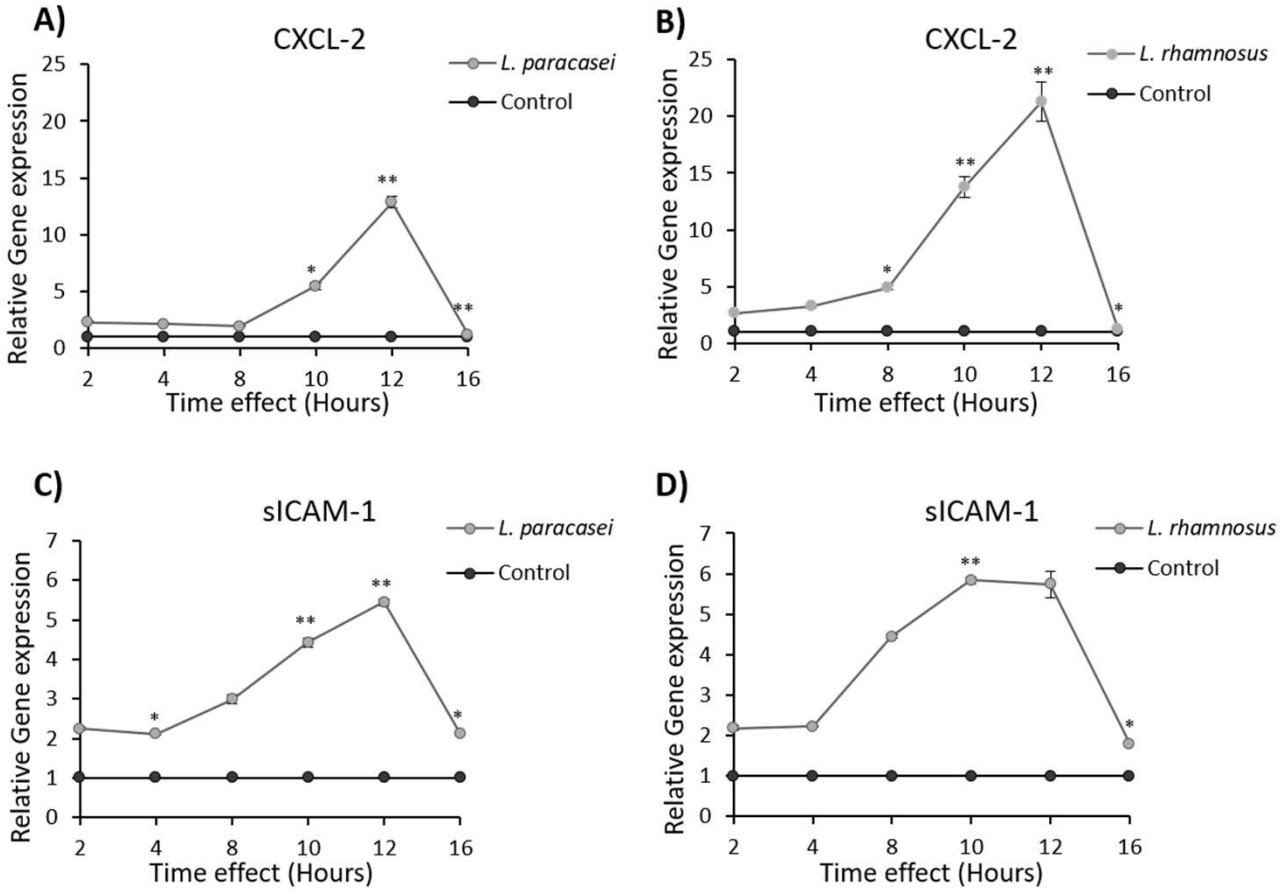
L. paracasei or PBS were administered in the air pouches of BALB/c mice, and after 3 h, the exudates were collected, and the supernatants were analyzed for cytokine/chemokine expression, using the Proteome Profiler TM Mouse Antibody Array Panel. The pairs of spots in the upper-left, upper-right, and 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 [b]no detection	
[c]downregulation	

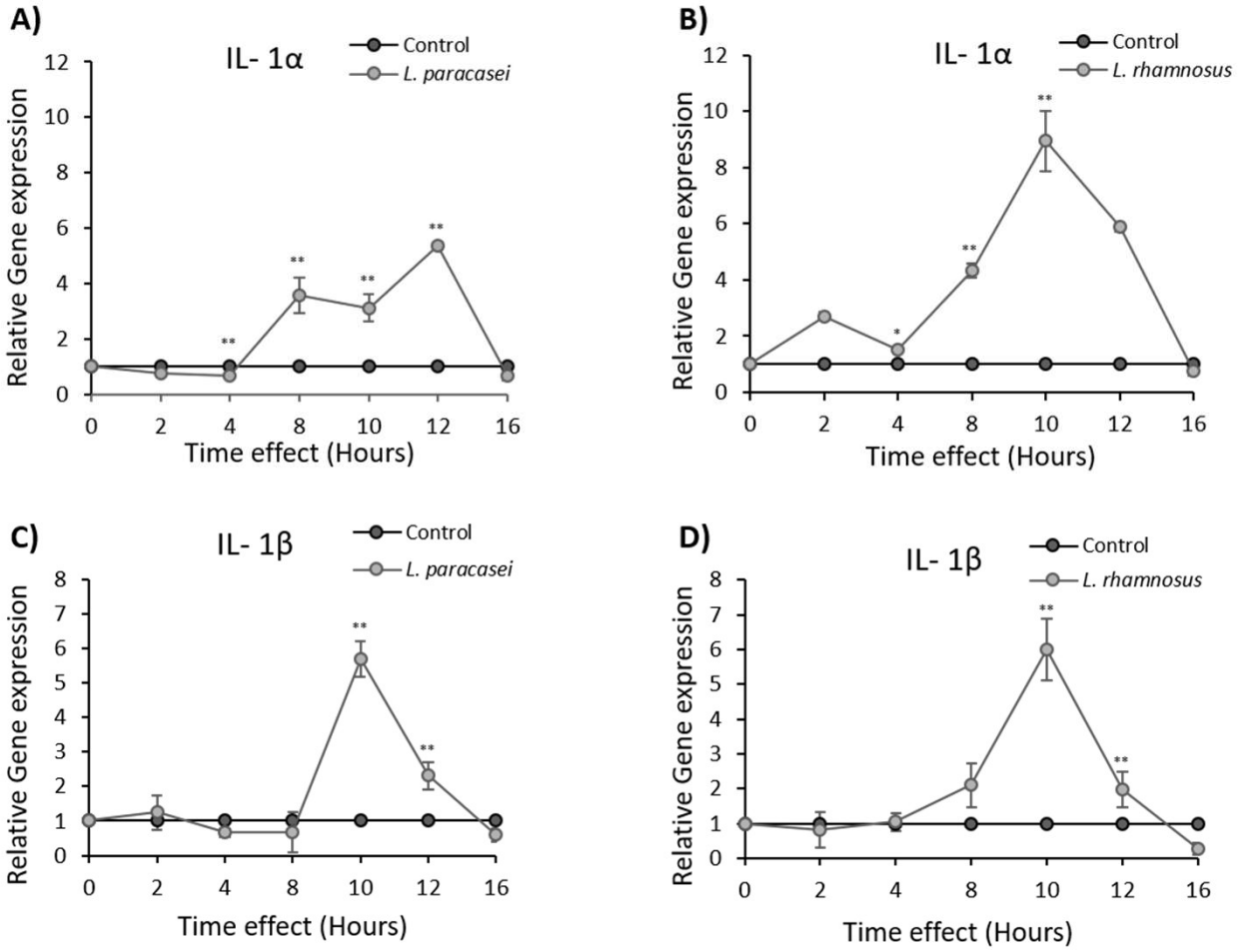
Health promoting properties of probiotic bacteria

Immunomodulatory Properties



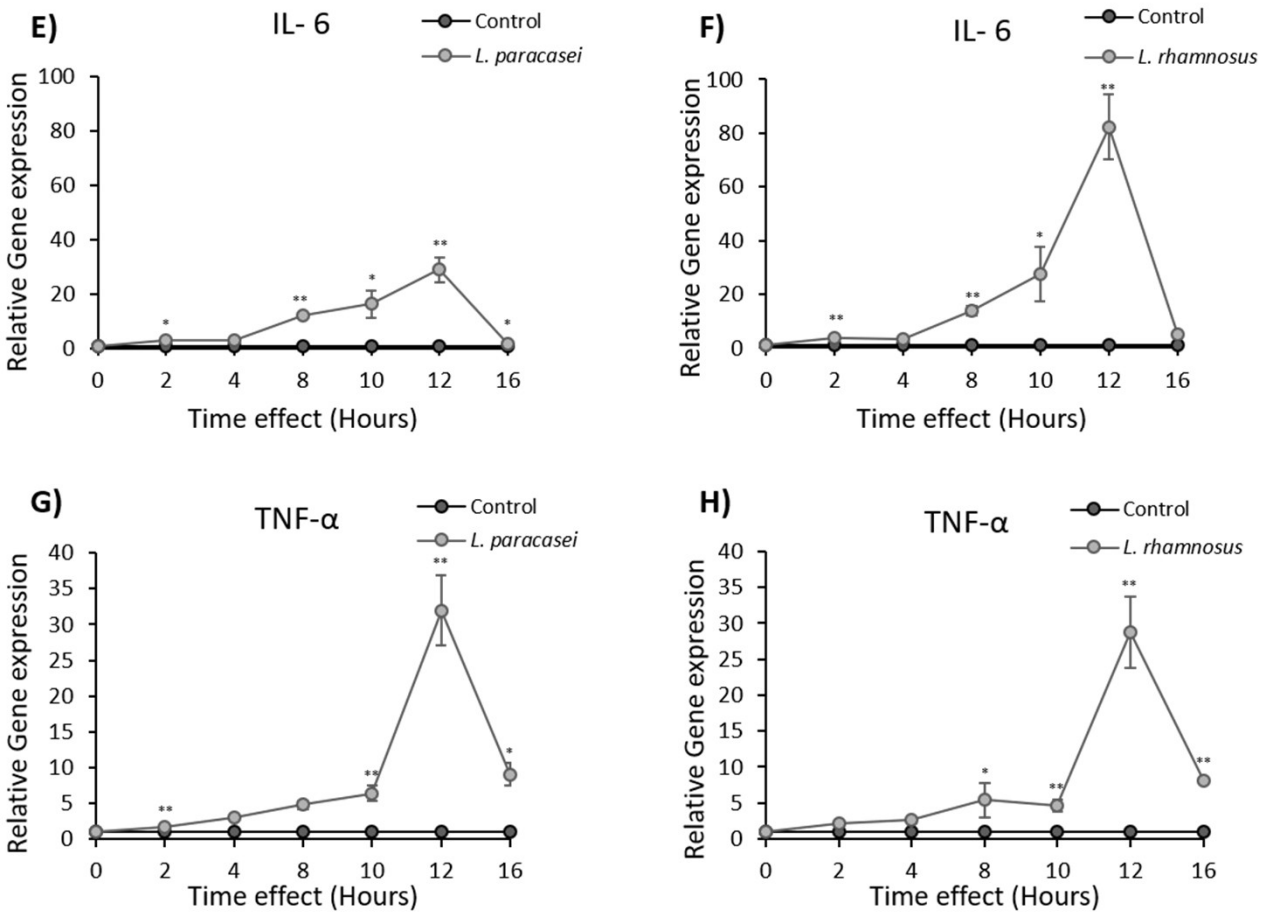
Health promoting properties of probiotic bacteria

Immunomodulatory Properties



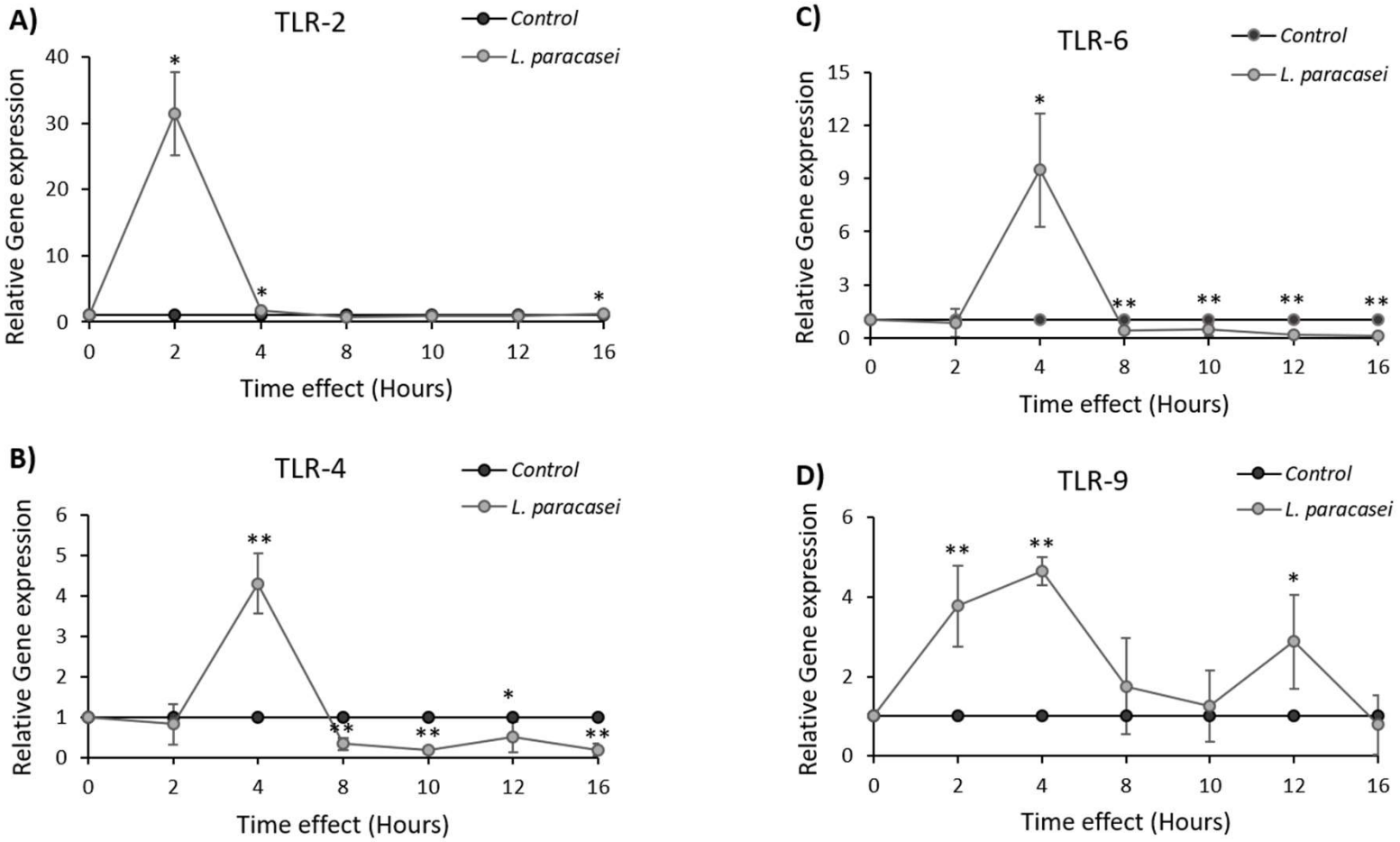
Health promoting properties of probiotic bacteria

Immunomodulatory Properties

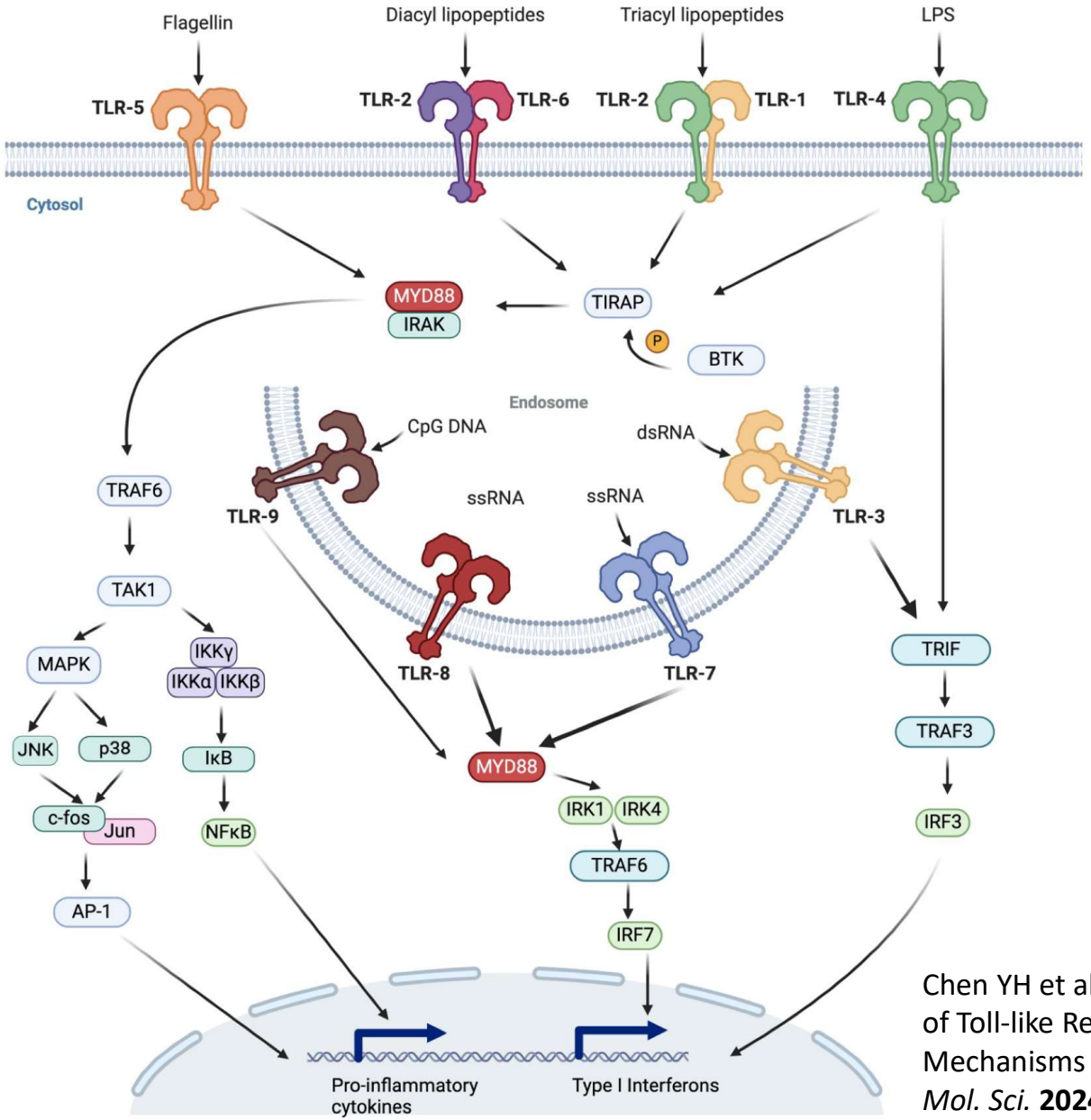


Health promoting properties of probiotic bacteria

Immunomodulatory Properties



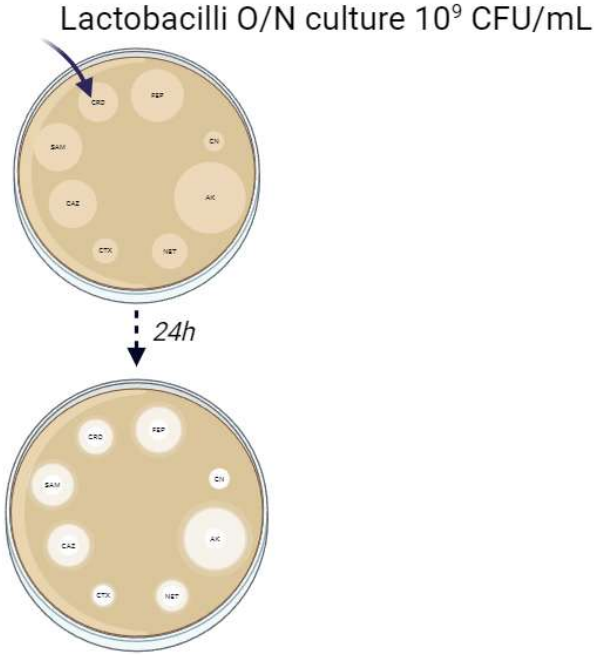
Toll-like receptors (TLRs) are a family of pattern recognition receptors (PRRs) that play a critical role in the innate immune system. They detect molecules shared by pathogens, known as pathogen-associated molecular patterns (PAMPs), as well as host-derived damage-associated molecular patterns (DAMPs) 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



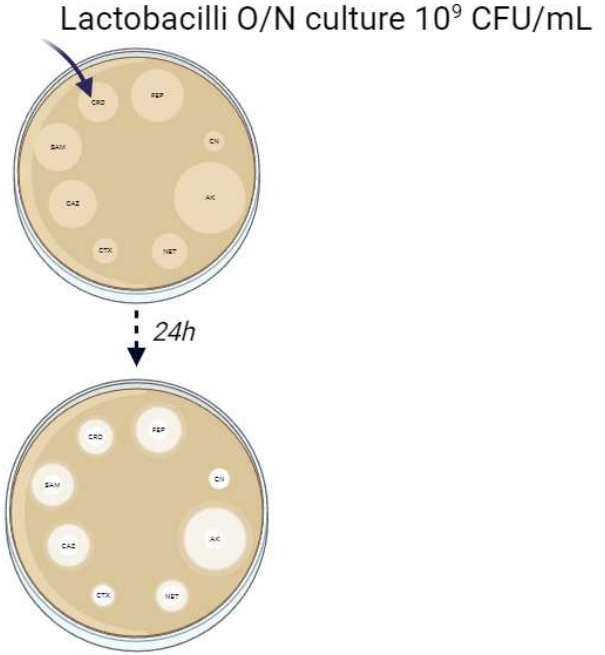
Agar well diffusion assay

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.

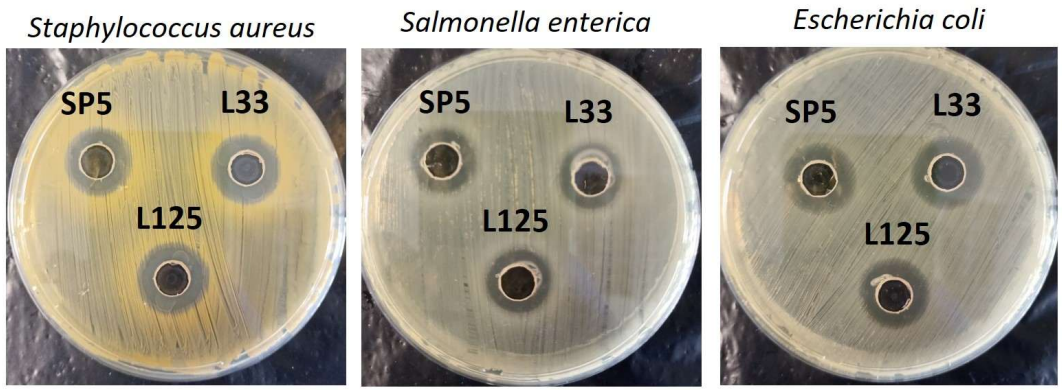
Sample application: The antimicrobial substance or extract is placed into the wells.

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

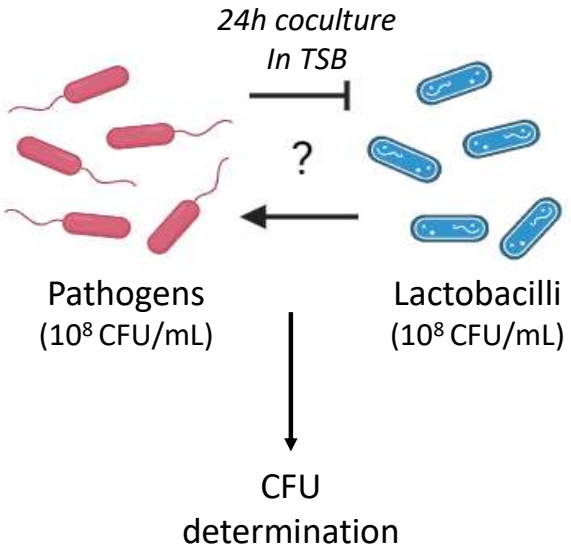
Genetic and phenotypic assessment of the antimicrobial activity of three potential probiotic lactobacilli against human enteropathogenic bacteria

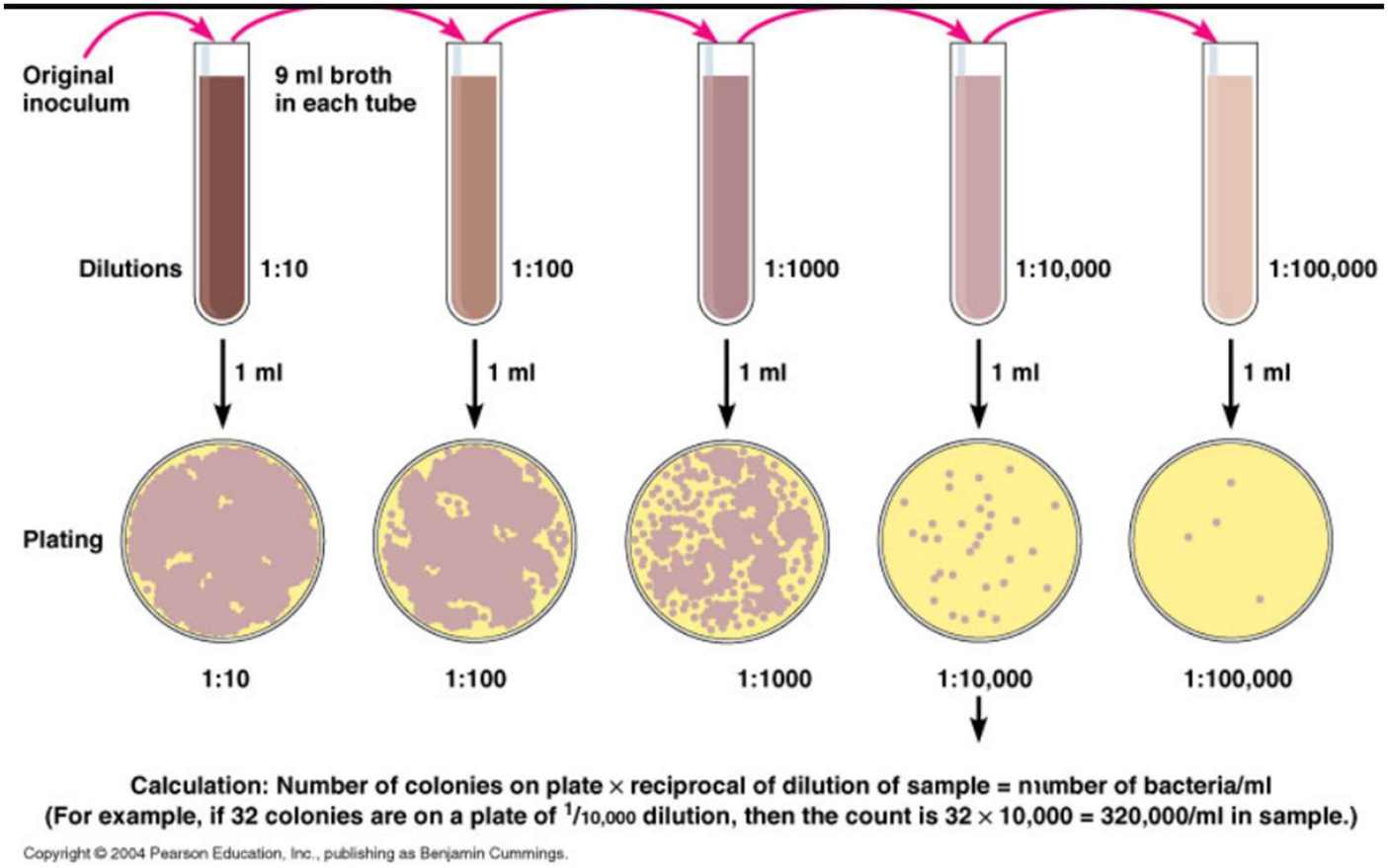
Despoina Eugenia Kiousi[1] Christos Efstathiou[1] Vasilis Tzampazlis[1]
Stavros Plessas[2] Maria Panopoulou[3] Maria Koffa[1] Alex Galanis[1*]

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

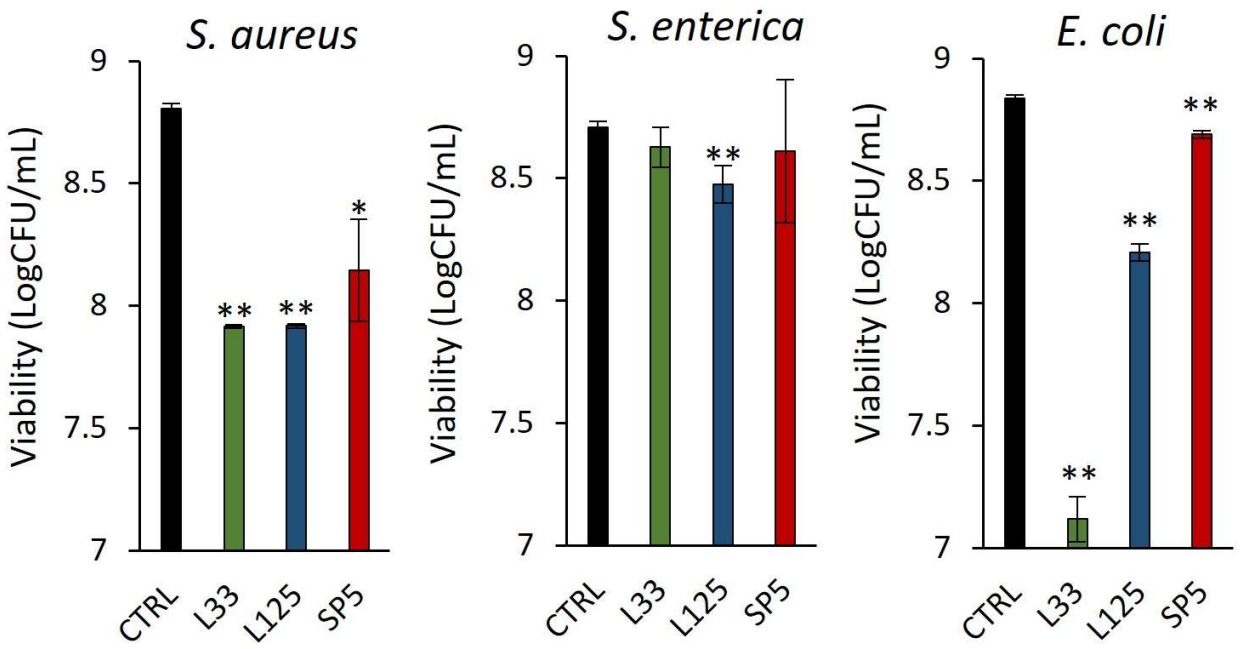
frontiers

Live lactobacilli affect planktonic pathogen growth in a strain-specific basis



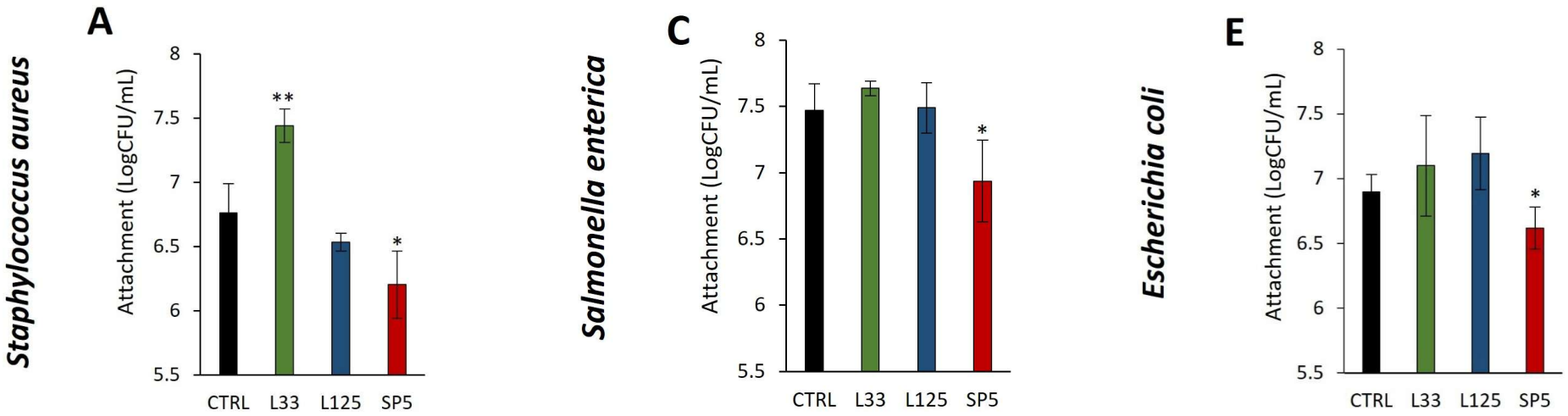


Live lactobacilli affect planktonic pathogen growth in a strain-specific basis



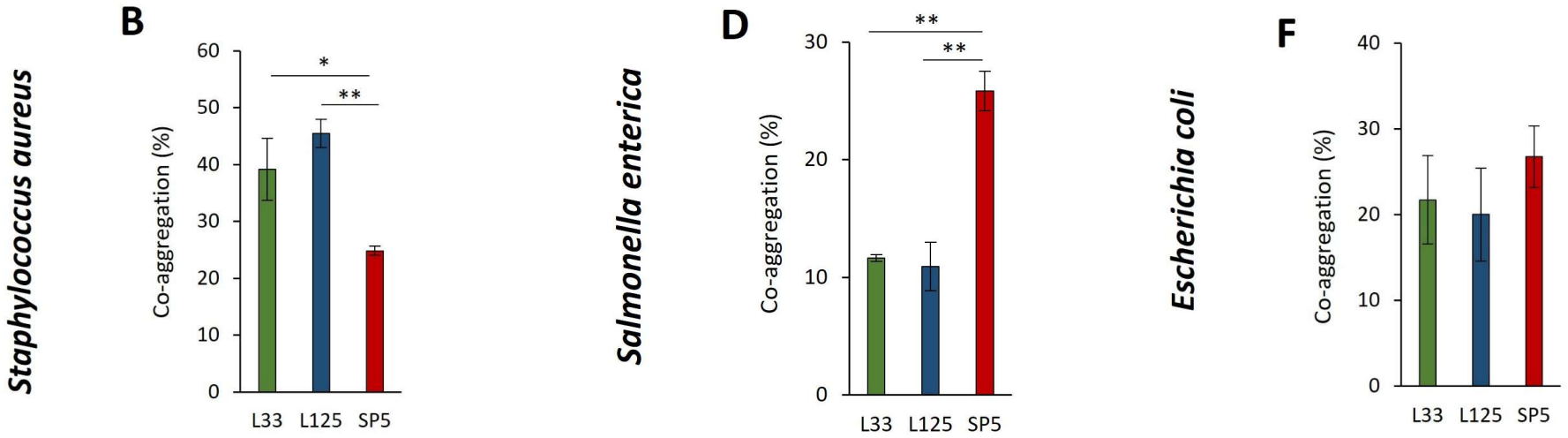
Pathogens (10^8 CFU/mL) and lactobacilli (10^8 CFU/mL) were co-incubated for 24 h, at 37°C under anaerobic conditions in TSB. The next day, the bacterial suspension was serially diluted in 1× Ringer's solution and spread on agar plates for colony enumeration.

Competition for adherence and coaggregation ability of lactobacilli



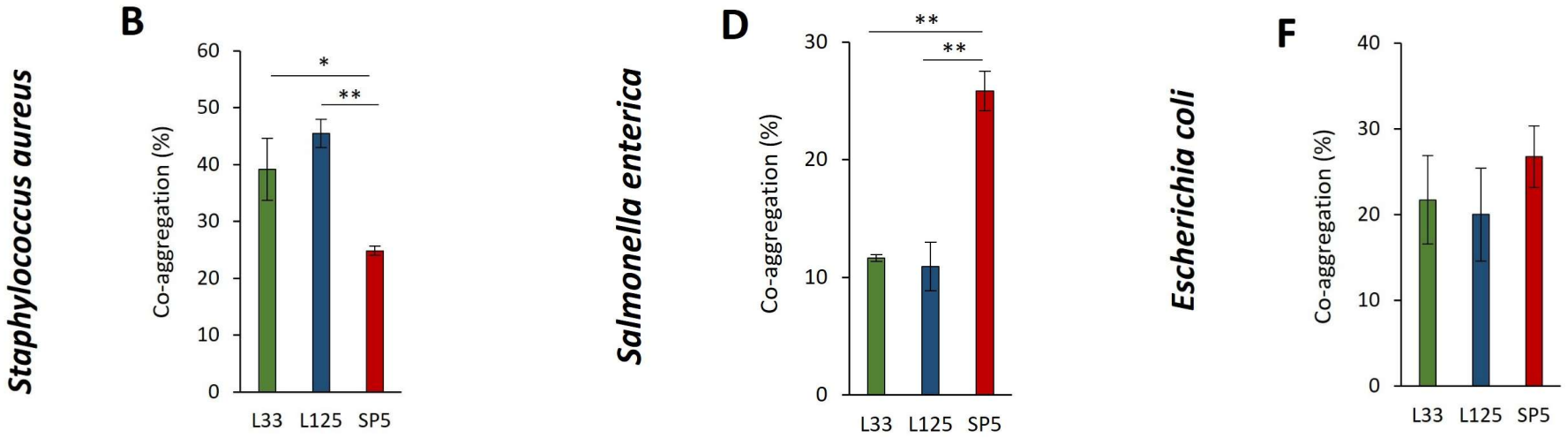
HT-29 cells were seeded in 24-well plates at a density of 4 $\times 10^5$ cells per well and were incubated until the formation of a monolayer (100% confluency). Then, cells were treated with lactobacilli and/or pathogens at a concentration of 10^8 CFU/mL for 4 h. Control samples were incubated with pathogens alone for 4 h. The monolayers were washed twice with (PBS) 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



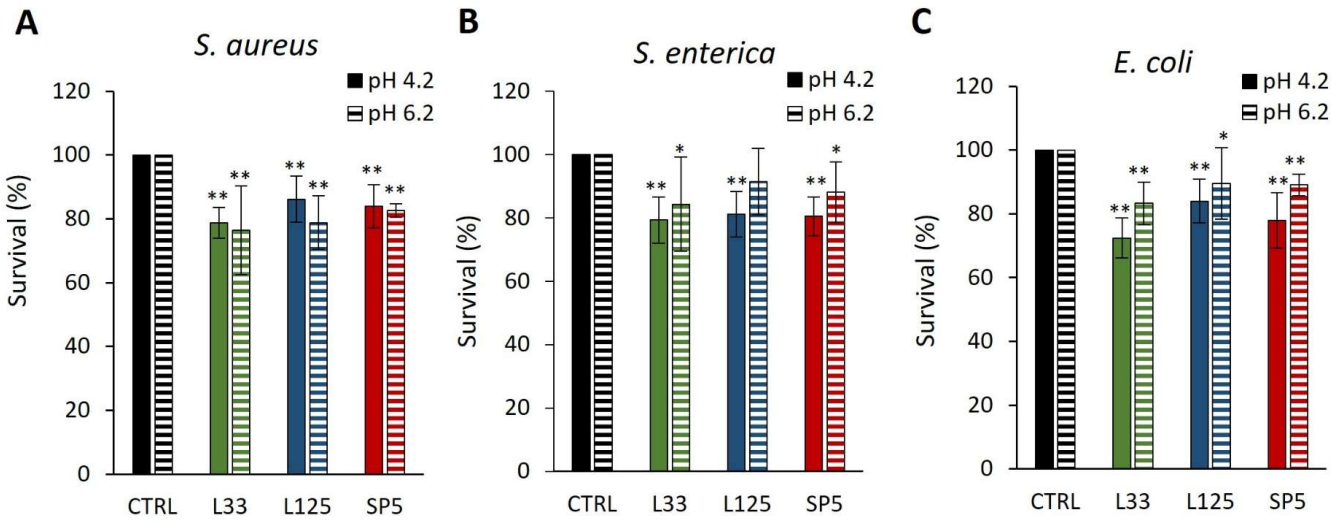
Why it's important

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.

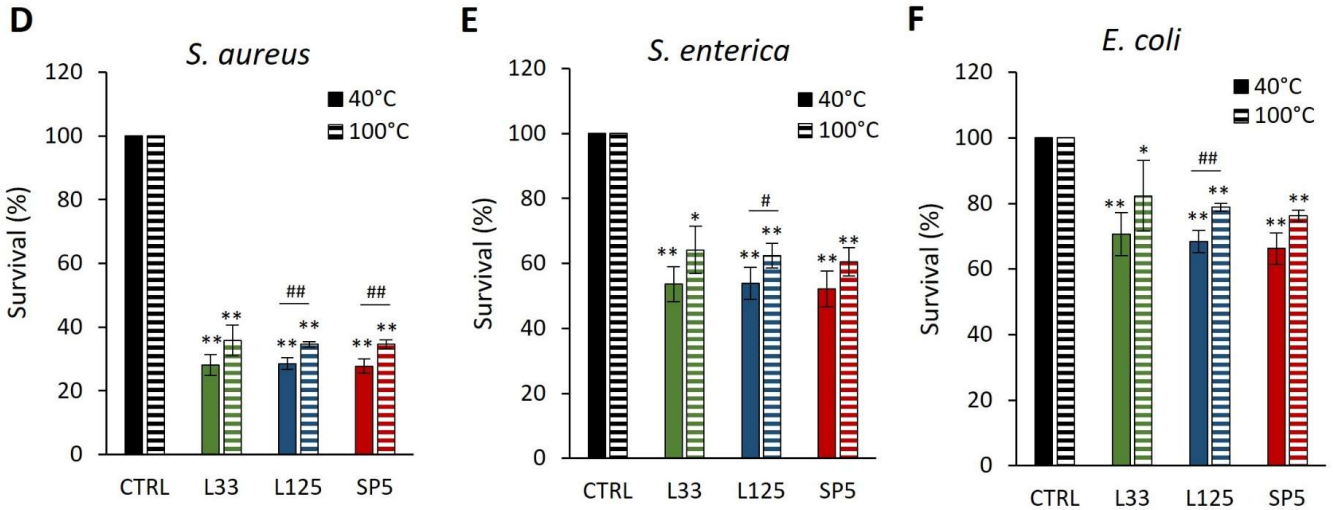
Pathogen inhibition: A probiotic's ability to co-aggregate with a pathogen can help prevent the pathogen from attaching to host tissues, as seen with the formation of a barrier in the gastrointestinal tract.

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

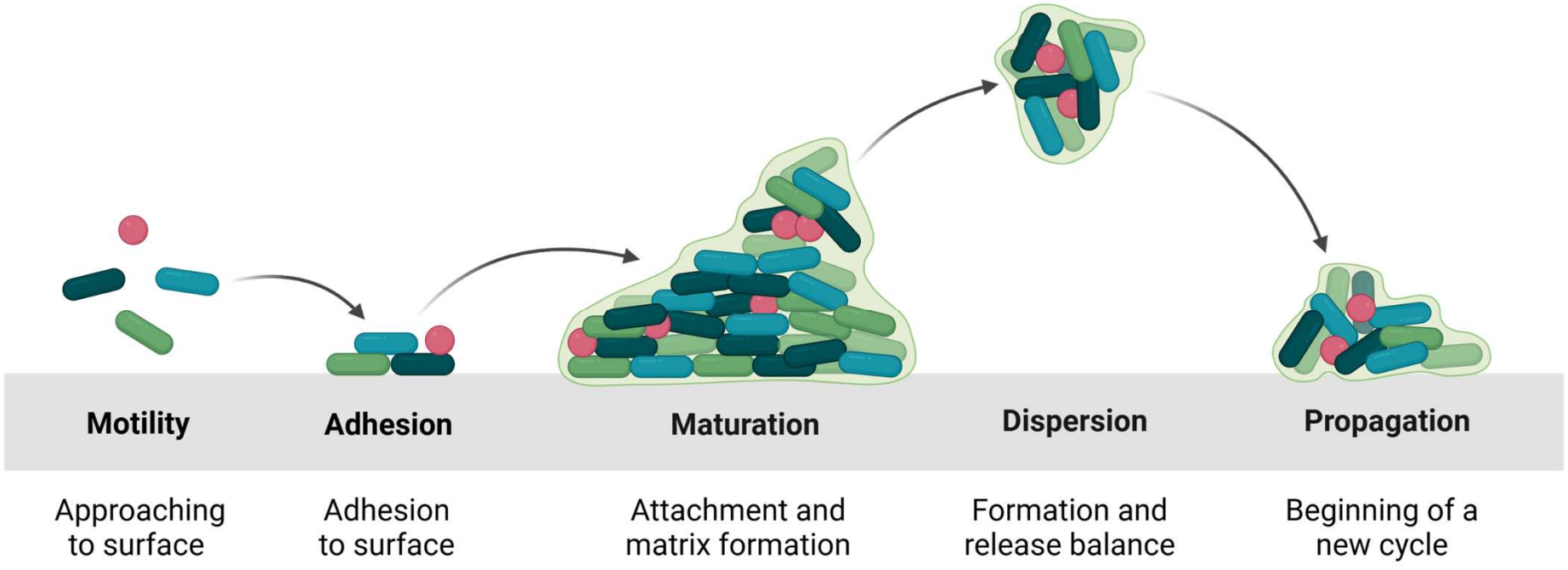


Heat-treated CFCS (pH 4.2) was utilized to determine the stability of the bioactive compounds responsible for the antimicrobial effects at denaturing temperature.

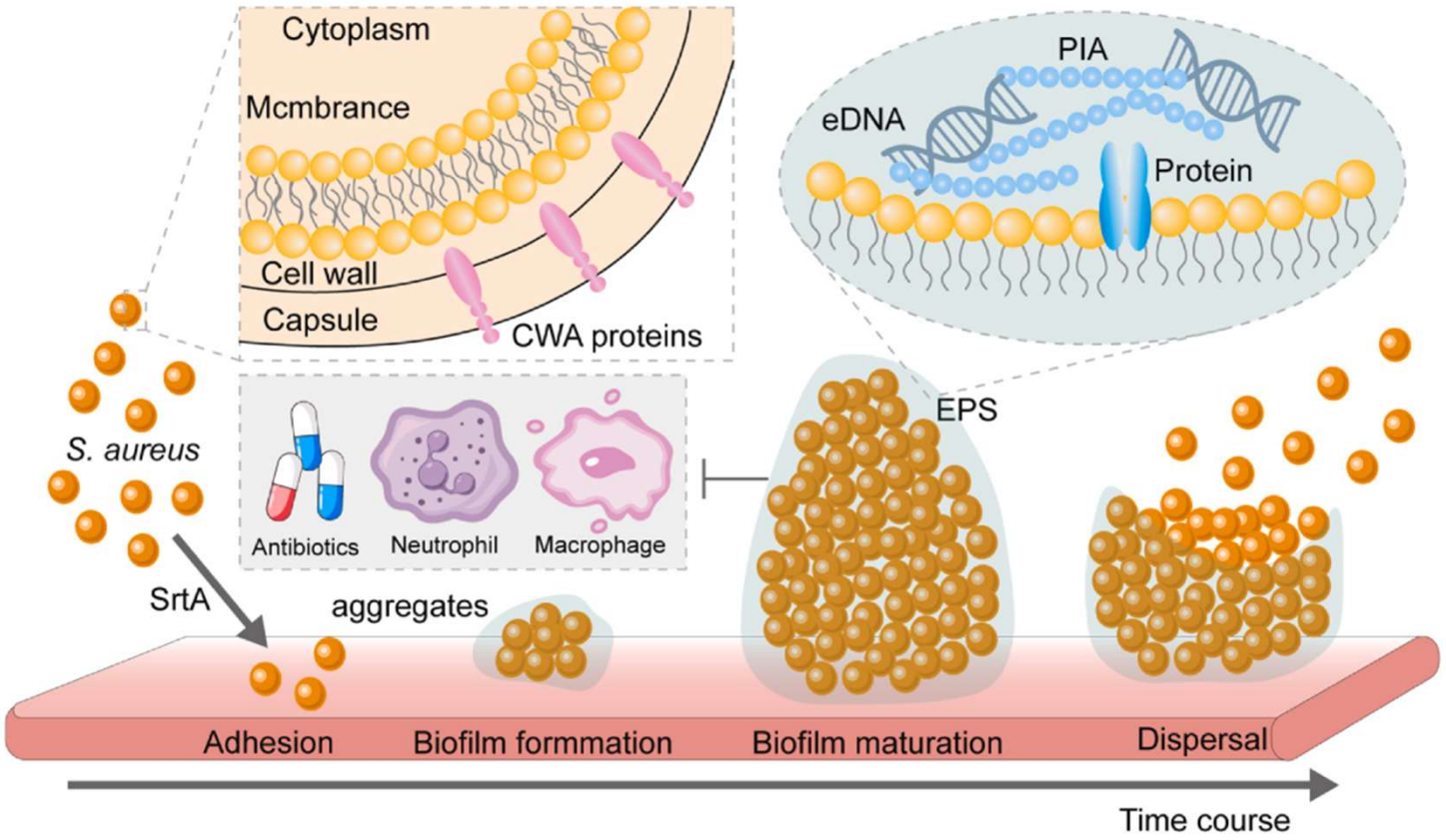
Pathogen biofilms

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**



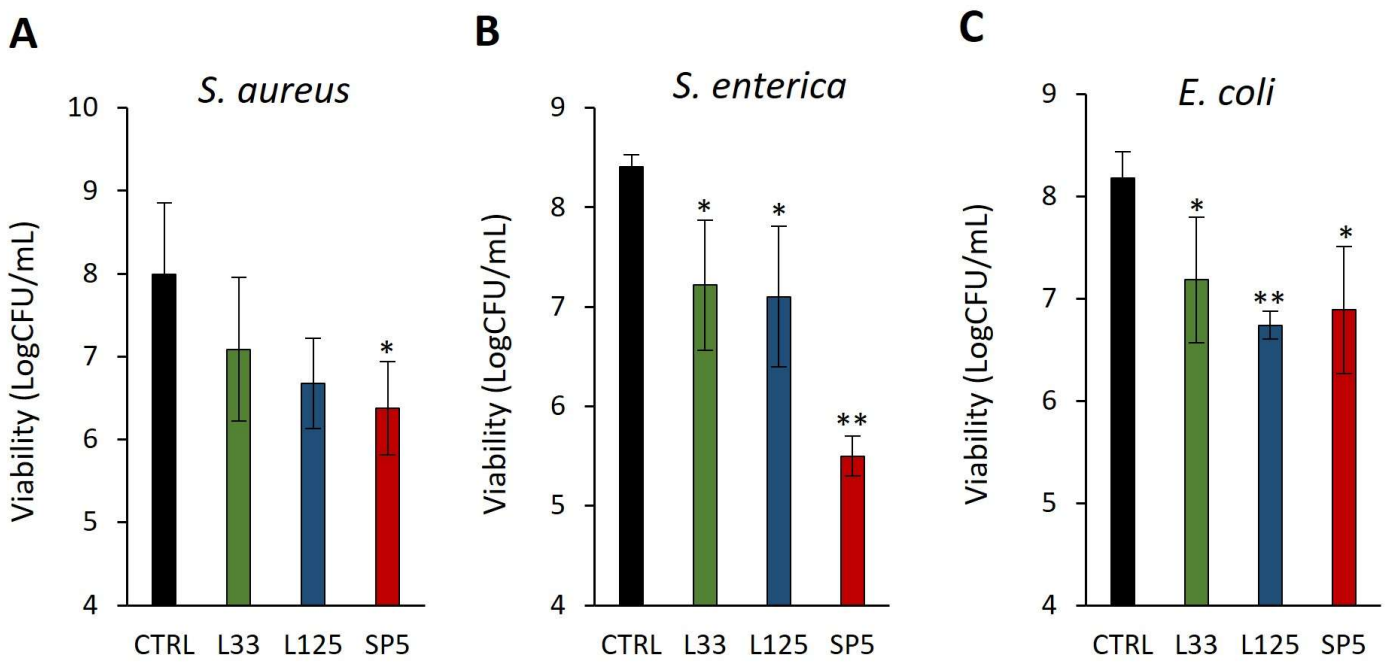
Pathogen biofilms



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.

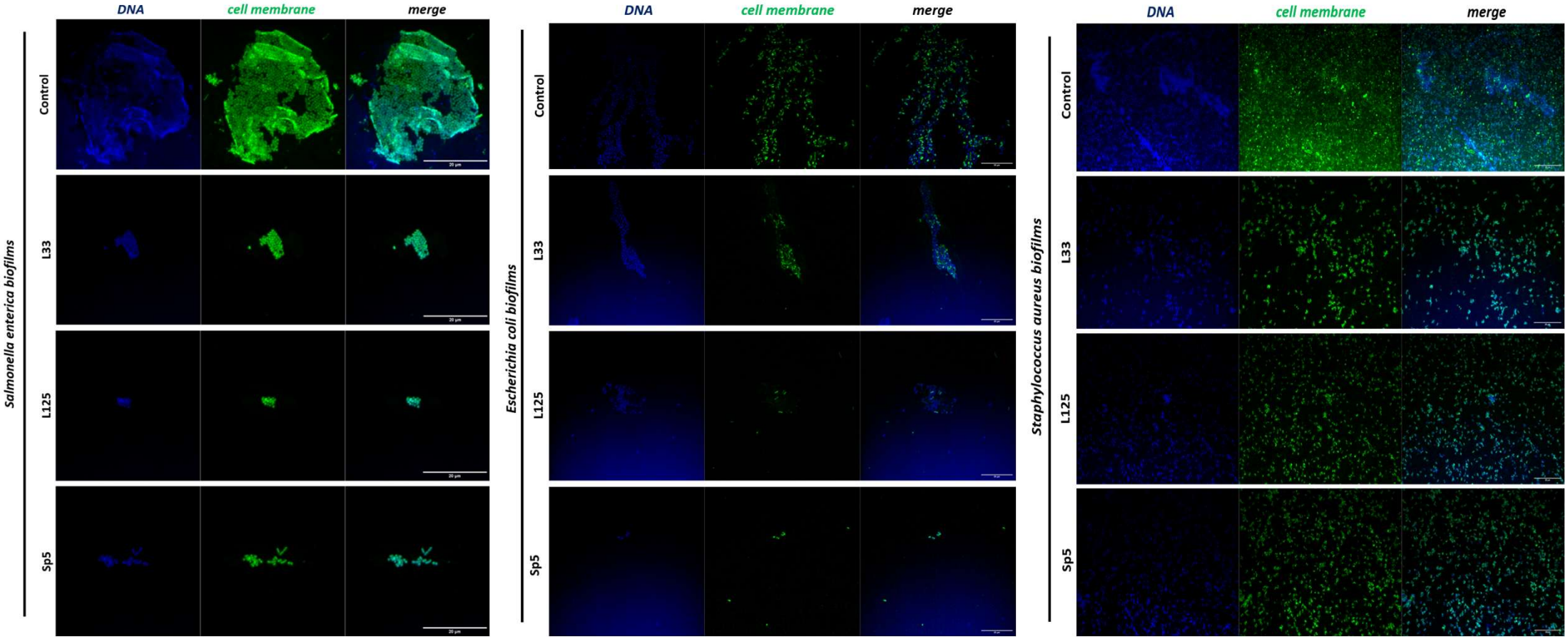
Wang et al., 2025, Animals and Zoonoses, Volume 1, Issue 2, June 2025, Pages 188-202

Antibiofilm activity of CFCS



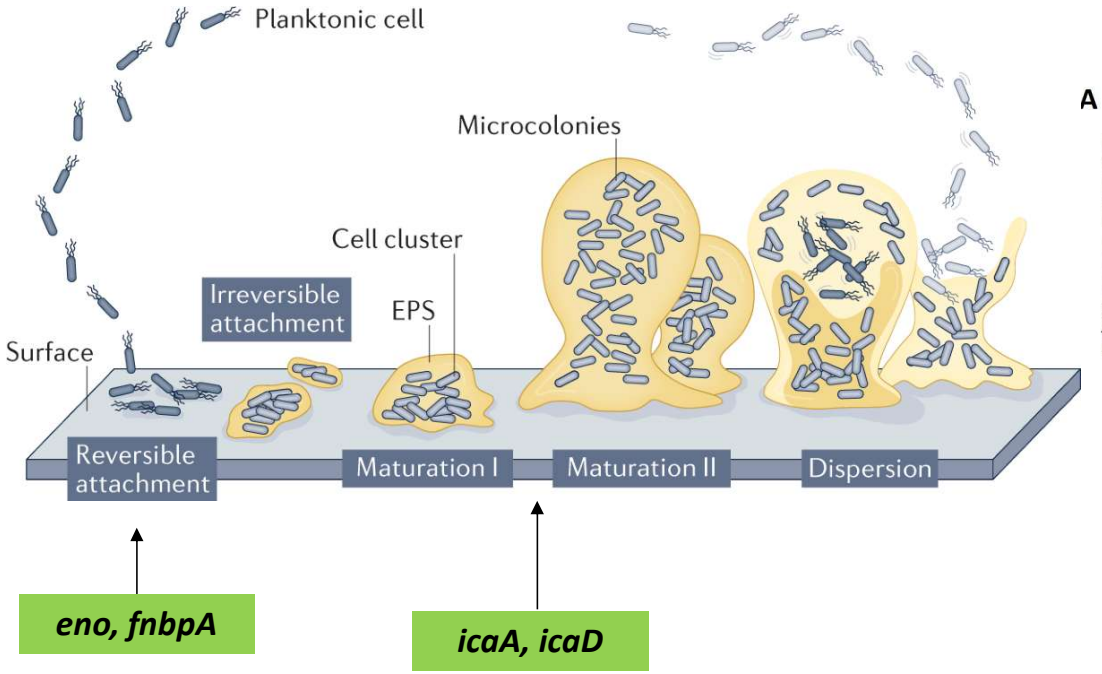
10^8 CFU/mL of fresh O/N cultures of pathogens (100 mL) were co-incubated with 100 mL CFCS in a 96-well plate for 24 h. For the estimation of viable cells, biofilms were washed twice post-treatment with PBS, and were mechanically disrupted. The suspension was serially diluted in 1× Ringer's solution 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.

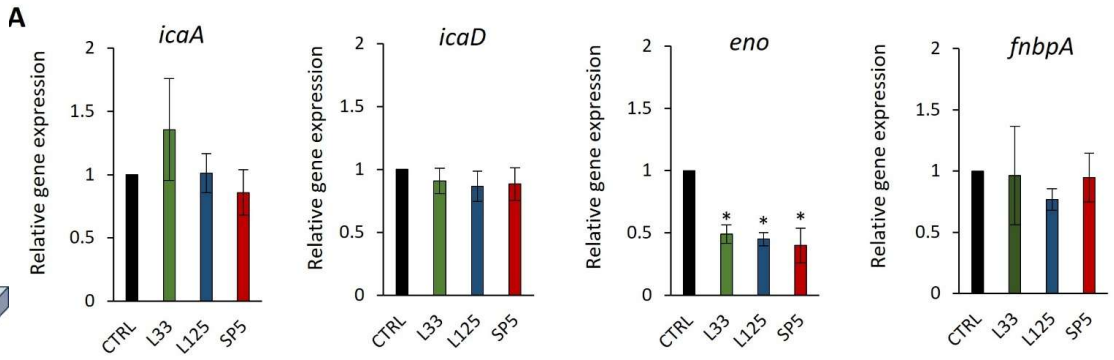
Antibiofilm activity of CFCS



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

Staphylococcus aureus

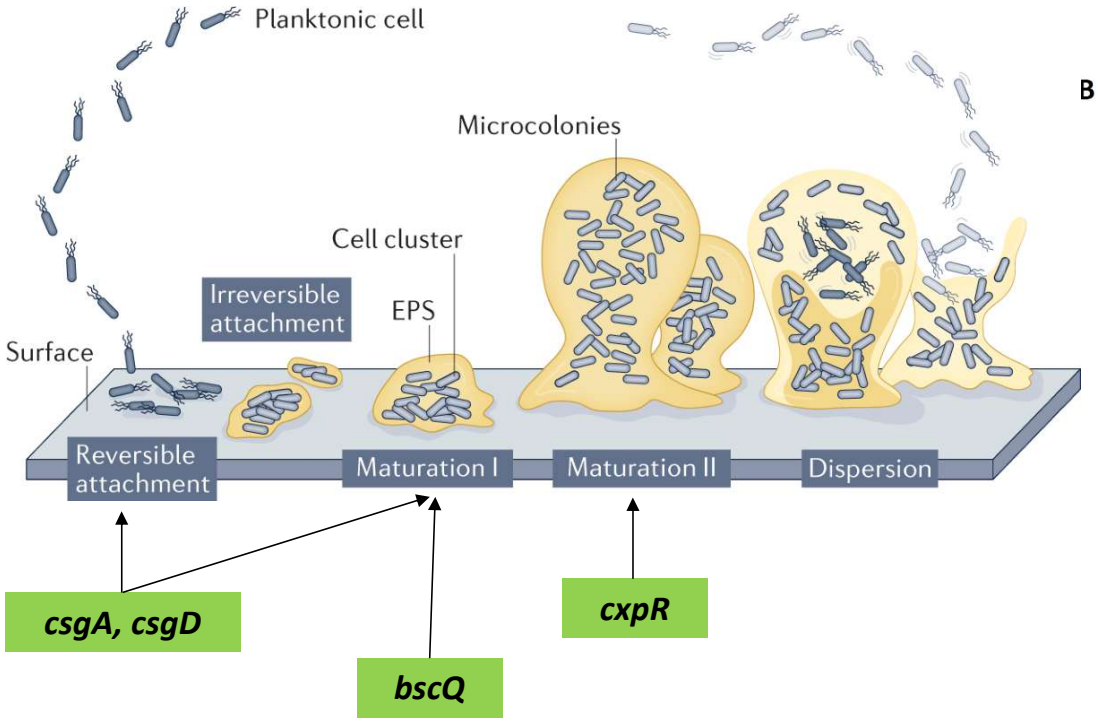


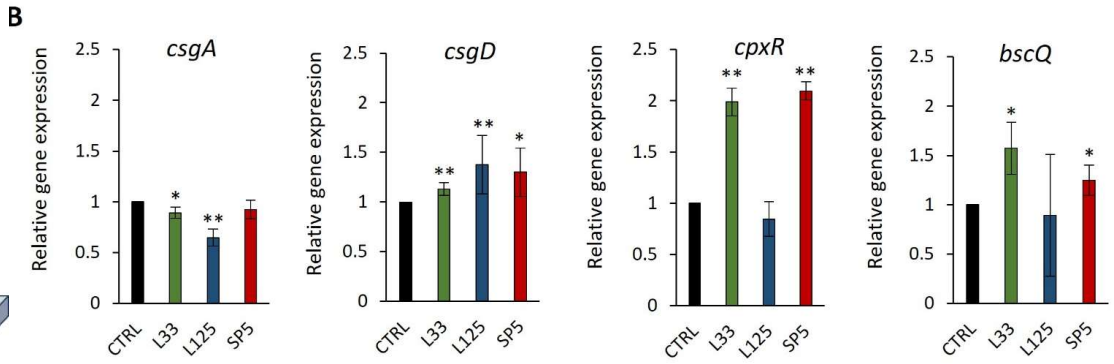


Enolase is an adhesion-related moonlighting protein that mediates attachment on inorganic and organic surfaces, inducing no significant effect on the expression levels of the biofilm formation regulator complex icaA/icaD or the FnbpA adhesin.

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

Salmonella enteritidis

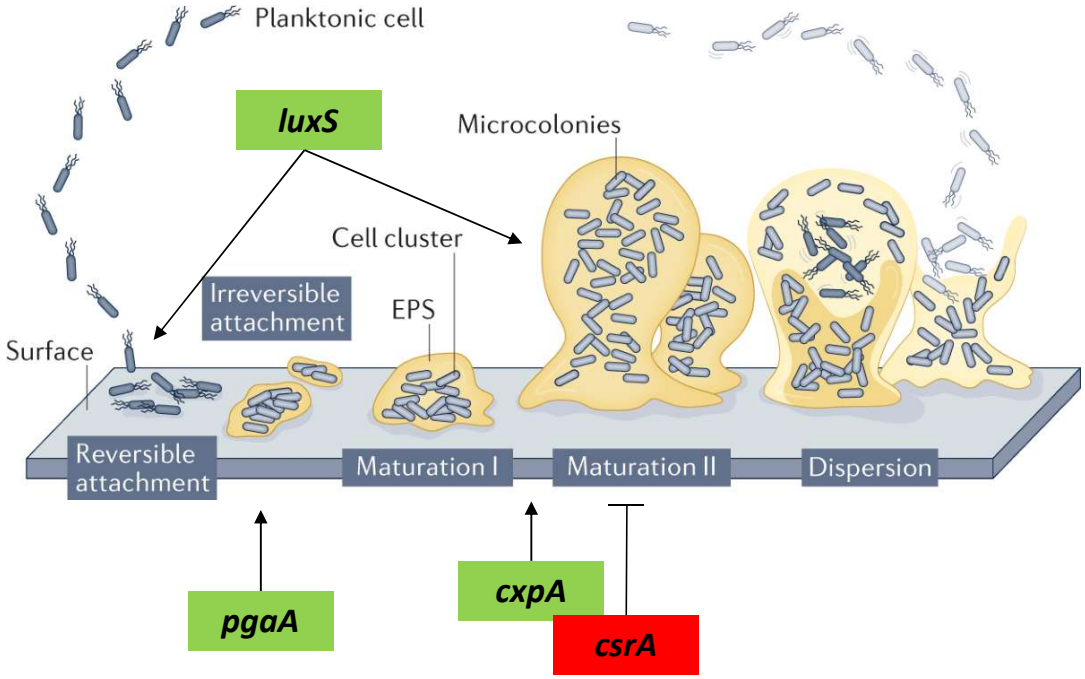


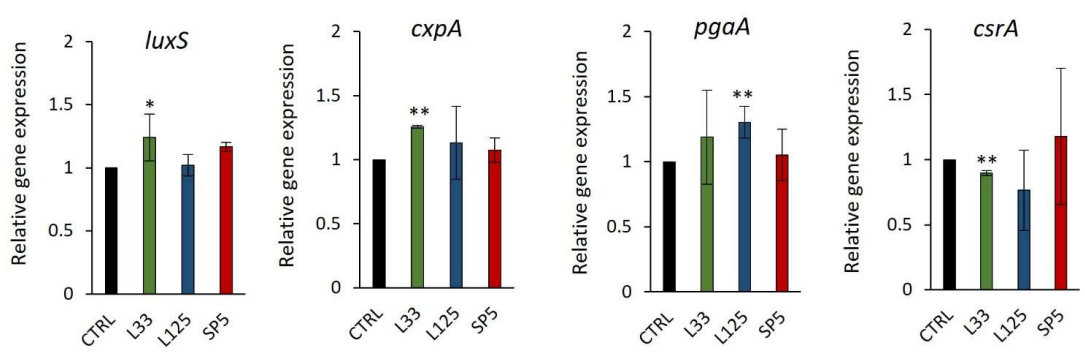


csgA is a gene involved in the production of the major Curli protein subunit. *csgD* and *cpxR* code for transcriptional regulators responsible for adhesion on hydrophobic surfaces and biofilm formation. *BscQ* encodes for cellulose synthase.

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

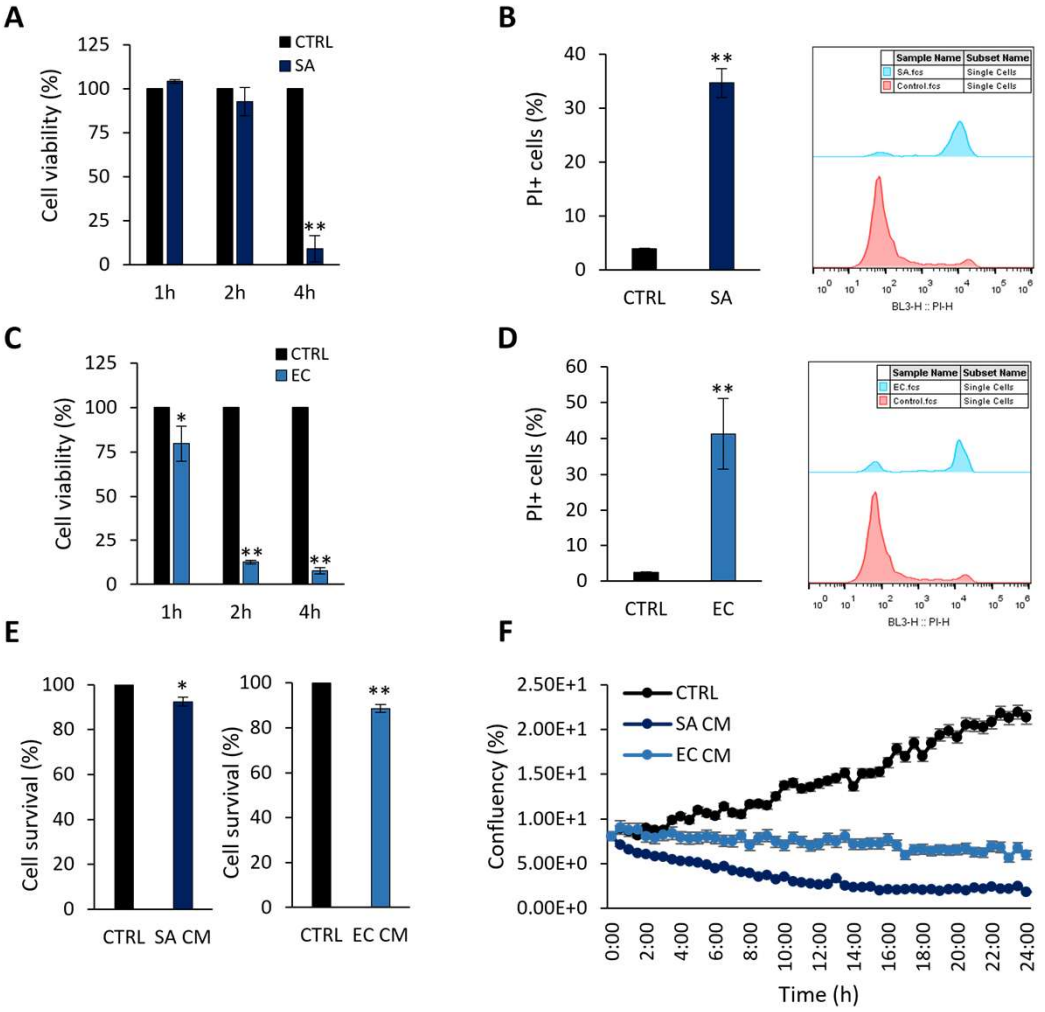
Escherichia coli





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*]

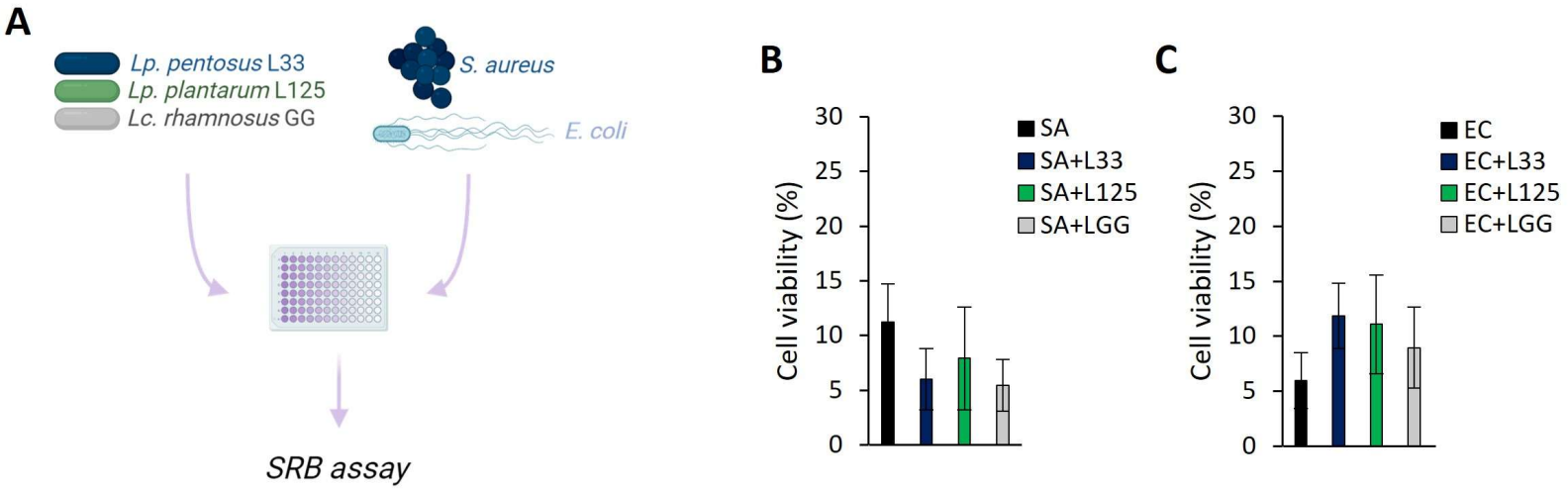
Front. Microbiol., 18 December 2024

Sec. Food Microbiology

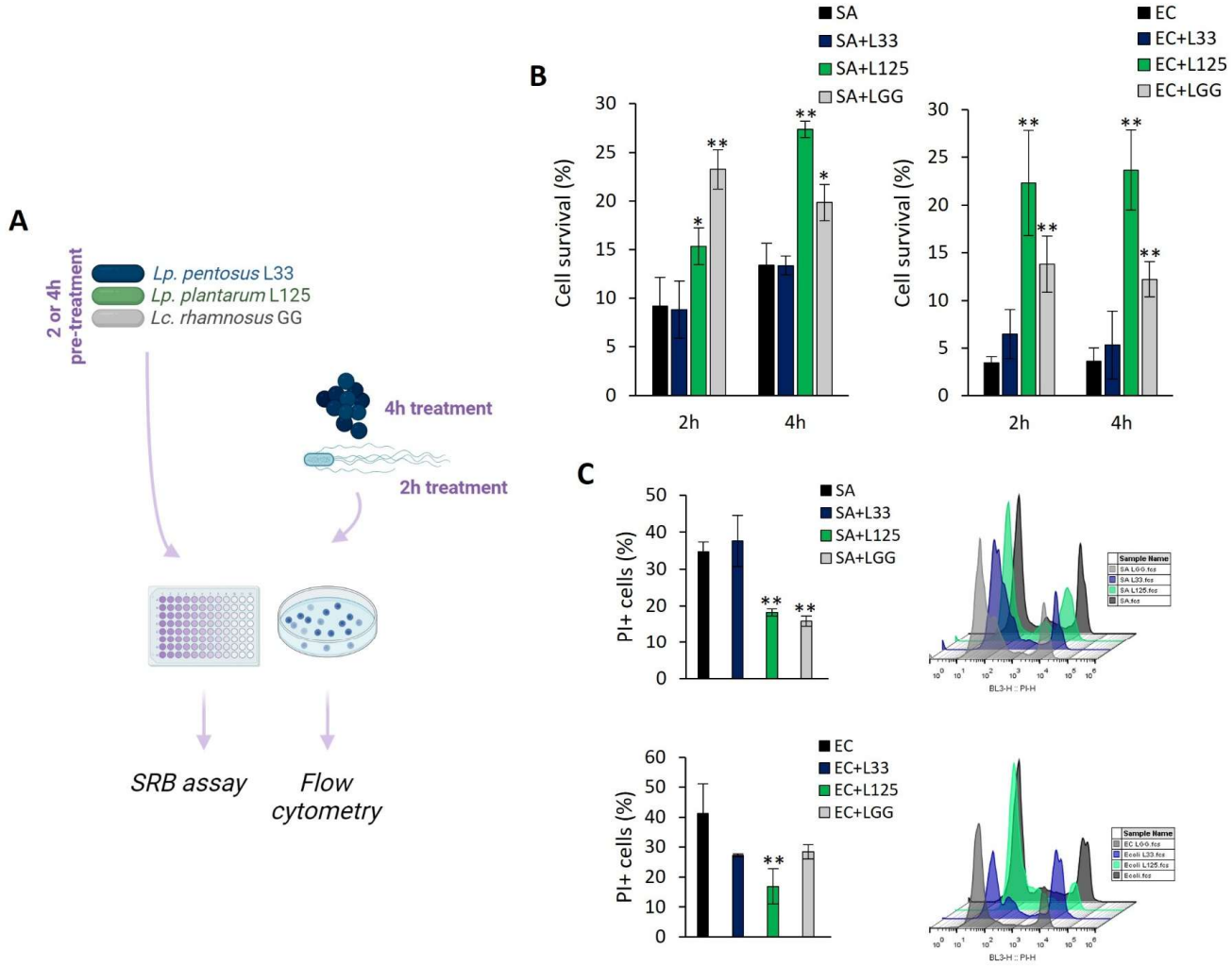
Volume 15 - 2024 |

frontiers

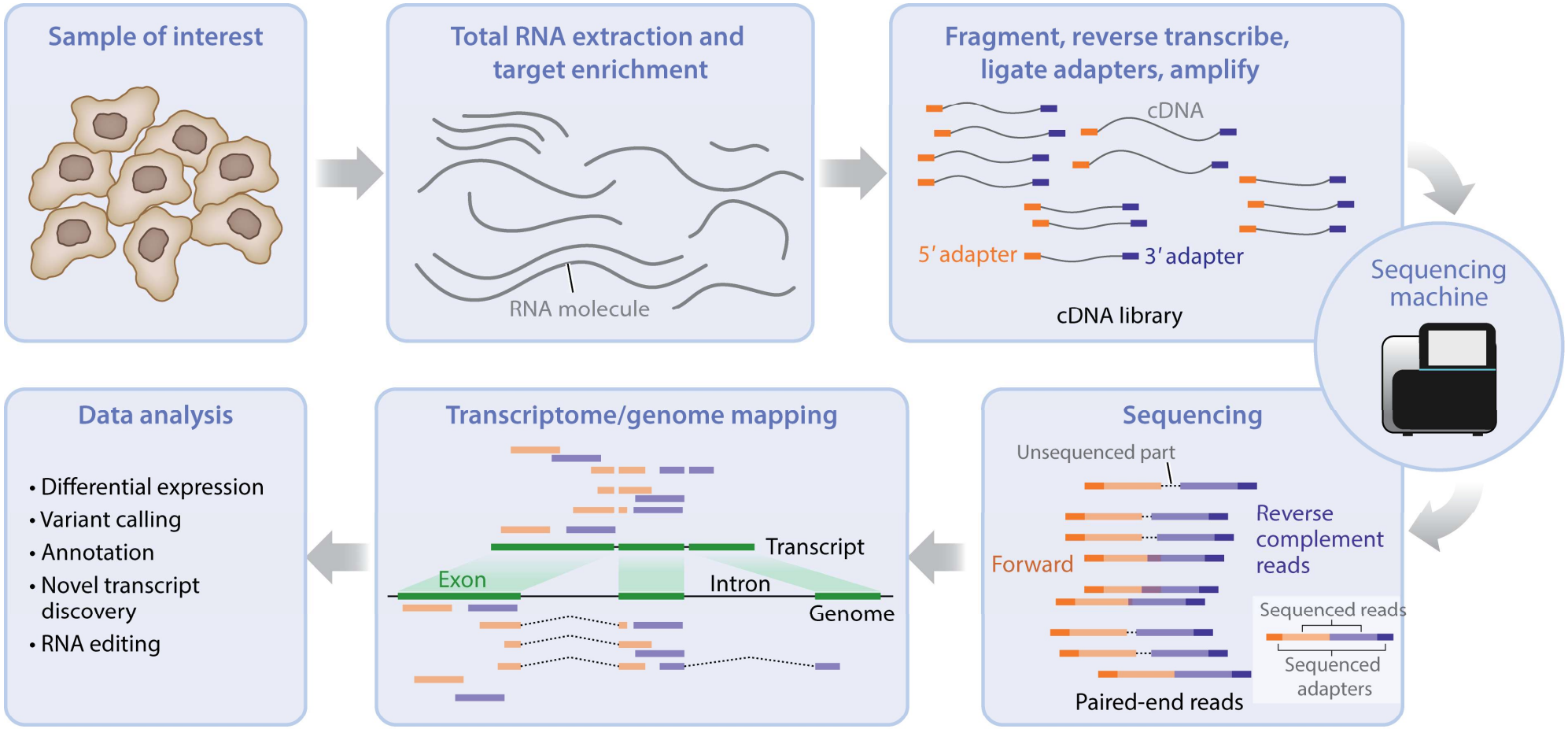
L125 pretreatment protects cells from pathogen-induced cytotoxicity



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

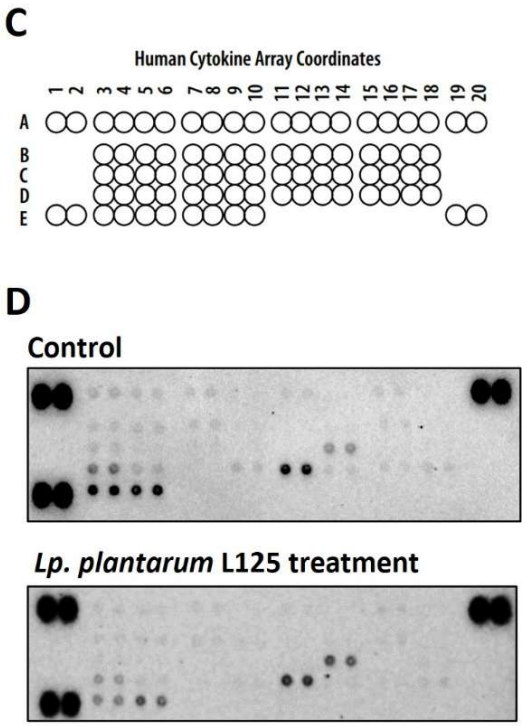
RNA seq was utilized to explore the capacity of L125 to prime antimicrobial responses in the host cell.

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.

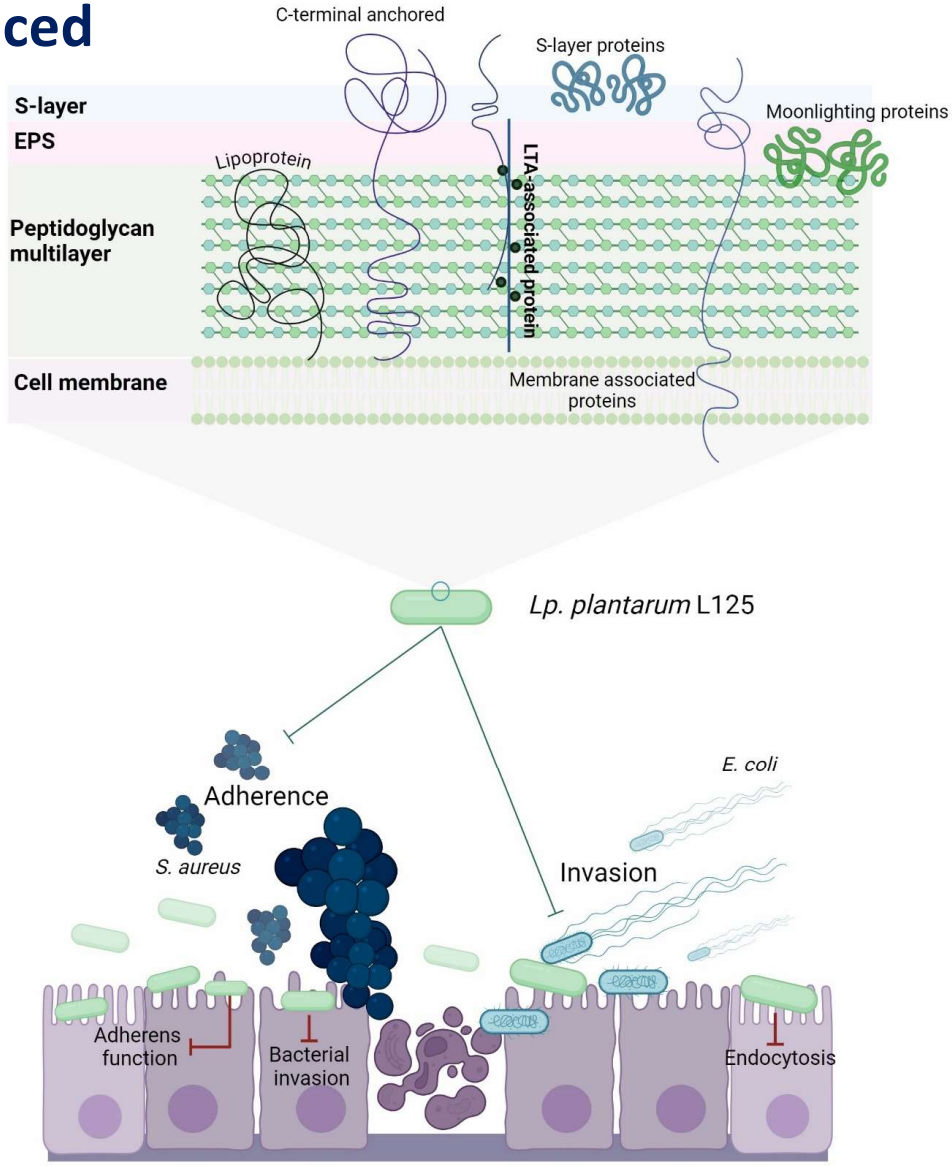


Schematic representation of a putative mechanism by which *L. plantarum* L125 prevents pathogen-induced cell death

L125 cell surface is decorated by C-terminal anchored proteins containing LPxTG, WxL, SH3 or LysM motifs, S-layer proteins carrying the SLH domain, and moonlighting proteins with adhesin function.

Adhesins form covalent bonds with cell wall or membrane components, while moonlighting proteins form reversible interactions based on their charge and hydrophobicity.

L125 pretreatments resulted in the formation of a protective layer on epithelial cells, preventing the invasion of *S. aureus* and *E. coli*. At the same time, L125 downregulated pathways involved in pathogen adhesion and internalization, endocytosis, cell–cell adherence, and actin cytoskeleton formation.



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