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Prebiotics and probiotics for biopackaging: a case study

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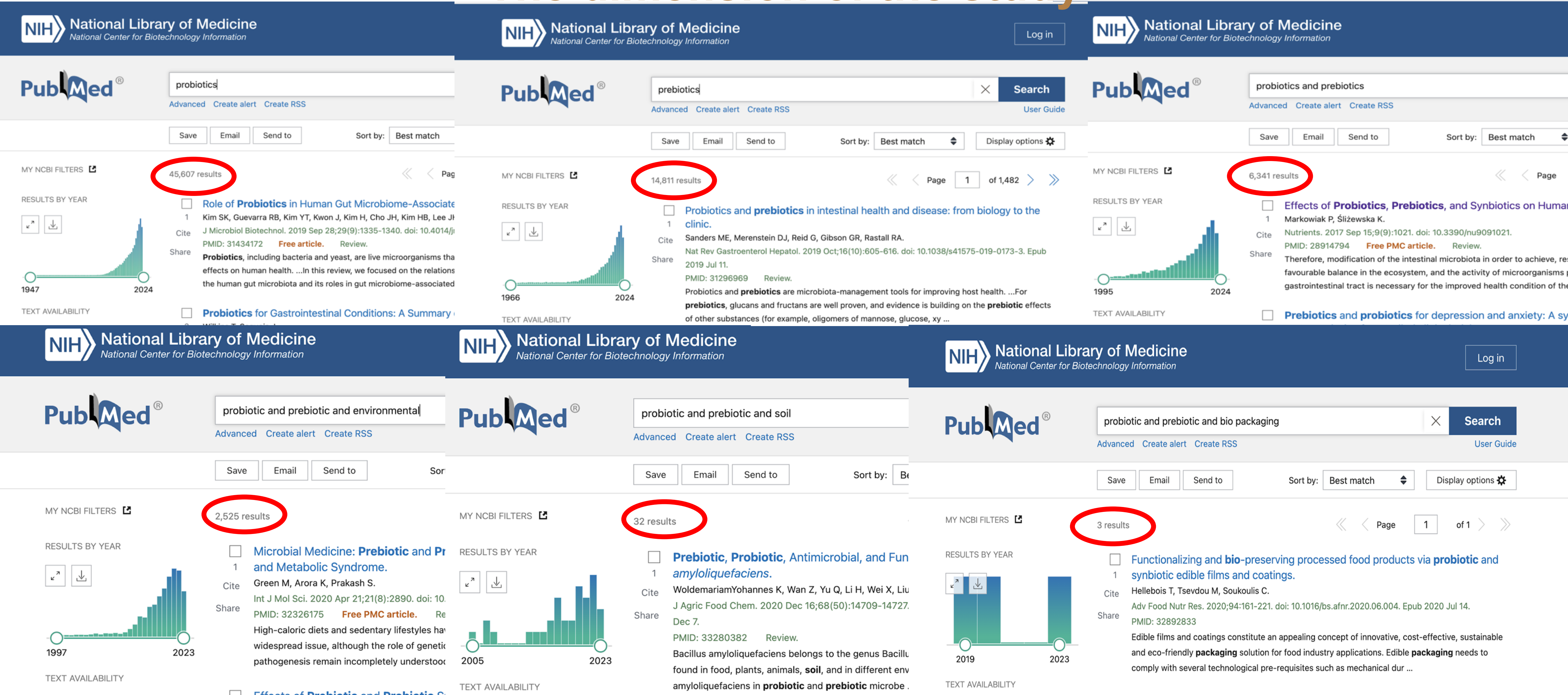
The dimension of the study



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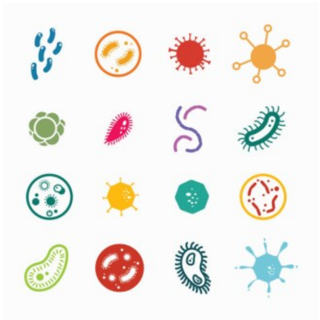


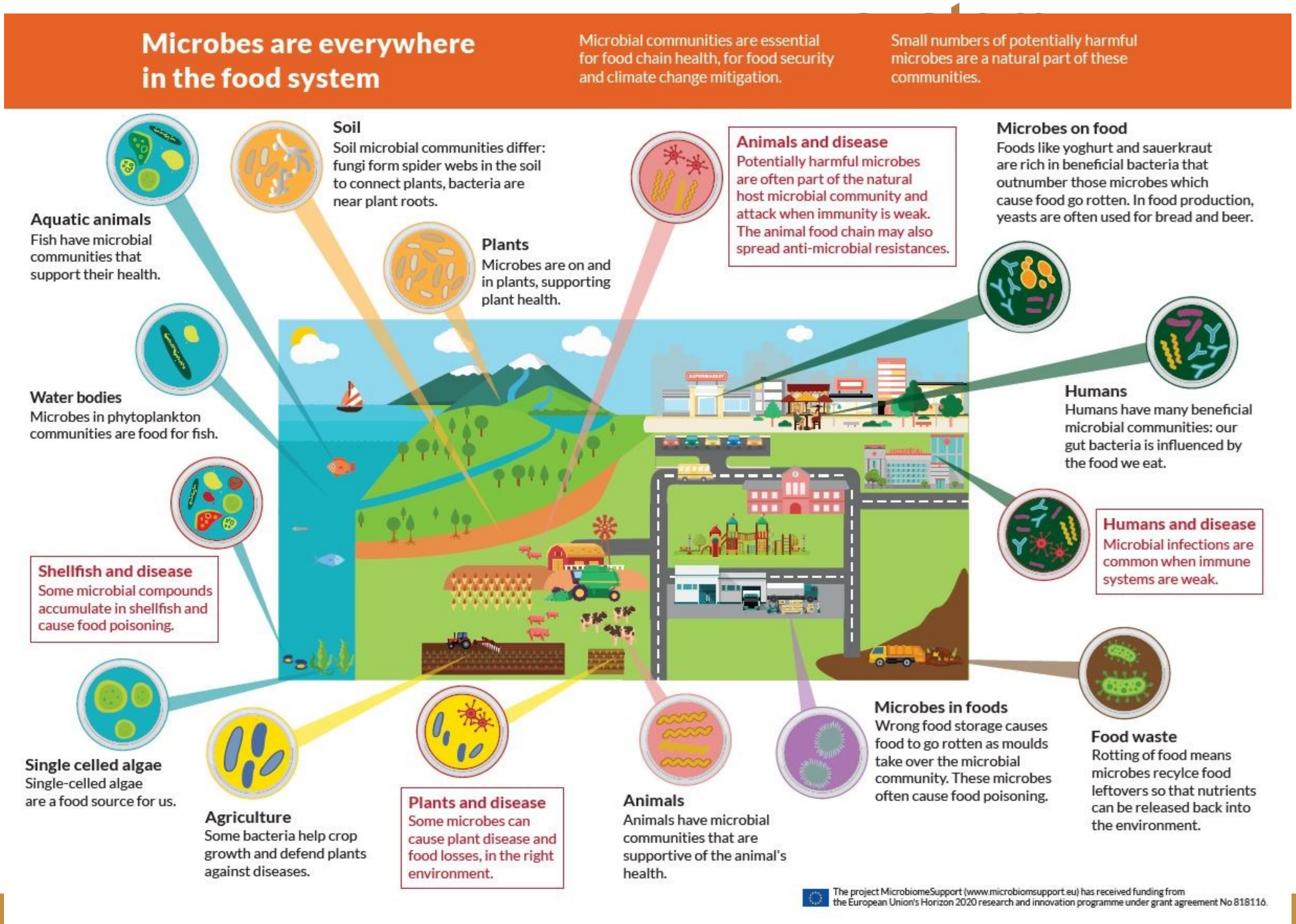
ISAPP Definitions for «Biotic»

ISAPP
International Scientific Association for PROBIOTICS and PREBIOTICS

ADVANCING SCIENTIFIC EXCELLENCE
in probiotics, prebiotics, synbiotics, postbiotics and fermented foods

1. Probiotic: «Live microrganisms which, when administered in adequate amounts, confer health benefit on the host”
2. Prebiotic: «A substrate that is selectively utilized by host microrganisms conferring a health benefit»
3. Synbiotic: «a mixture comprising live microrganisms and substrate(s) selectively utilized host microorganisms that confers a health benefit on the host»
4. Postbiotic: «Preparation of inanimate microrganisms and/or their components that confers a health benefit on the host»

The spread of microorganisms in the food system



The main challenges

Producing more and at the same time improving agricultural practices to reduce environmental impact, efficiently using scarce natural resources

Microbiomes found everywhere throughout the entire food system.

taken from : Microbiome Support Action https://www.microbiomesupport.eu/wp-content/uploads/2020/06/A4-infographic.jpg).

MICROORGANISMS and their NATURAL ENVIRONMENT

In nature, cells live in association with other cells

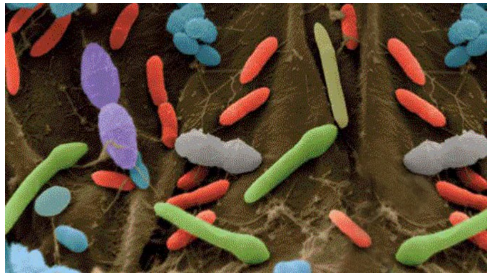
↓

POPULATIONS

NUMEROUS ORGANISMS OF THE SAME SPECIES

Composed of groups of cells that derive from successive cell divisions starting from a single parental cell

A habitat is defined as the place where a microbial population lives



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MICROBIAL CONSORTIUM

Several populations that occupy the same habitat and are metabolically related

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MICROBIAL COMMUNITIES

They are made up of different microbial populations (consortia) that interact with each other in a given environment.

The components and the number of cells that make up a microbial community depend on the resources and conditions present in that particular habitat

ECOSYSTEM

is made up of living organisms together with the chemical and physical constituents of the environment of which they are part

Over time, an ECOSYSTEM gradually changes, both CHEMICALLY and PHYSICALLY through microbial transformations of nutrients

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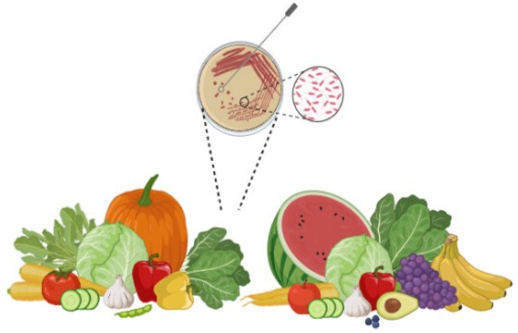
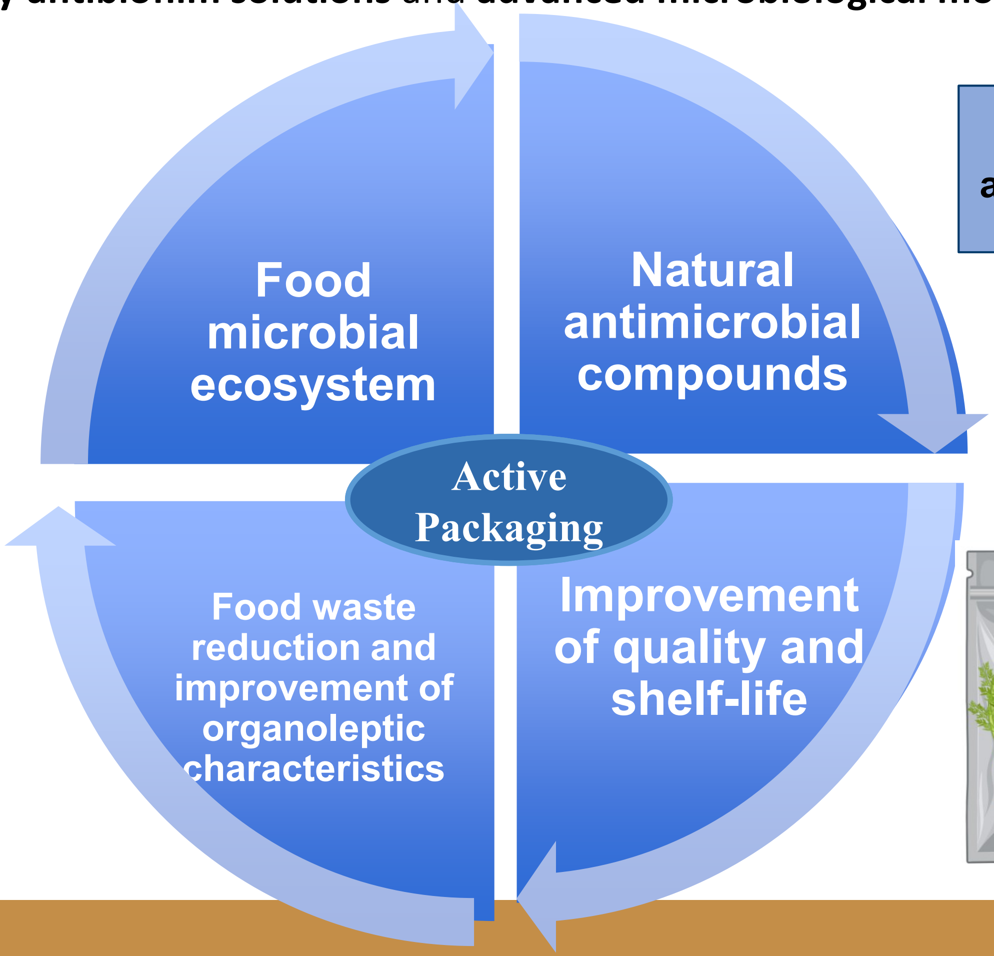
Food microbial ecosystem

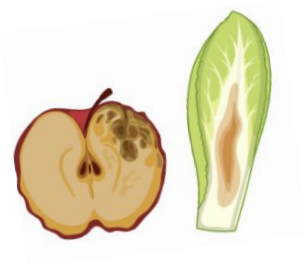
The increasing focus on **packaged and ready-to-eat products** has enhanced the risks associated with foodborne illness, demanding the development of **innovative** and **eco-friendly antibiofilm solutions** and **advanced microbiological monitoring systems**



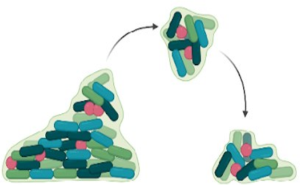


Essential oils and other natural compounds

- Quality
- Safety
- Sustainability
- Consumer approval



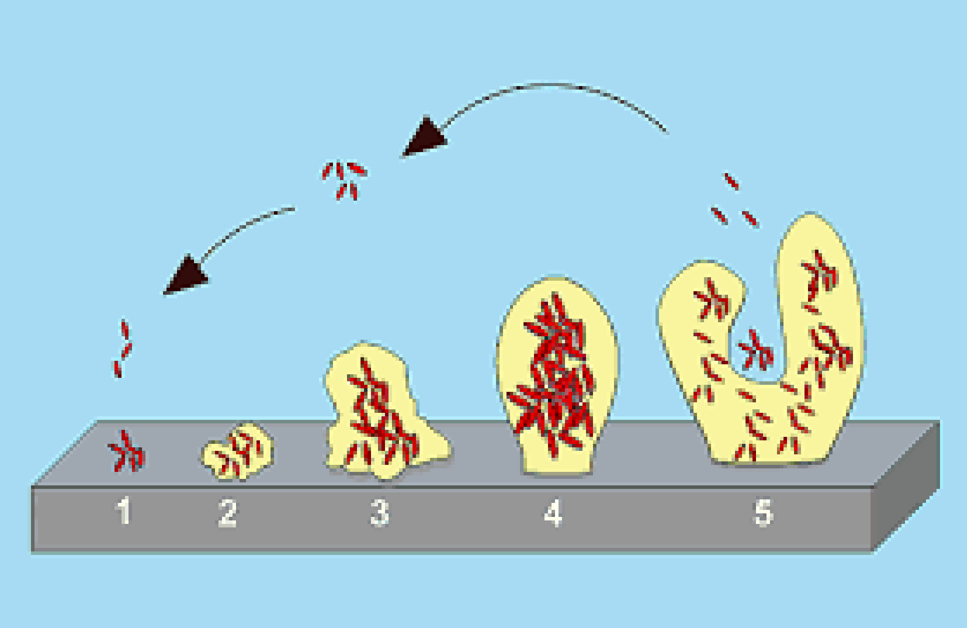
Biofilm



Microbial Biofilm

A biofilm is an aggregate of associated microbial cells to a surface and embedded in a polymer matrix extracellular produced by them.

Once a biofilm has formed and the exopolysaccharide matrix is was secreted by the sessile cells, the resulting structure is highly viscoelastic with characteristics of rubbery material



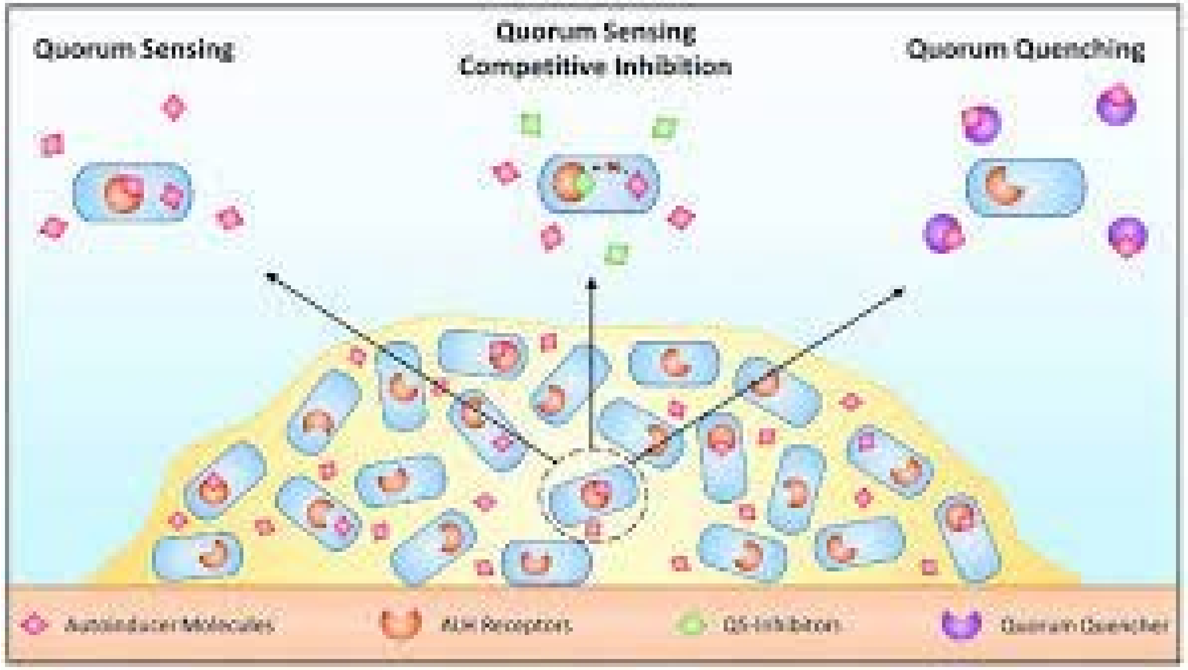
Caracteristic of the biofilm

Microbial biofilms exhibit:

- **a distinct phenotype**
- **a gene transcription**
- **a growth rate different from microorganisms in planktonic form.**

Biofilms develop specific mechanisms:

- **for initial adhesion to the surface,**
- **for development in structured communities, therefore small ecosystems microbial,**
- **and for detachment from the substrate.**

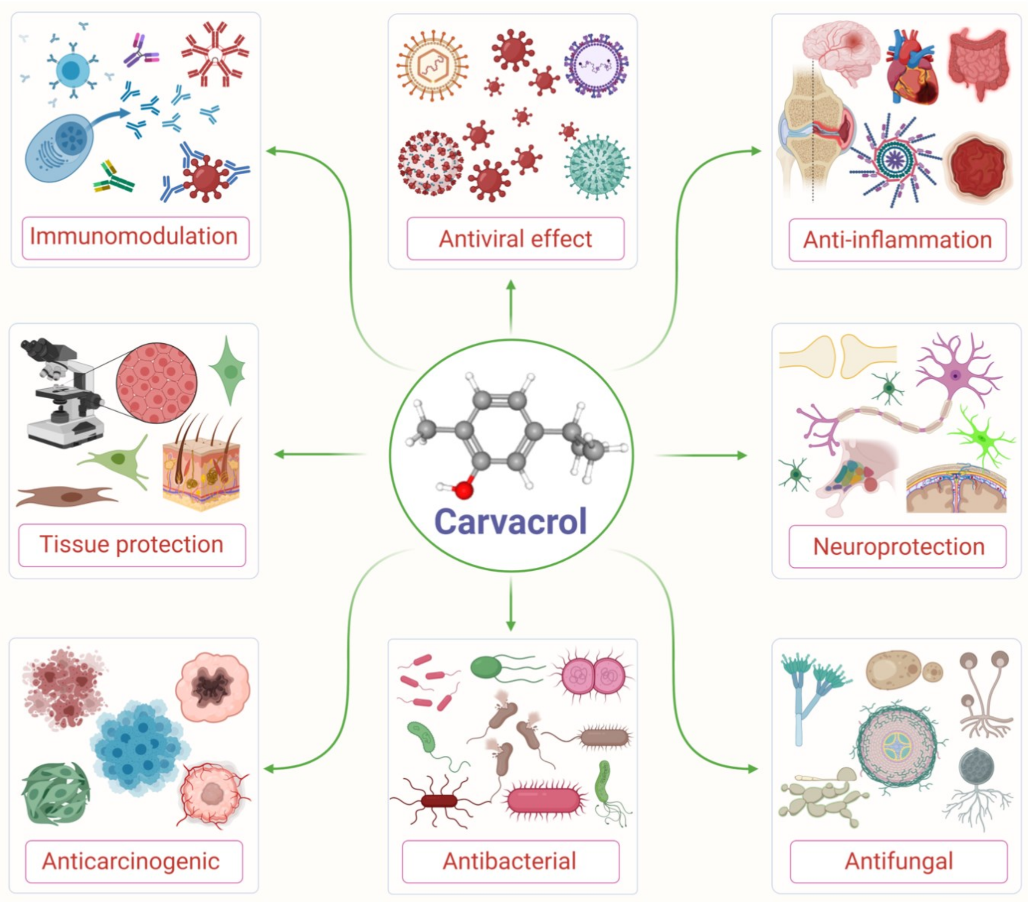


Why Carvacrol?

Carvacrol, or cymophenol,
$C_6H_3(CH_3)(OH)C_3H_7$
is a monoterpenoid phenol.

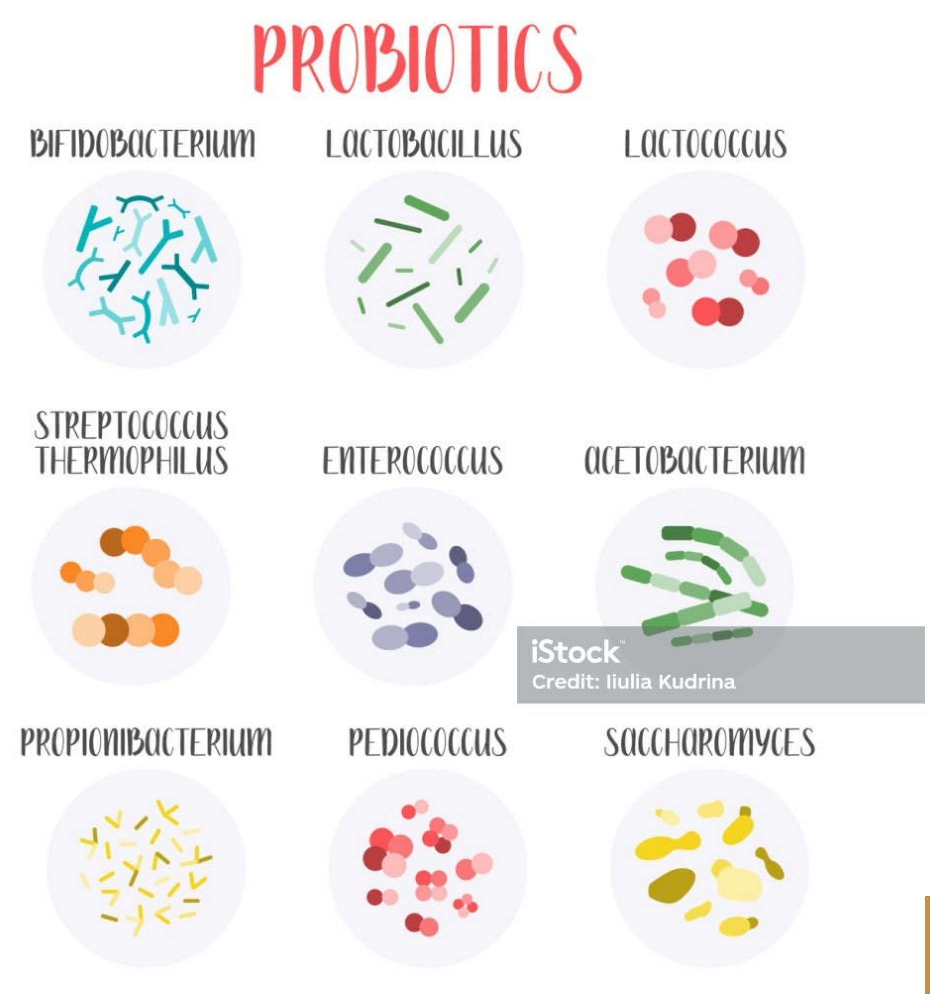
Carvacrol has the capacity to selectively target biofilm-signaling pathways that regulate quorum sensing (QS), extracellular polymeric substance (EPS) synthesis, biofilm-related gene expression, microbial motility, adhesion, and dispersion.

Despite the potential, wider adoption in the food industry is limited due to the characteristics (e.g. volatility and strong aromas) of EOs and their components, which can be addressed through advanced techniques (e.g. encapsulation, spray drying, and solid lipid nanoparticles)



Javed et al. 2021 https://doi.org/10.3389/fpls.2020.601335

MICROORGANISMS INVOLVED IN THE PRODUCTION OF BIOACTIVE COMPOUNDS:
lactic acid bacteria and their multifunctional role in foods



LACTIC ACID BACTERIA

Food fermentations
STARTER CULTURES
Ensure the technological result of the production process

Biological conservation
PROTECTIVE CULTURES
Control of undesirable microorganisms

Probiotic
Beneficial health effects

Alteration
Unwanted changes in food products

Diseases
Some strains are potential opportunistic pathogens in some clinical infections

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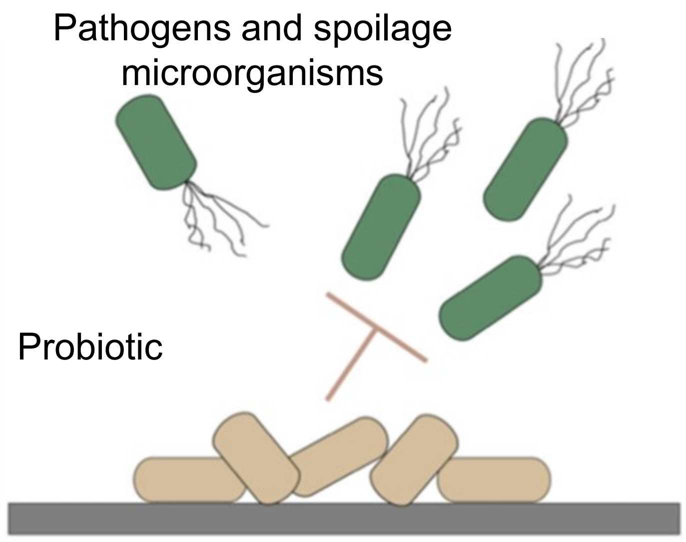
Why Lactic Acid Bacteria?

A feasible approach to reducing this natural compound concentration could be the combination with other natural solutions. Lactic acid bacteria (LAB) represent one of the promising possibilities for the natural control of biofilms, composed of high antimicrobial resistance and associated risks for foodborne disease spread

Organic acids synthesis, such as lactic and acetic acids, is the main responsible for their antagonistic activity against pathogens, by acidifying intracellular pH, and generating an unfavourable local microenvironment for pathogenic bacteria



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A CASE STUDY: *Lactiplantibacillus plantarum* monolayer enhanced bactericidal action of carvacrol: biofilm inhibition of viable foodborne pathogens and spoilage microorganisms

This study aims to investigate the *in vitro* efficacy of combined effect of **carvacrol** and a **pre-formed biofilm monolayer** of the **probiotic** *Lactiplantibacillus plantarum* DSM 20174 on both planktonic and sessile cells of **food pathogenic** and **spoilage strains** using culture-based and flow-cytometry approaches.

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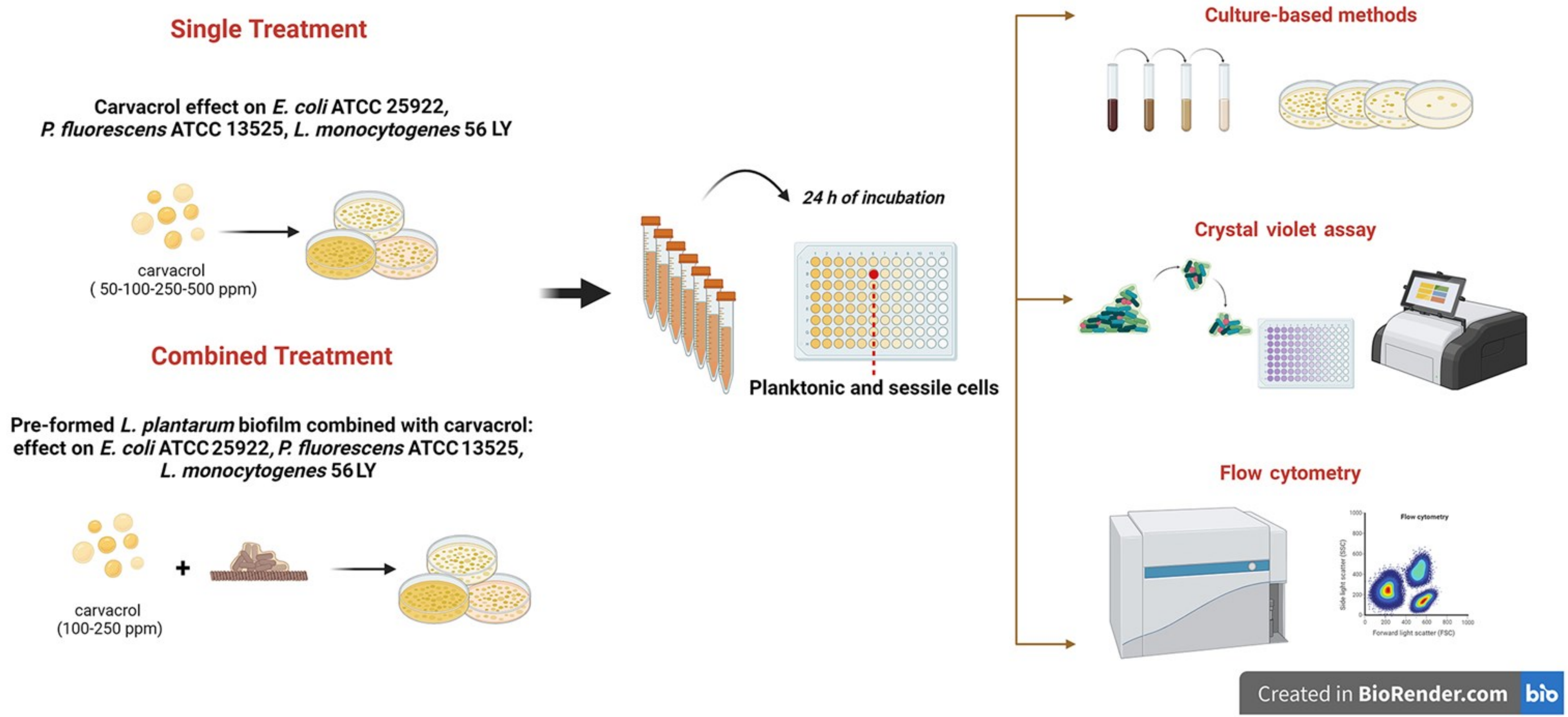
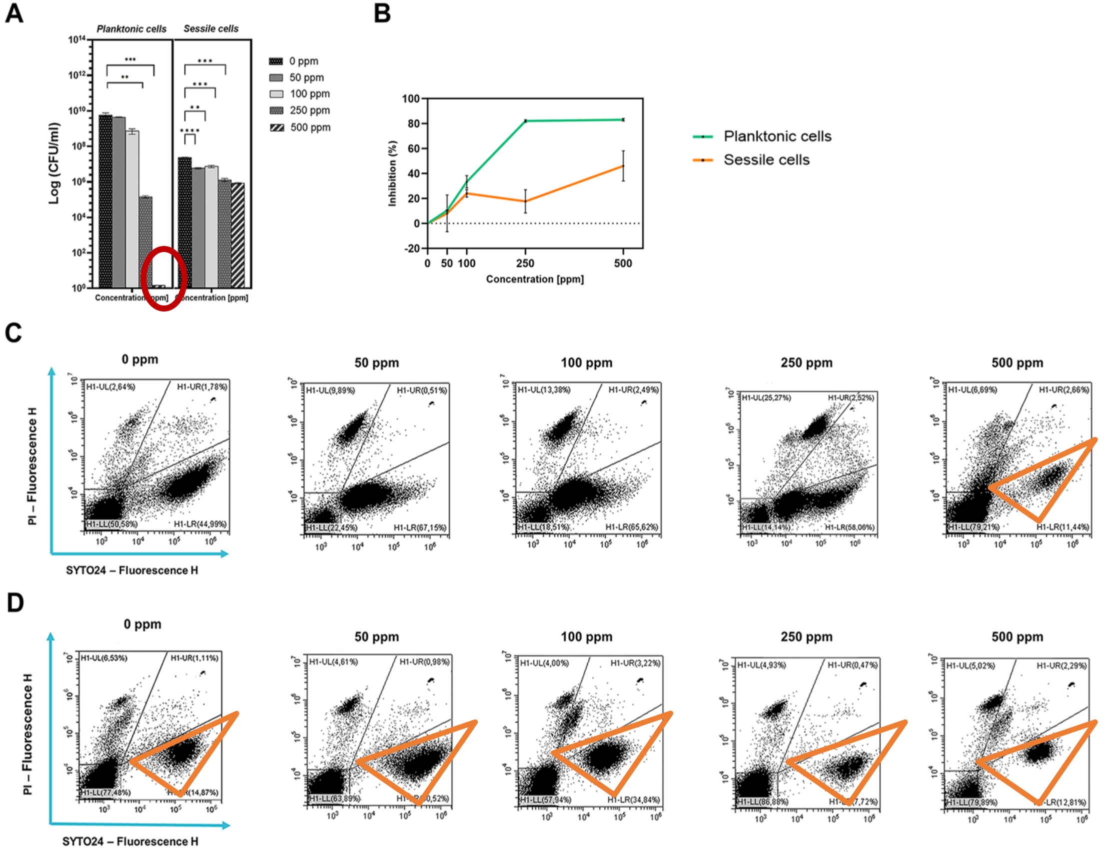
Experimental Set-up



Carvacrol effect on L. plantarum DSM 20174 probiotic strain

Planktonic cells. The highest carvacrol concentration tested (500 ppm) promoted a total loss of cultivability. Nevertheless, the FCM results showed that after carvacrol 500 ppm treatment, **42% of the cell population was still viable** despite with no further ability to replicate on culture media, suggesting cells transition into the **VBNC state** treatment-induced.

Sessile cells. *L. p.* sessile fraction was less sensitive to carvacrol antimicrobial treatment, even at higher concentrations.



Poscente et al. 2023, Frontiers in Microbiology - Food Microbiology

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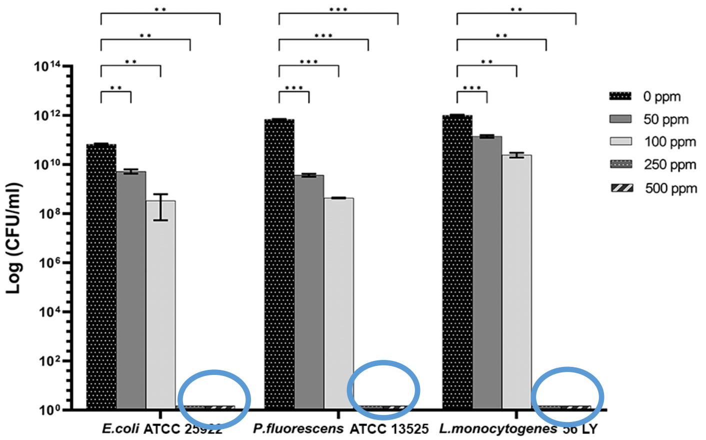
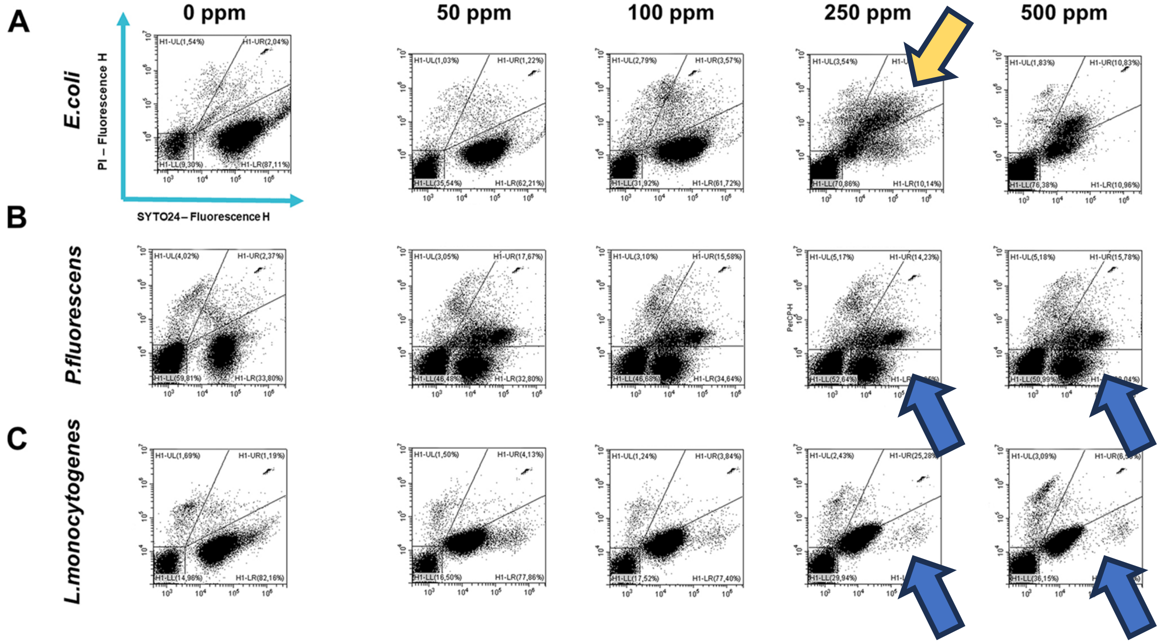
Carvacrol effect on *E. coli, P. fluorescens, and L. monocytogenes* planktonic cells



- Carvacrol treatment had a significant antimicrobial action on *E.c., P.f.,* and *L.m.* planktonic cells for all tested concentrations ($p < 0.05$), compared to the control
- 250 ppm and 500 ppm concentrations promoted a total loss of culturability in each strain.

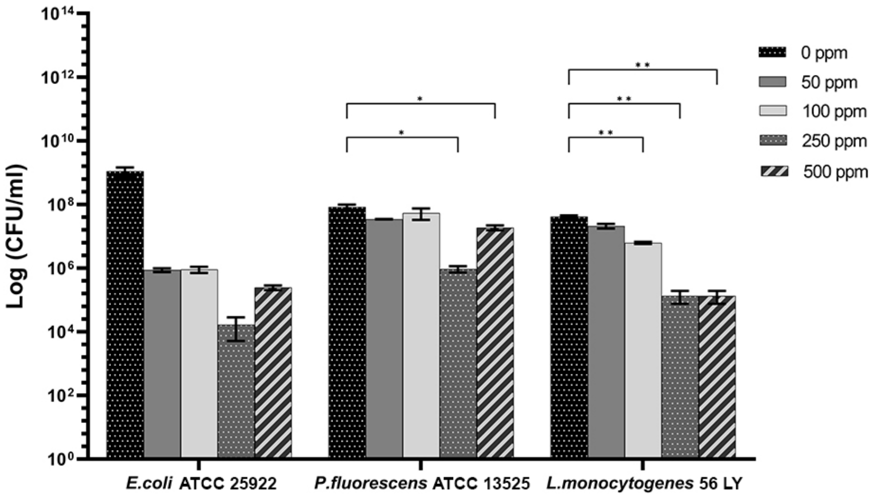


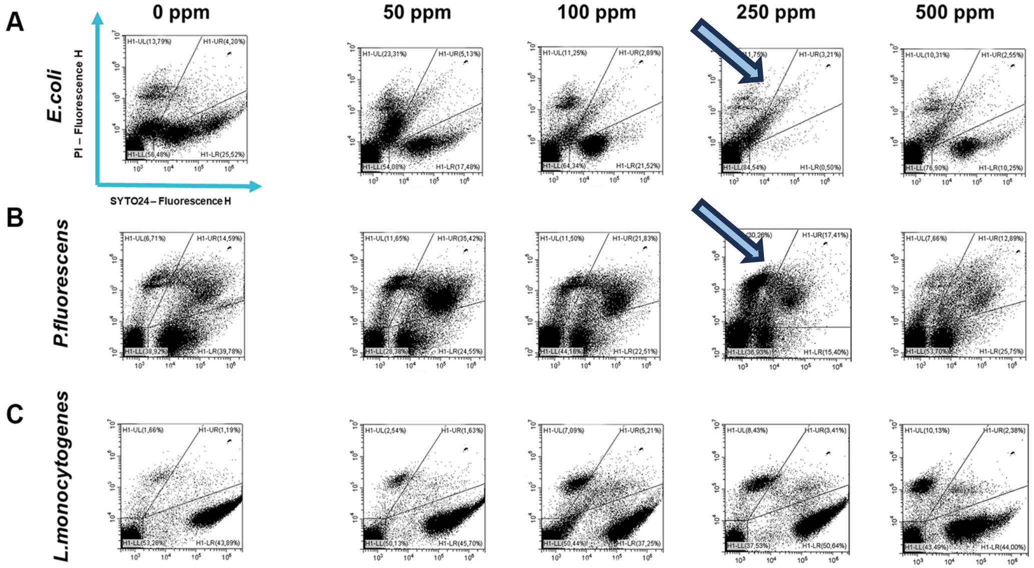
E. coli planktonic cells: proportional damage degree up to 250 ppm carvacrol, with non-culturable but still viable state (VBNC) at 250 and 500 ppm.

(P.f.) and (L.m.) showed relatively stable percentages of viable cells across different carvacrol concentrations.

Poscente et al. 2023, Frontiers in Microbiology - Food Microbiology

Carvacrol effect on *E. coli, P. fluorescens, and L. monocytogenes* biofilm cells





The 250 ppm carvacrol concentration demonstrated higher efficacy in inhibiting biofilm-forming adherent cells, while the increase of concentration (500 ppm) did not enhance the antimicrobial effect, significantly.

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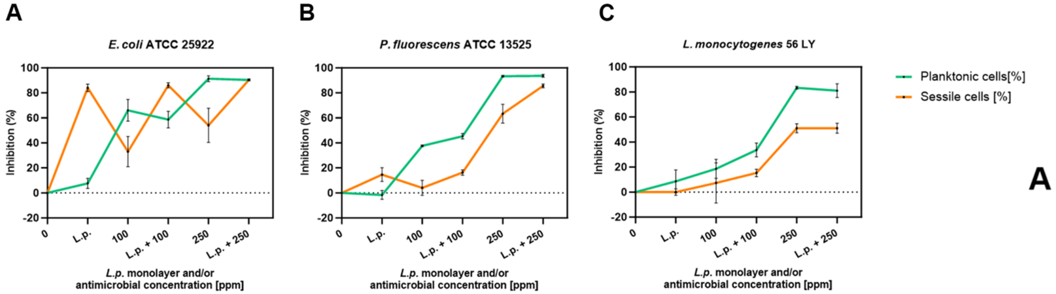
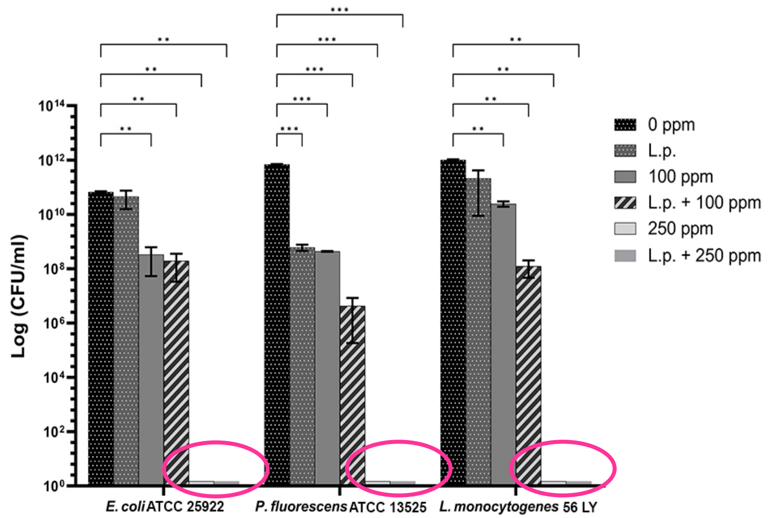
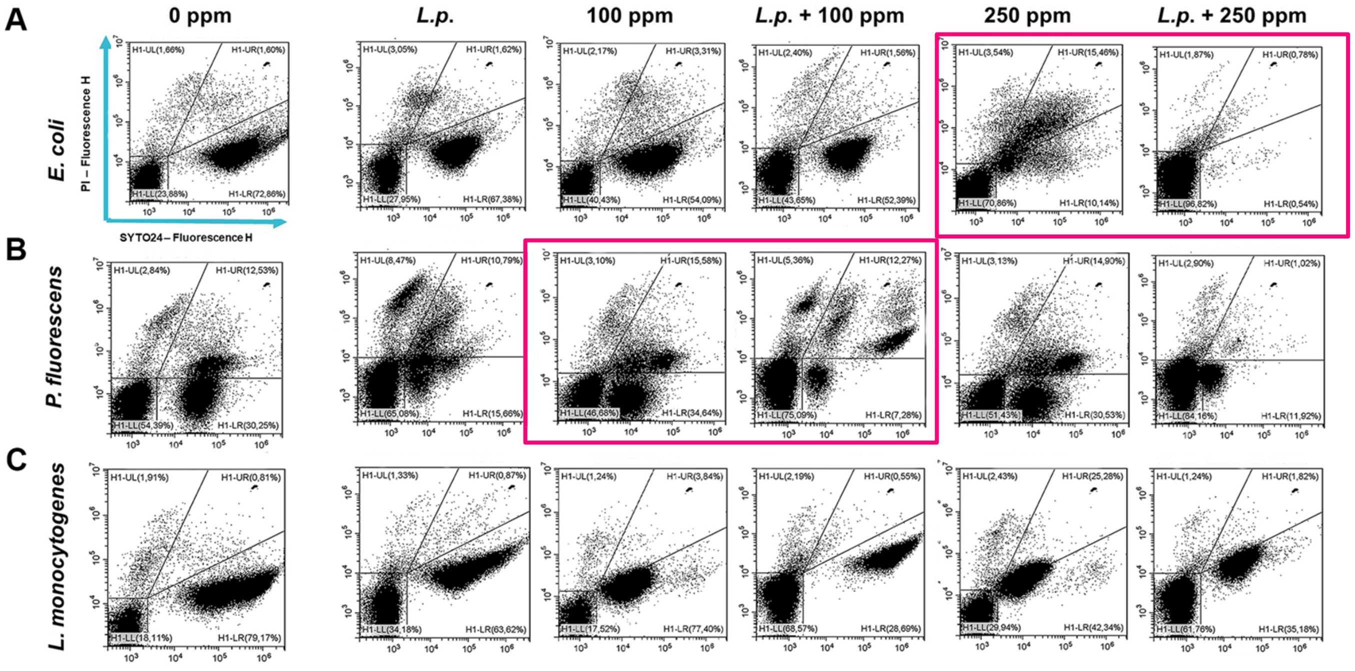
Pre-formed L. plantarum biofilm combined with carvacrol against *E. coli, P. fluorescens, and L. monocytogenes* : Planktonic cells







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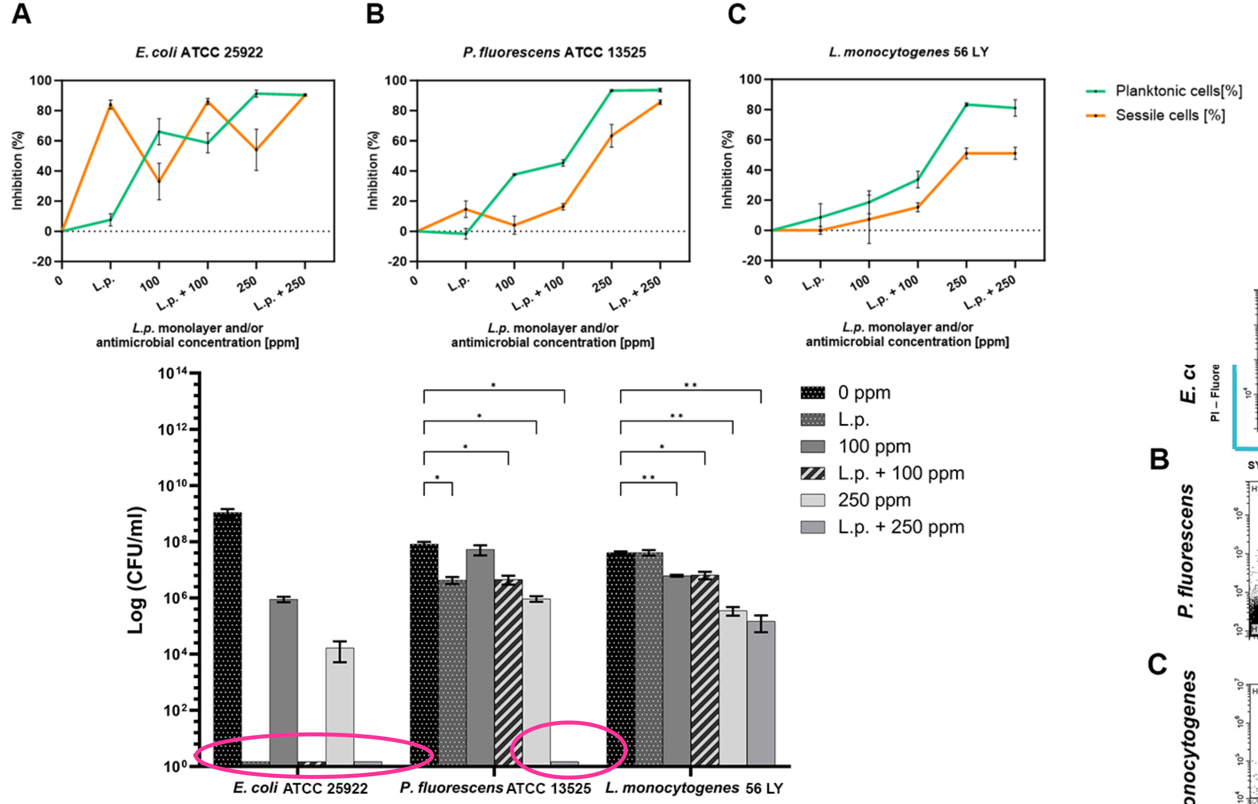
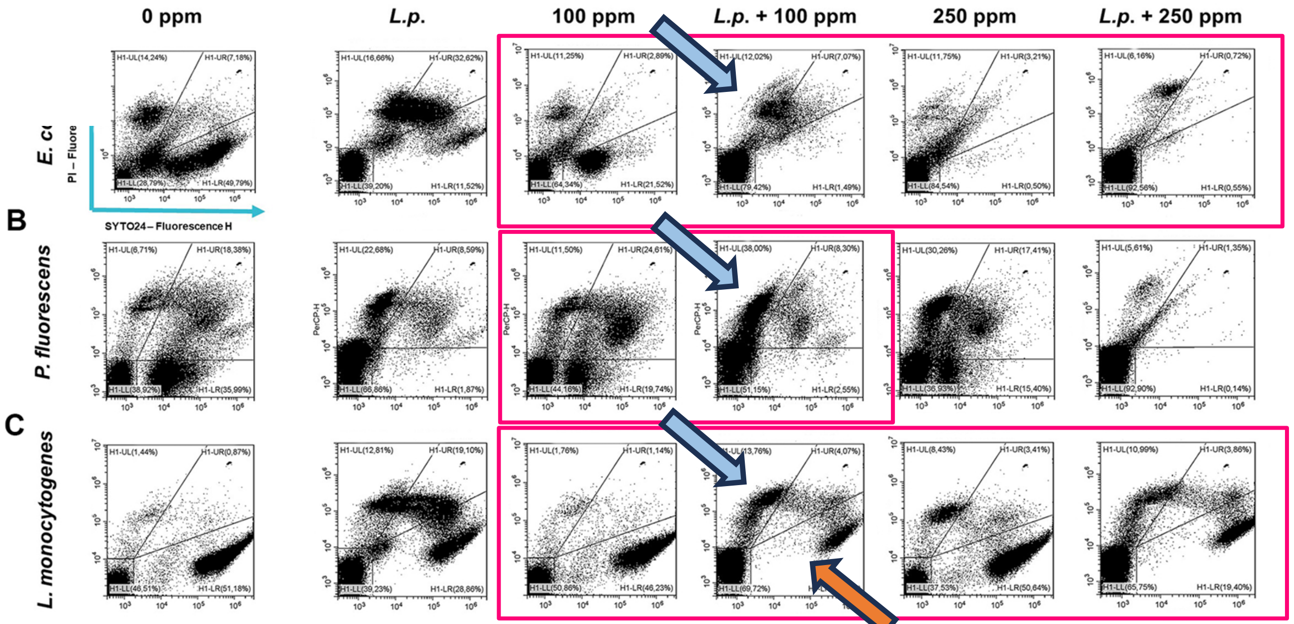
Pre-formed L. plantarum biofilm combined with carvacrol against *E. coli, P. fluorescens, and L. monocytogenes* : biofilm cells



Biofilm cells. The biofilm monolayer presence allowed to use lower concentrations of carvacrol (100 ppm) to achieve massive damage to bacterial physiology. *L. monocytogenes* exhibited the most negligible efficacy among all strains.



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Take Home Messages

- Application of lactic acid bacteria (LAB) and carvacrol, represents a promising solution for the natural control of food packaging pathogenic and spoilage foodborne biofilms.

- *L. plantarum* biofilm monolayer and carvacrol together showed an **enhanced antibiofilm action**, acting on the adhesion process.

- The combined treatment with LAB, which create an acidic environment, fostered the interaction and dissolution of carvacrol in the cell membrane lipids of target bacteria, thus allowing his use at sub-inhibitory concentrations, nevertheless achieving an increased efficacy.

- The FCM analysis gave a comprehensive investigation of cell 'sub-populations' distribution based on the physiological state, providing information of viability vs. culturability, and highlighting the overestimation of the treatment success if considering the culture-based approaches only.

Future Perspectives

To test the antibiofilm combination against other pathogenic bacteria

To perform *in vivo* analysis, evaluating the impact of LAB biofilm monolayer plus carvacrol on product shelf-life and characteristics, by enclosing them within a matrix or inert material (e.g., PLA, PLA + Chitosan).

Considering the expansion of functional food market, due to increased “green consumers” demand for natural, nutritional, and healthy food products, it will be essential to **implement food packaging and design successful delivery systems for such bioactive compounds.**

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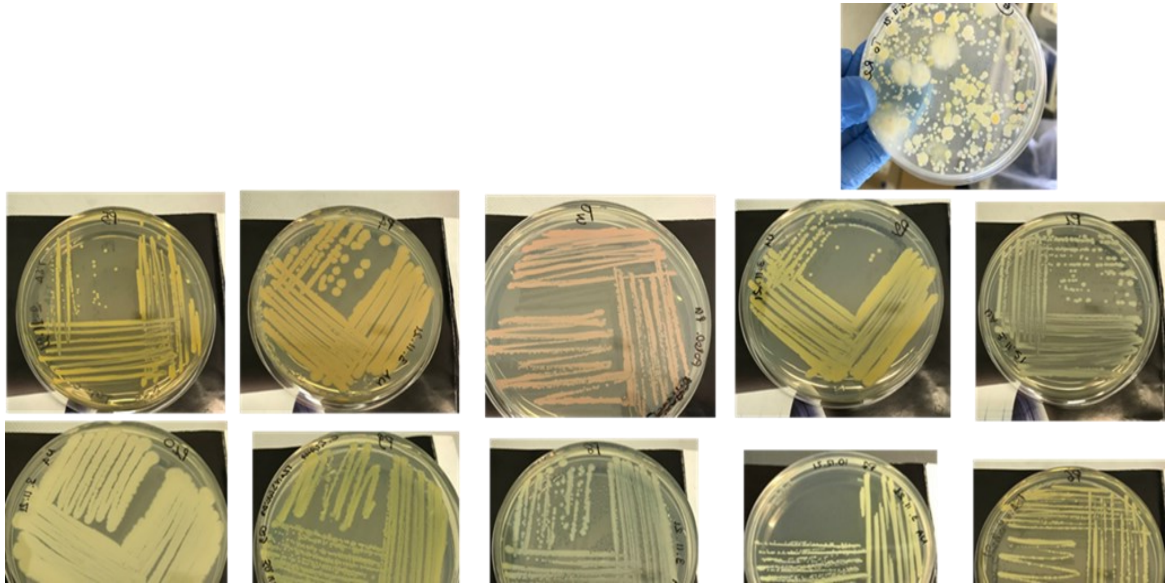

Thanks for your attention!

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