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Prebiotics and probiotics for biopackaging: a case study

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ENEA SSPT-BIOAG-SOQUAS

November 28, 2023

Hybrid course

c/o ENEA CR Casaccia

Sala Blu, Via Anguillarese 301, Roma





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The dimension of the study

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45,607 results

RESULTS BY YEAR

1947 2024

TEXT AVAILABILITY

Role of Probiotics in Human Gut Microbiome-Associate
Kim SK, Guevarra RB, Kim YT, Kwon J, Kim H, Cho JH, Kim HB, Lee JI
J Microbiol Biotechnol. 2019 Sep 28;29(9):1335-1340. doi: 10.4014/jmb.1903.03017. Free article. Review.
PMID: 31434172

Probiotics for Gastrointestinal Conditions: A Summary

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14,811 results

RESULTS BY YEAR

1966 2024

TEXT AVAILABILITY

Probiotics and prebiotics in intestinal health and disease: from biology to the clinic.
Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastal RA.
Nat Rev Gastroenterol Hepatol. 2019 Oct;16(10):605-616. doi: 10.1038/s41575-019-0173-3. Epub 2019 Jul 11.
PMID: 31296969 Review.
Probiotics and prebiotics are microbiota-management tools for improving host health. ...For prebiotics, glucans and fructans are well proven, and evidence is building on the prebiotic effects of other substances (for example, oligomers of mannose, glucose, xy ...

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6,341 results

RESULTS BY YEAR

1995 2024

TEXT AVAILABILITY

Effects of Probiotics, Prebiotics, and Synbiotics on Human Health
Markowiak P, Śliżewska K.
Nutrients. 2017 Sep 15;9(9):1021. doi: 10.3390/nu9091021. PMID: 28914794 Free PMC article. Review.
Therefore, modification of the intestinal microbiota in order to achieve, re-favourable balance in the ecosystem, and the activity of microorganisms in the gastrointestinal tract is necessary for the improved health condition of the host.

Prebiotics and probiotics for depression and anxiety: A Systematic Review

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2,525 results

RESULTS BY YEAR

1997 2023

TEXT AVAILABILITY

Microbial Medicine: Prebiotic and Probiotic and Metabolic Syndrome.
Green M, Arora K, Prakash S.
Int J Mol Sci. 2020 Apr 21;21(8):2890. doi: 10.3390/ijms21082890. Free PMC article. Review.
High-caloric diets and sedentary lifestyles have become widespread issues, although the role of genetic factors in the pathogenesis remain incompletely understood.

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32 results

RESULTS BY YEAR

2005 2023

TEXT AVAILABILITY

Prebiotic, Probiotic, Antimicrobial, and Functional Properties of Bacillus amyloliquefaciens.
Woldemariam Yohannes K, Wan Z, Yu Q, Li H, Wei X, Liu J.
J Agric Food Chem. 2020 Dec 16;68(50):14709-14727. doi: 10.1021/acs.jafc.0c4727. Epub 2020 Dec 7.
PMID: 33280382 Review.
Bacillus amyloliquefaciens belongs to the genus Bacillus and is a Gram-positive, rod-shaped, soil-dwelling bacterium. It is a member of the phylum Firmicutes and the class Bacilli. It is found in food, plants, animals, soil, and in different environments. It is a probiotic and prebiotic microorganism.

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3 results

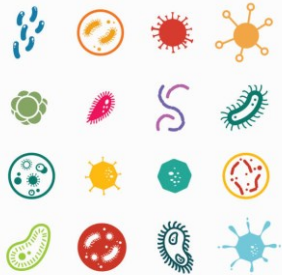
RESULTS BY YEAR

2019 2023

TEXT AVAILABILITY

Functionalizing and bio-preserving processed food products via probiotic and synbiotic edible films and coatings.
Hellebois T, Tsevdou M, Soukoulis C.
Adv Food Nutr Res. 2020;94:161-221. doi: 10.1016/bs.afnr.2020.06.004. Epub 2020 Jul 14.
PMID: 32892833
Edible films and coatings constitute an appealing concept of innovative, cost-effective, sustainable and eco-friendly packaging solution for food industry applications. Edible packaging needs to comply with several technological pre-requisites such as mechanical durability, barrier properties, and compatibility with the food product.

ISAPP Definitions for «Biotic»

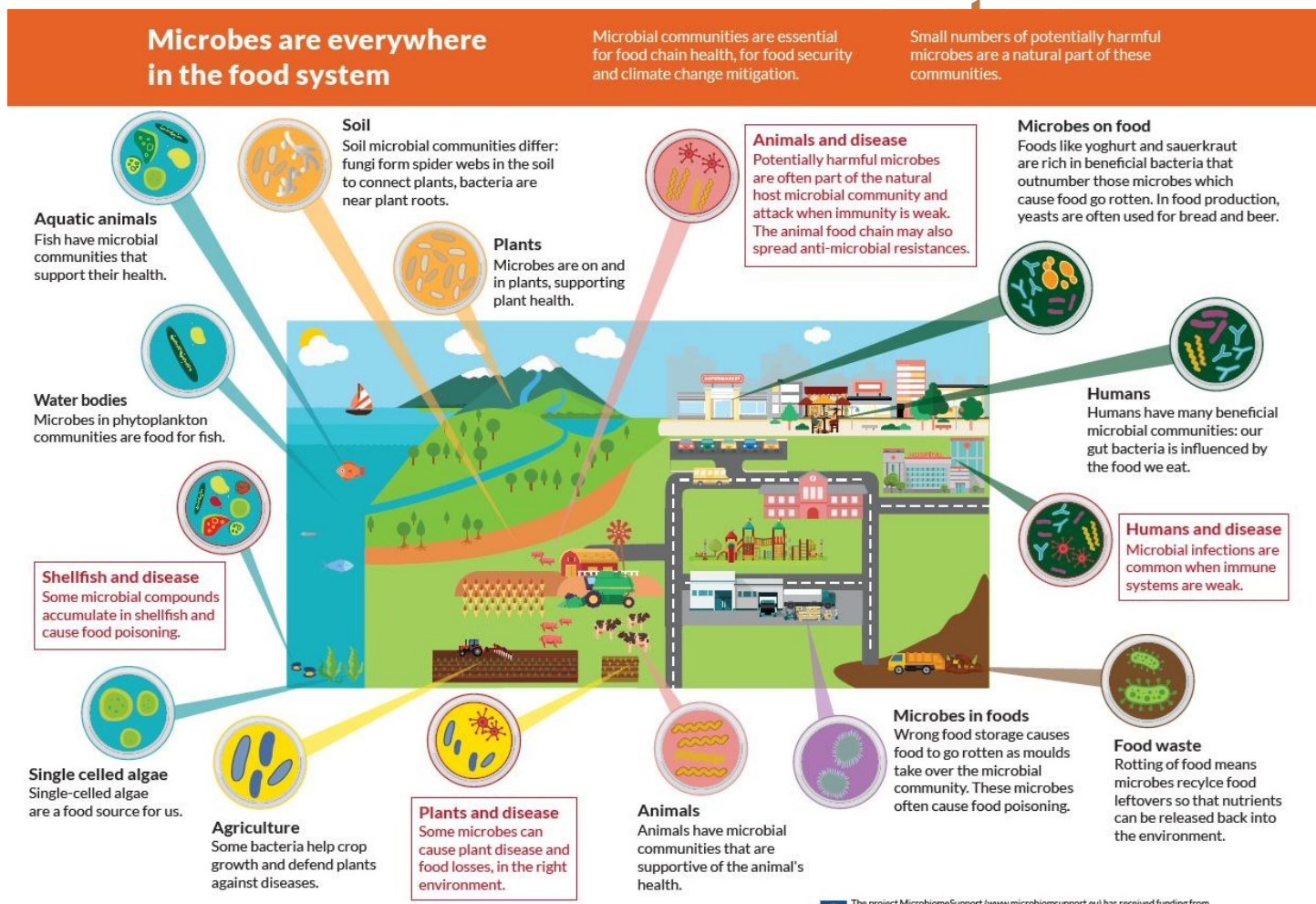


ADVANCING SCIENTIFIC EXCELLENCE
in probiotics, prebiotics, synbiotics, postbiotics and fermented
foods



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

The spread of microorganisms in the food



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.



MICROORGANISMS and their NATURAL ENVIRONMENT

In nature, cells live in association with other cells



POPULATIONS



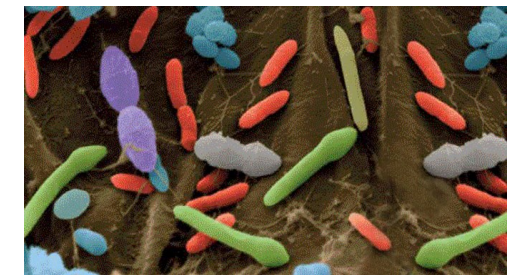
NUMEROUS ORGANISMS OF THE SAME SPECIES

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



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

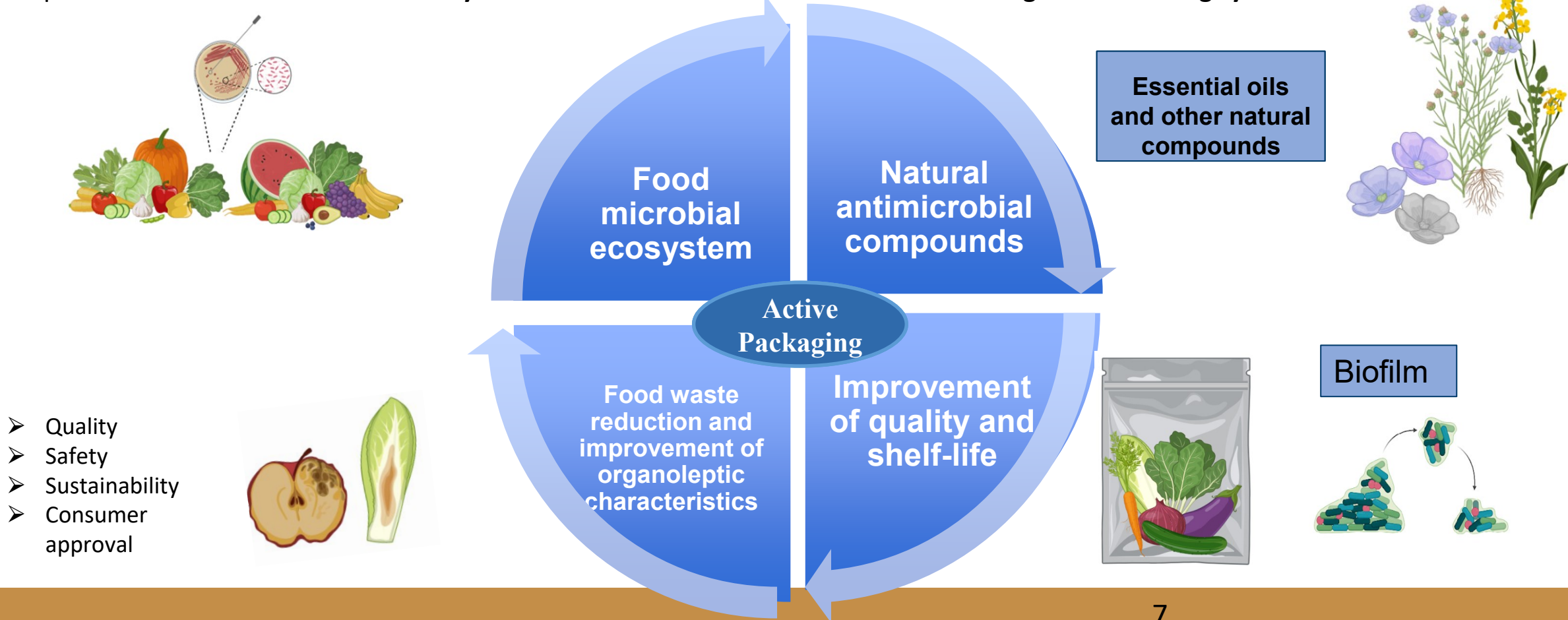


is made up of living organisms together with the chemical and physical constituents of the environment of which they are part

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

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**





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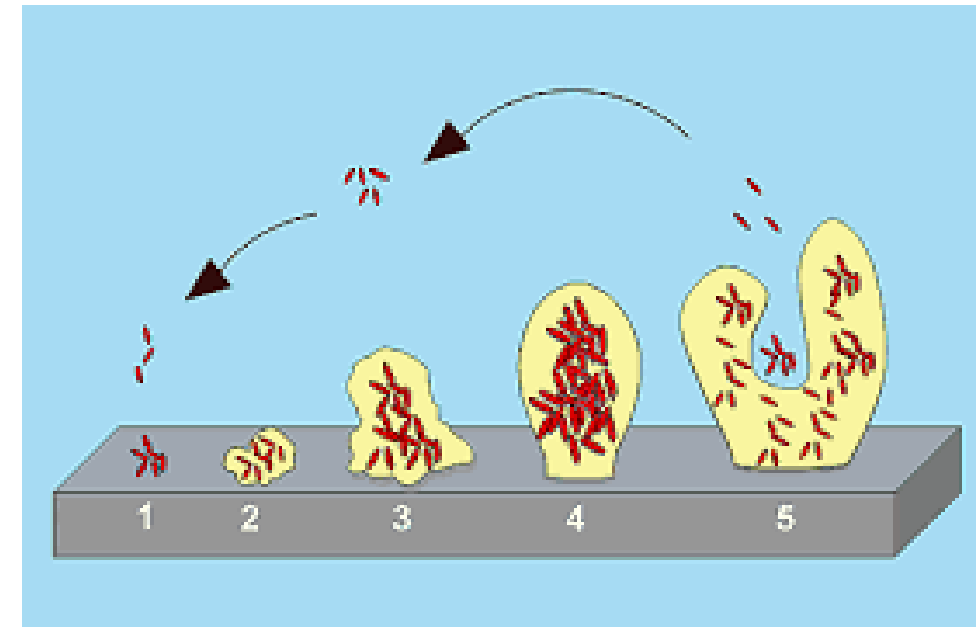
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Microbial Biofilm

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 secreted by the sessile cells, the resulting structure is highly viscoelastic with characteristics of rubbery material





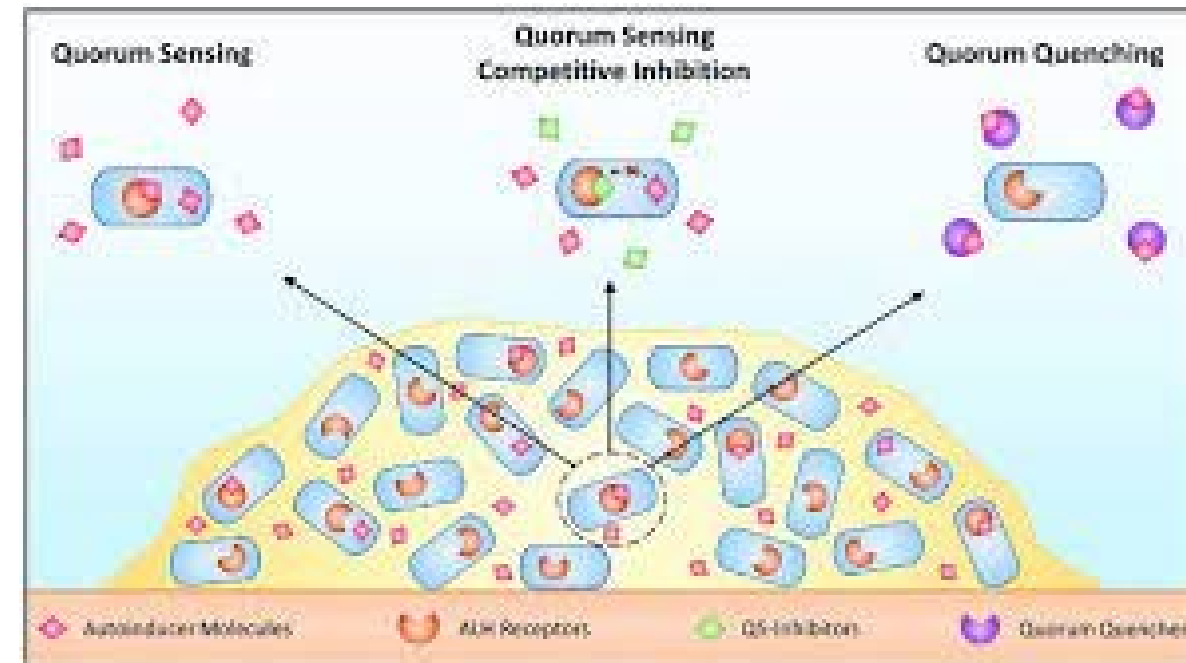
Characteristic of the biofilm

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.

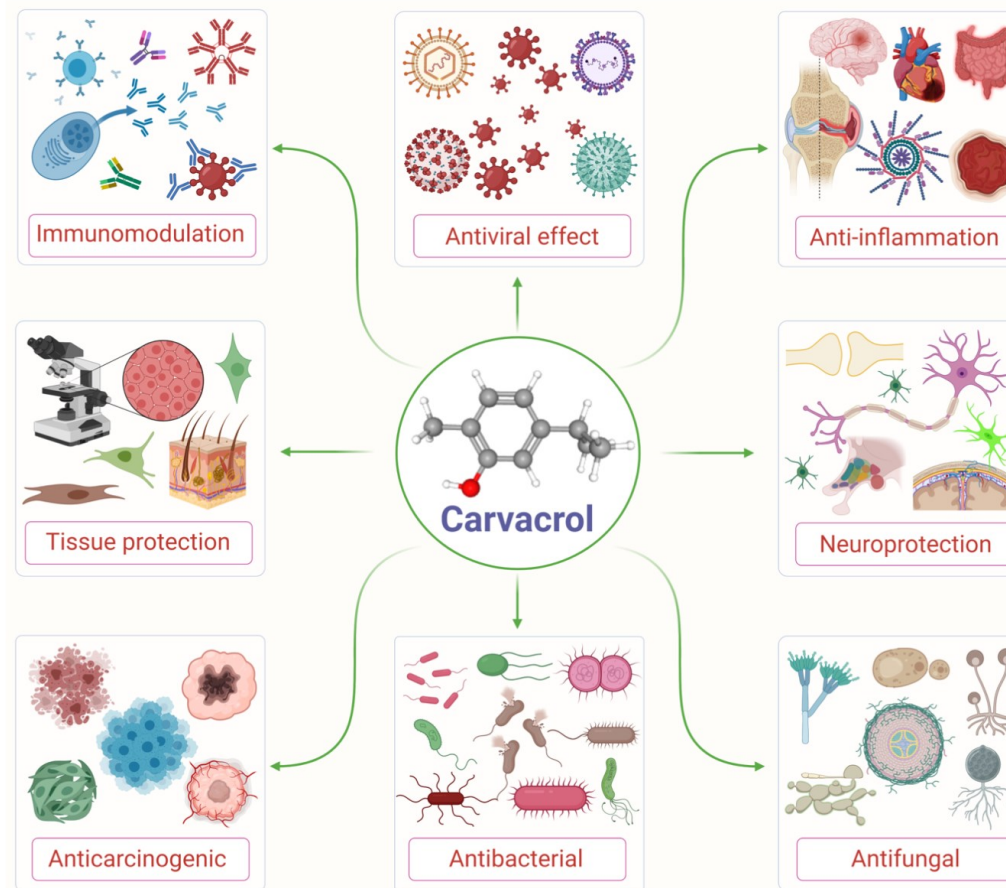


Carvacrol, or cymophenol,
 $C_6H_3(CH_3)(OH)C_3H_7$
is a monoterpene phenol.

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)

Why Carvacrol?



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

PROBIOTICS

BIFIDOBACTERIUM



LACTOBACILLUS



LACTOCOCCUS



STREPTOCOCCUS THERMOPHILUS



ENTEROCOCCUS



ACETOBACTERIUM



iStock
Credit: Iuliia Kudrina

PROPIONIBACTERIUM



PEDIOCOCCUS



SACCHAROMYCES



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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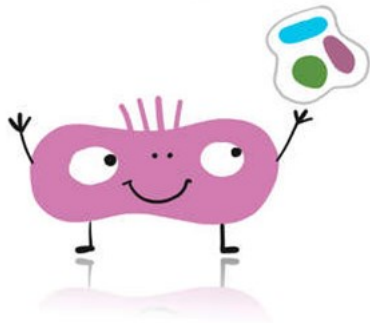


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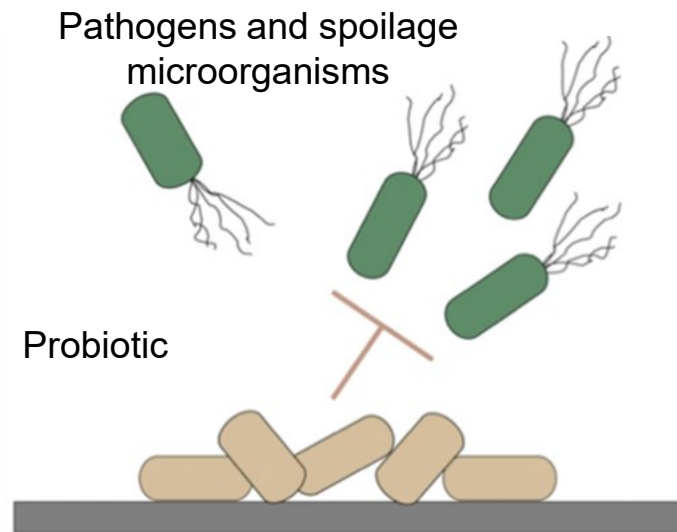
Why Lactic Acid Bacteria?

PROBIOTIC



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.

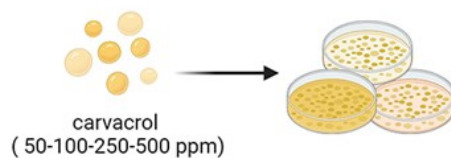




Experimental Set-up

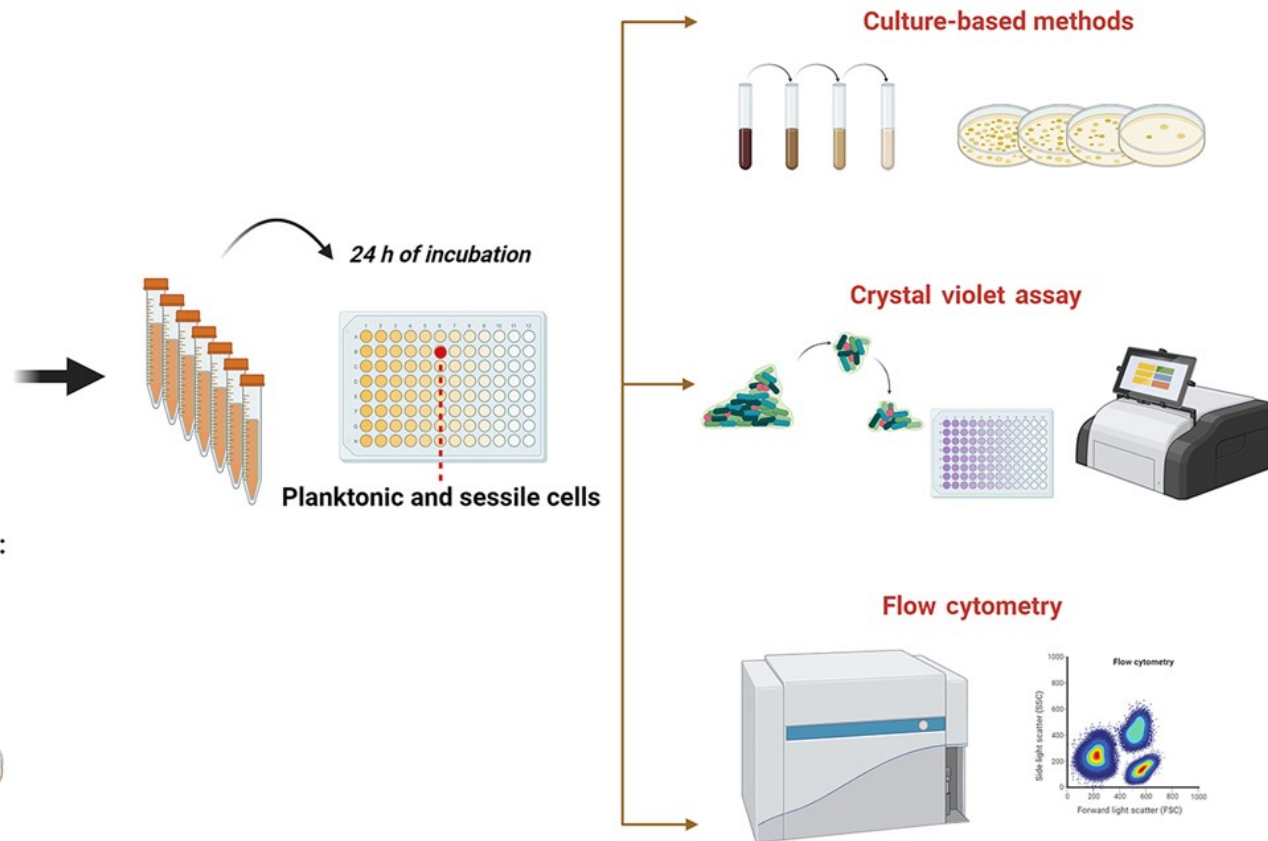
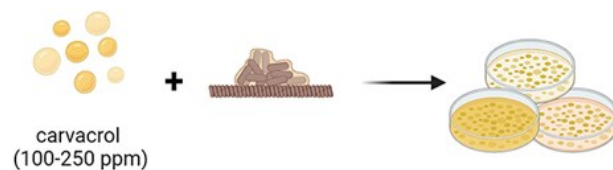
Single Treatment

Carvacrol effect on *E. coli* ATCC 25922, *P. fluorescens* ATCC 13525, *L. monocytogenes* 56 LY



Combined Treatment

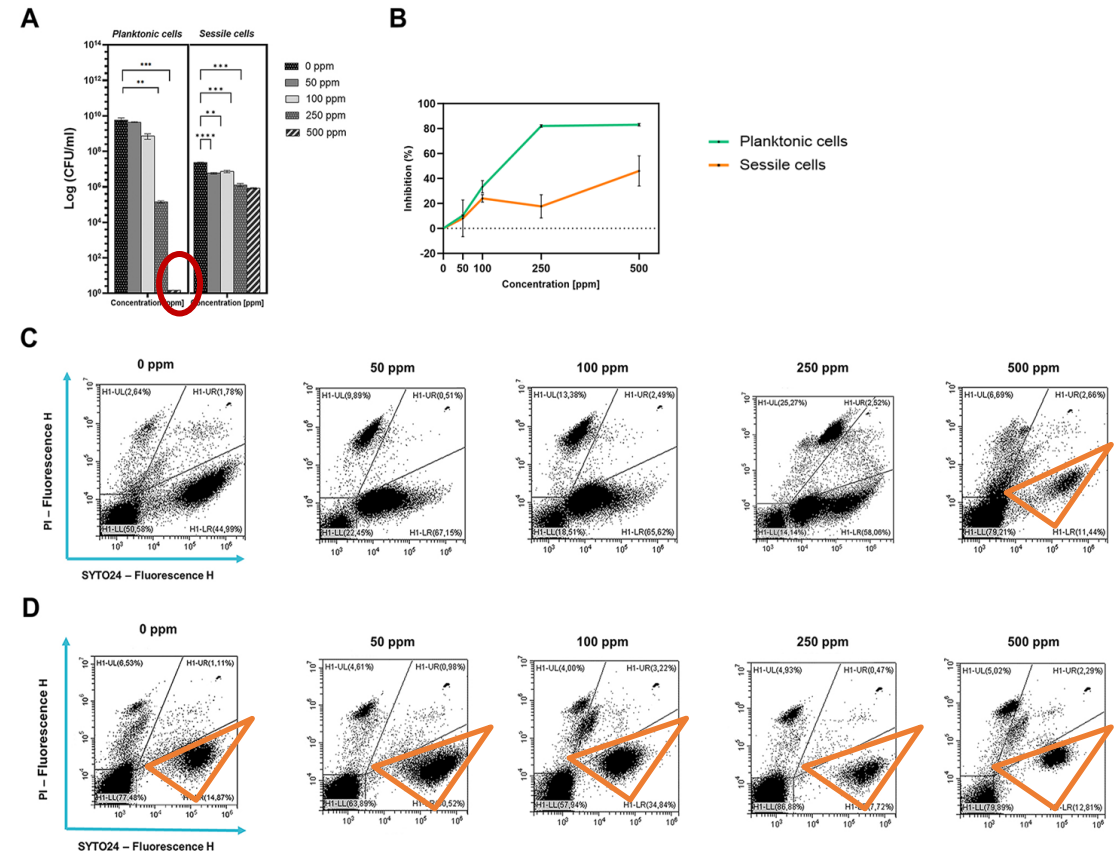
Pre-formed *L. plantarum* biofilm combined with carvacrol: effect on *E. coli* ATCC 25922, *P. fluorescens* ATCC 13525, *L. monocytogenes* 56 LY



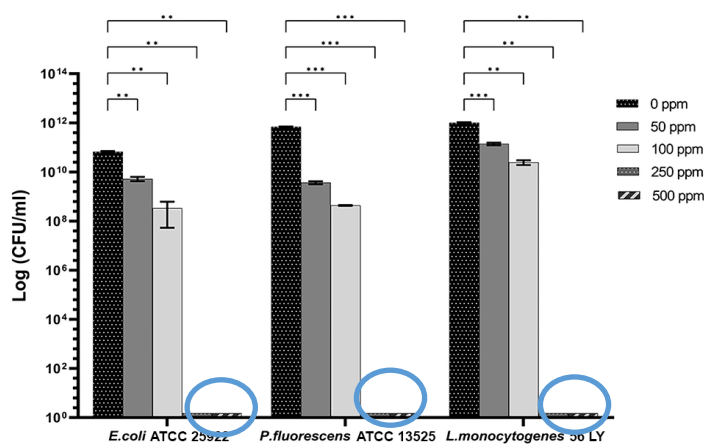
Carvacrol effect on *L. plantarum* DSM 20174 probiotic strain

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.

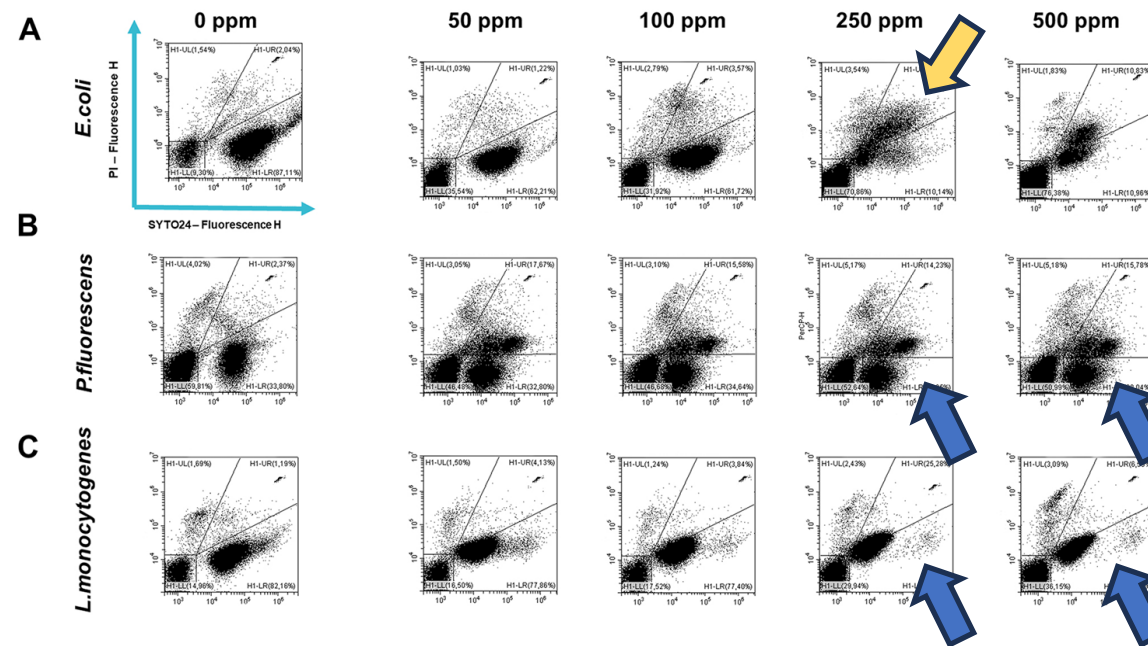
Sessile cells. *L. p.* sessile fraction was less sensitive to carvacrol antimicrobial treatment, even at higher concentrations.



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.

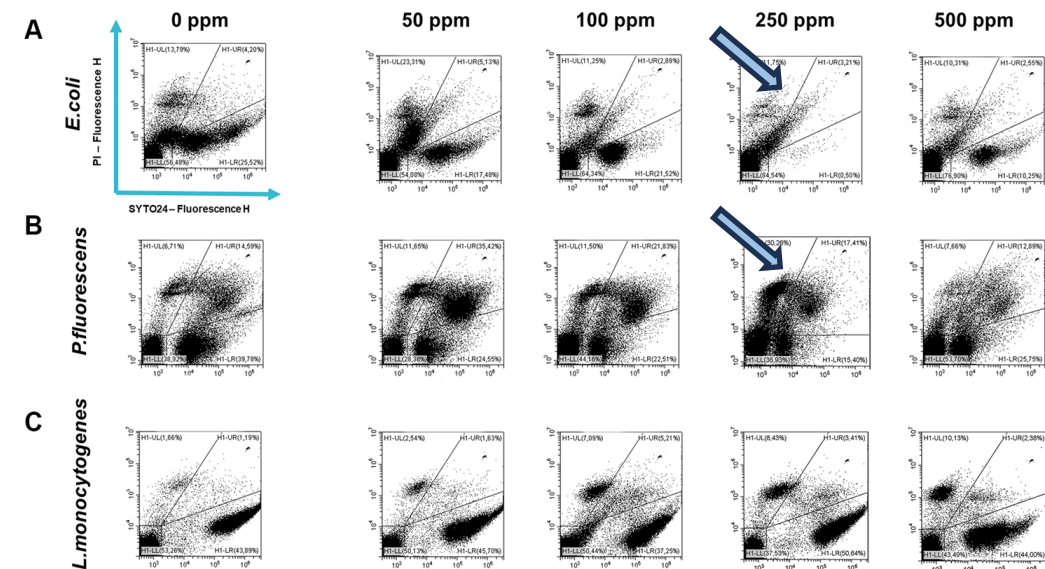
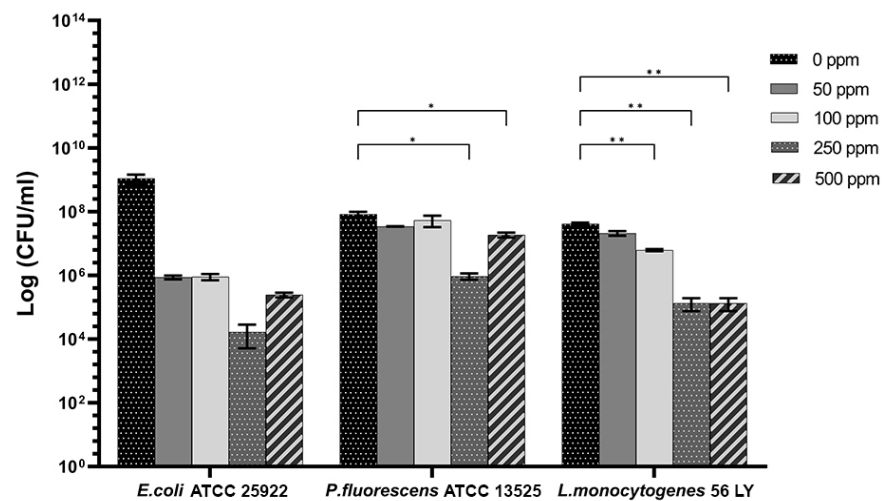


E. coli planktonic cells: proportional damage degree up to 250 ppm carvacrol, with non-culturable but still viable state (VBNC) at 250 and 500 ppm.

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

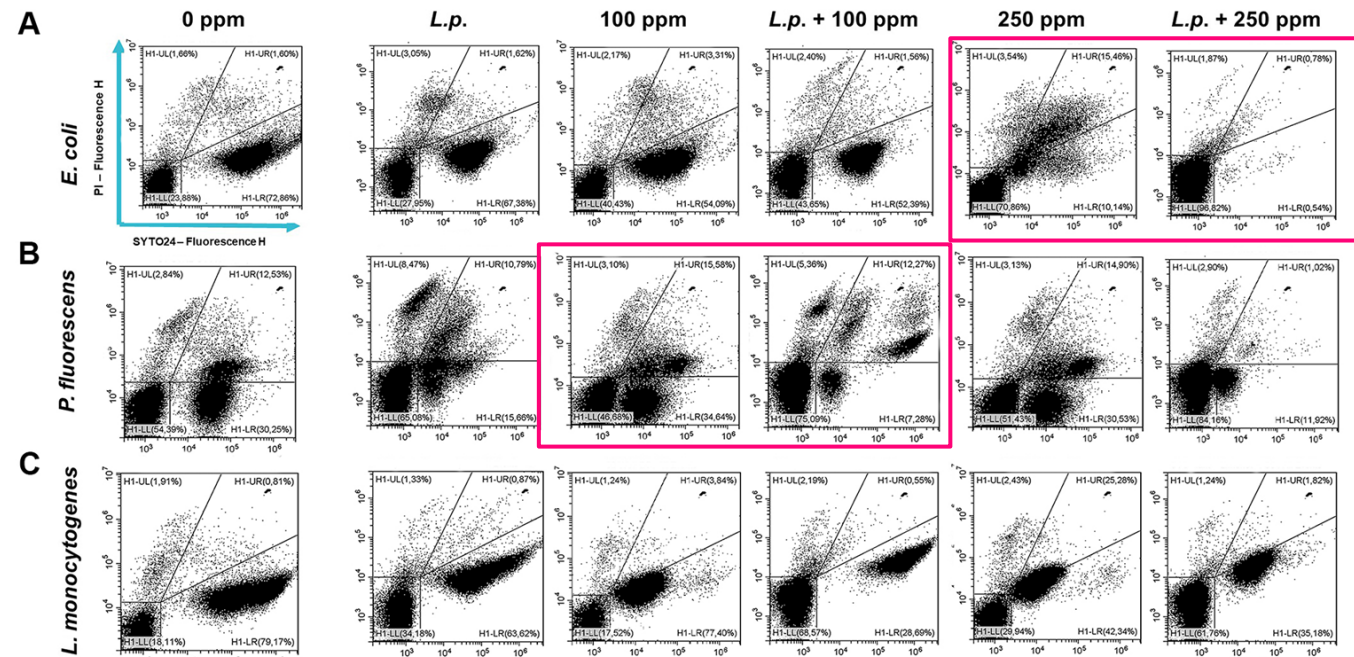
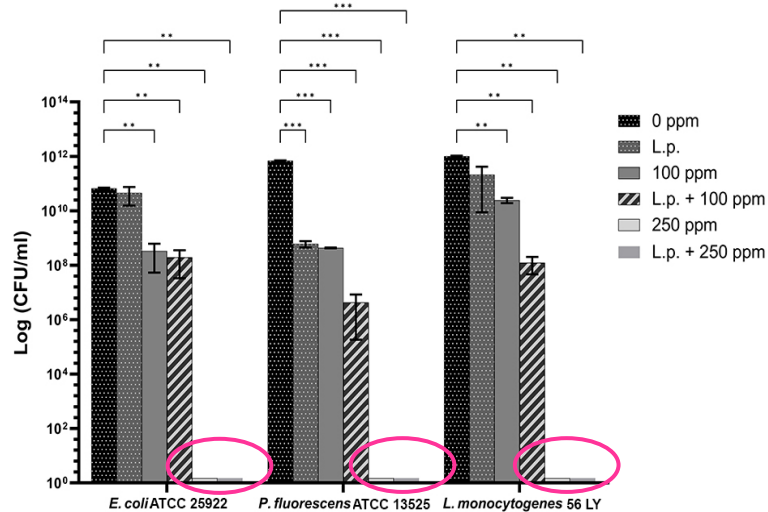
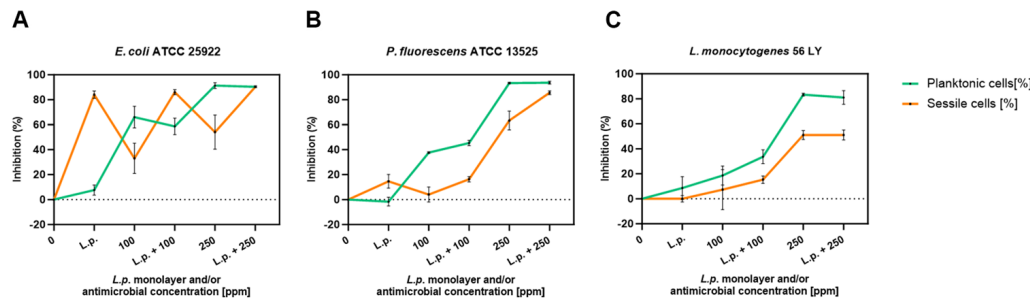


Carvacrol effect on *E. coli*, *P. fluorescens*, and *L. monocytogenes* biofilm cells

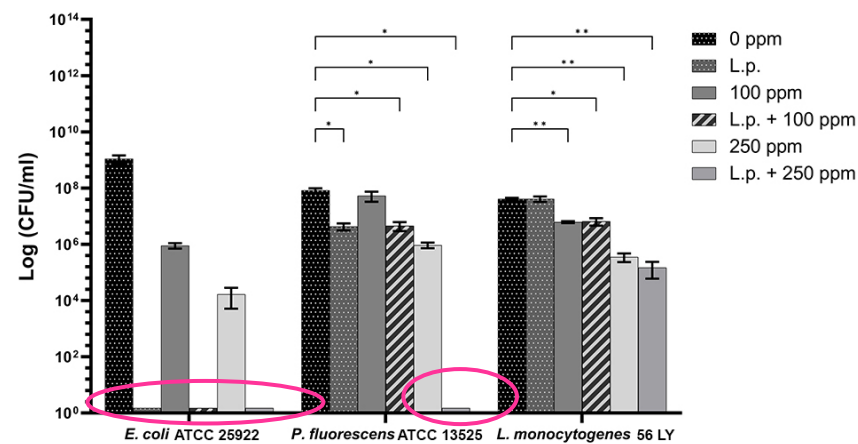
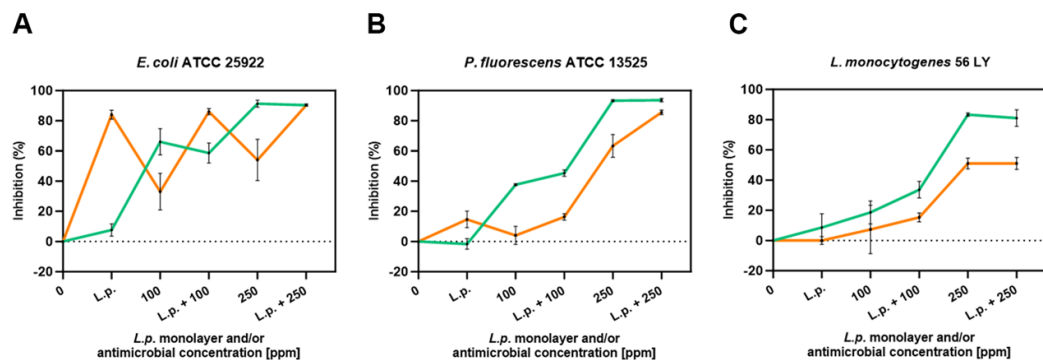


The 250 ppm carvacrol concentration demonstrated higher efficacy in inhibiting biofilm-forming adherent cells, while the increase of concentration (500 ppm) did not enhance the antimicrobial effect, significantly.

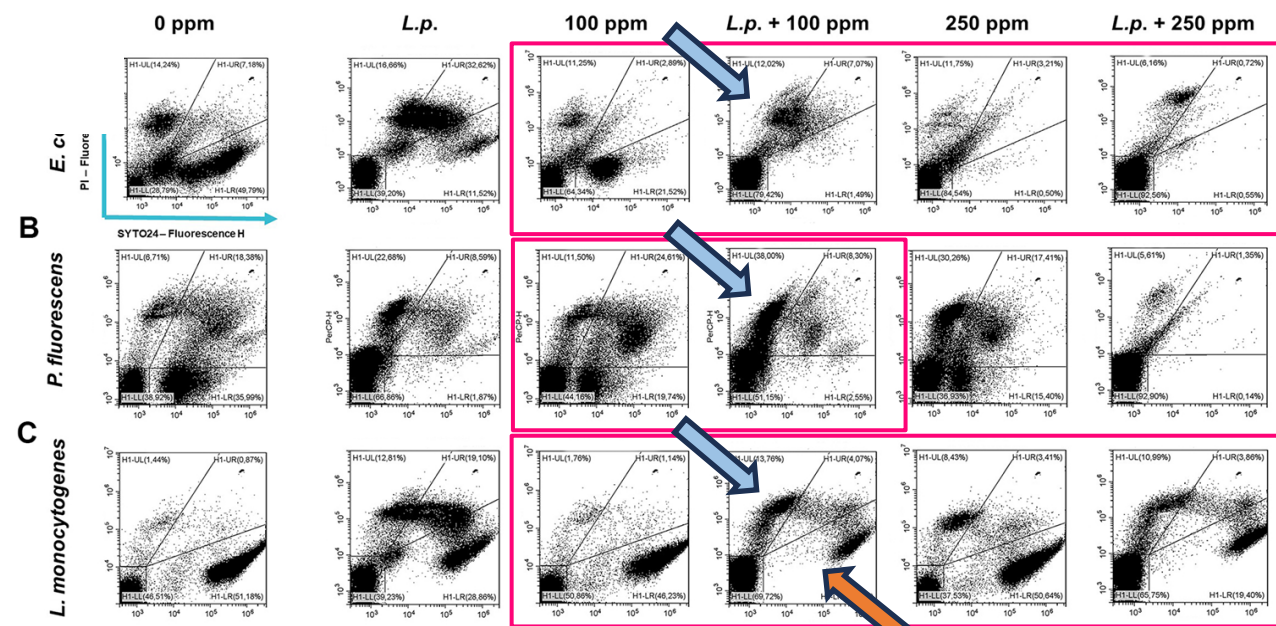
Pre-formed *L. plantarum* biofilm combined with carvacrol against *E. coli*, *P. fluorescens*, and *L. monocytogenes* : Planktonic cells



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.



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



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.





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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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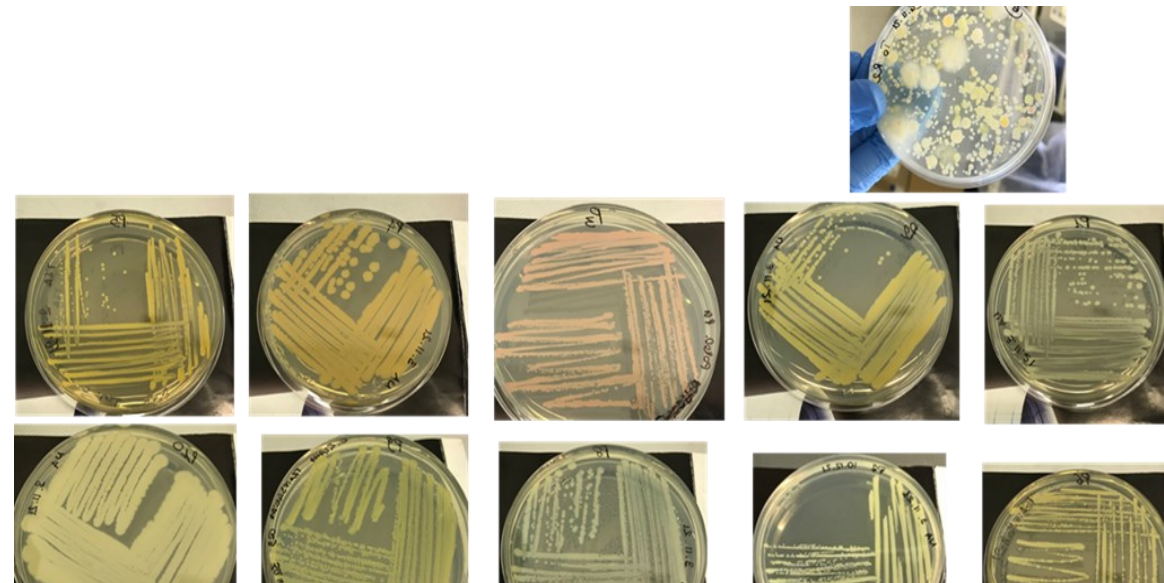
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Sostenibilità, Qualità e Sicurezza
Produzioni Agroalimentari



Thanks for your attention!

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