

Biofilm forming reduction by combining *L. plantarum* monolayer with carvacrol in antimicrobial-functionalized food packaging

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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. The combined action of probiotics with natural bioactive compounds incorporated in active packaging represents an extremely promising alternative to prevent food spoilage and microbial contamination for extending products' shelf-life.

This work aimed at investigating the *in vitro* combined efficacy of carvacrol with a pre-formed biofilm monolayer of *Lactobacillus plantarum* DSM 20174 against planktonic and sessile cells of food pathogenic (*Escherichia coli* ATCC 25922 and *Listeria monocytogenes* 54ly) and spoilage (*Pseudomonas fluorescens* ATCC 13525) strains. Traditional culture-based and flow cytometry (FCM) methods were applied to perform the first screening of several natural antimicrobials (Thyme essential oil (EO), Origanum EO, Basil EO, Citrus Limon EO, carvacrol, limonene, nisin) and to evaluate the combined action of carvacrol with the preformed biofilm of *L. plantarum* against target strains.

The results showed a great efficacy of the combined treatment, suggesting that the *L. plantarum* preformed biofilm enhanced the antimicrobial effect of carvacrol determining a bactericidal action, while the treatment alone induced the viable but nonculturable (VBNC) cell state only. Furthermore, an overestimation of the dead population was observed using the culture-based method, while FCM showed the prevalence of VBNC cells. Overall, the incorporation of carvacrol into the preformed biofilm of *L. plantarum* represents a future perspective for antimicrobial-functionalized ready-to-eat packaging.

Keywords: food packaging, bioactive compounds, pre-formed biofilm, probiotics, flow cytometry

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Acknowledgements.

This work was supported by ILIP S.r.l and partially funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Award Number: Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP I83C22001790001, Project title "ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods".