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Antibacterial activity of plasma-treated polypropylene membrane functionalized with living Carnobacterium divergens in cold-smoked salmon

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Abstract :

In recent years, bacteriocins produced by lactic acid bacteria (LAB) have shown great potential for food safety preservation, especially for ready-to-eat products. In this study, bio-protective membrane was made from plasma-treated polypropylene film and functionalized with Carnobacterium divergens V41 (bacteriocin-producing strain) for the purpose of inhibiting Listeria monocytogenes growth in culture media and cold-smoked salmon (CSS) at refrigerated temperatures. In semi solid Brain Heart Infusion (BHI) agar, bio-protective plastic membrane led to a 3-Log reduction in L. monocytogenes count compared to the control, after 14 days of aerobic incubation at 8 °C. In vacuum-packed CSS, L. monocytogenes growth was inhibited by bio-protective plastic membrane after 7 days of storage at 4 °C and 21 days at 8 °C. Antilisterial activity of plastic membrane was even better than C. divergens cells added in CSS by direct spraying. Stability test has shown that bio-protective plastic membrane stored for 42 days at 4 °C still exerted antimicrobial activity against L. monocytogenes on BHI agar (2-Log reduction compared to the control). These preliminary results demonstrate that bio-protective plastic membrane can be used to control pathogenic bacteria in food products with potential industrial development.

Highlights

► Modification of polypropylene surface by plasma treatment in order to fix bioprotective *Carnobacterium divergens* bacteria. ► Antilisterial activity of bio-protective plastic film tested in BHI and smoked salmon. ► Assessment of stability of bio-protective polypropylene film in BHI over storage time. ► Potentiality of development of a new active food packaging

Keywords : Bacteriocin, Polypropylene membrane, Plasma, Carnobacterium divergens, Listeria monocytogenes, Cold-smoked salmon

1- **Introduction**

Contamination with *Listeria monocytogenes* is a major concern in food industry because of its ubiquitous occurrence and its ability to survive and persist in unfavorable environments. *L. monocytogenes* is responsible for listeriosis, a disease lethal in 20-30% of the case especially for immune deficient people, elderly and pregnant women. The number of case in Europe has been increasing steadily since 2006 to reach 5.6 cases of listeriosis/million inhabitants in 2019 (ANSES, 2020). This microorganism is present in a wide range of raw and ready-to-eat food products (Hartmann, et al., 2011; Løvdal, 2015) and cold-smoked salmon (CSS) is not an exception. Indeed, salting and smoking steps contribute to minimize spoilage and pathogenic bacterial contamination but do not totally eliminate *L. monocytogenes*. Its prevalence in retail CSS is highly variable, from 0 to 61% giving an average value of

9.8% according to publications post year 2000 (Duffes, 1999; Løvdal, 2015). Although generally present at low concentration just after processing, *L. monocytogenes* can grow during the storage at refrigerated temperature under air or vacuum-packaging and sometimes overpass the 100 CFU/g European regulatory limit (Commission Regulation (EC) No 2073/2005). Therefore, inhibition of *L. monocytogenes* growth contributes to minimize life-threatening health hazards and extend the shelf life of CSS.

Many lactic acid bacteria (LAB) are widely used in food preservation (known as biopreservation) owing to the capacity of production of a large variety of antimicrobial substances (organic acids, bacteriocins, hydrogen peroxide…) (Hartmann et al., 2011). *Carnobacterium* sp., frequently found in a large number of food including fish, meat and some dairy products, is a promising genus as many strains produce bacteriocins with antilisterial activity (Aymerich et al., 2019; Begrem et al., 2021), do not acidify flesh and have few negative impact on the quality of food (Joffraud et al., 2001; Stohr et al., 2001; Leisner et al., 2007; Leroi, 2010; Casaburi et al., 2011). Among the twelve reported species, *Carnobacterium divergens*, *Carnobacterium maltaromaticum* (formerly *Carnobacterium piscicola*) and *Carnobacterium inhibens* have gained a lot of attention as protective cultures in order to inhibit growth of *L. monocytogenes* in seafood products in last two decades (Duffes, 1999; Duffes et al.,1999a; Tahiri et al, 2009; Wiernasz et al., 2020; Passerini et al, 2021). A wide range of studies have used the strain *C. divergens* V41 as a protective culture in CSS and shrimp against *L. monocytogenes* (Duffes et al., 1999b, 2000; Brillet et al., 2004; Saraoui et al., 2017). This strain has been isolated from salmon viscera by Pilet et al. (1995). Its antilisterial activity is due to the production of the extensively studied class IIa bacteriocin called divercin V41, which exerts its activity by forming pore in target cell membrane (Connil et al., 2002; Metivier et al., 1998; Richard et al., 2003, 2004; Rihakova et al, 2009). Previous study has shown that the effect of the semi-purified divercin V41 had an immediate listericidal effect in CSS which did not last during the 4 weeks of storage at chilled temperature. Introduction of alive *C. divergens* V41 by spraying the pre-culture onto the surface inhibited growth of *L. monocytogenes* till the end of shelf-life (usually 4 weeks) (Duffes et al., 1999b). However, spraying bacteria tends to introduce water in the product, which may favor growth of undesirable bacteria. Development of active packaging with protective bacteria or their metabolites is a new trend to control the development of

pathogens in food (Mauriello et al 2005; Concha-Meyer et al., 2011; Degli Esposti et al., 2018). In CSS industry, polypropylene membranes are sometimes used to separate the slices for a convenient purpose. The study aimed to produce bio-protective polypropylene membranes with alive *C. divergens* V41 and evaluate its antibacterial activity against *L. monocytogenes* in both culture media and CSS stored at chilled temperatures.

2- Materials and methods

2.1- Bacterial strains and culture media

Carnobacterium divergens V41 and *Listeria monocytogenes* RF191 used in this work were provided by EM^{3}B laboratory (Ifremer, France). *C. divergens* V41 (co-property Ifremer/Oniris, Nantes, France) was isolated from trout intestine and characterized by Pilet et al. (1995). This strain produces divercin V41 which is a heat-stable, 4.5 kDa class IIa bacteriocin (Metivier et al., 1998). Elliker and BHI broth with 20% sterile glycerol added (*v/v*) were used to prepare *C. divergens* and *L. monocytogenes* aliquots which were stored at -20°C until use, respectively. Overnight bacterial cultures were prepared by transferring aliquots to fresh Elliker or BHI broth (1%, *v/v*) and incubated at 30°C or 37°C for *C. divergens* and *L. monocytogenes*, respectively.

2.2 - Preparation of bio-protective plastic membrane from alive Carnobacterium divergens

2.2.1 - Improvement of adhesive property of polypropylene membrane

Polypropylene films similar to those used in food industry packaging were selected (purchased at a French smokehouse). Those membranes, in form of plastic film, are commonly used to separate CSS slices for convenience. The membranes are in form of rectangle (6 cm width and 12 cm length) and were used as a carrier for *C. divergens* in production of bio-protective plastic films. In order to enhance adhesive property of polypropylene membrane, cold plasma treatment was chosen to insert OH groups on plastic surface and change its property from hydrophobic to hydrophilic. Plasma treatment was performed at Centre de Transfert de Technologie (CTTM, Le Mans University, France) using oxygen (O_2), argon (Ar) and nitrogen (N_2) gases under the following parameters:

- O_2: 10 standard cm^3/min (sccm) sweeping for 3 min at 0 W and then treating for 7 min at 200 W.

- Combination of Ar and O_2: Ar, 10 sccm sweeping for 3 min at 0 W and then treating for 5 min at 200 W. Afterwards, O_2, 10 sccm sweeping for 3 min at 0 W and then treating for 5 min at 200 W.

- Combination of Ar and N_2: Ar, 10 sccm sweeping for 3 min at 0 W and then treating for 5 min at 200 W. Afterwards, N_2 10 sccm sweeping for 3 min at 0 W and then treating for 5 min at 200 W.

Both sides of the membrane were treated one by one. Result of plasma treatment was evaluated by measuring contact angle with water, diiodomethane and ethylene glycol. The experiment was performed in triplicates.

2.2.2 - Preparation of bio-protective polypropylene membrane

4200 mL of an overnight *C. divergens* culture in Elliker (approximately 10^8 CFU/mL) was centrifuged at 8000 g at 4°C for 10 min in order to collect bacterial cells. The pellet was re-suspended in 420 mL of fresh sterile Elliker broth to obtain a suspension containing about 10^9 CFU/mL. The suspension was transferred to 12 sterile square Petri dishes (120×120 mm) (35 mL/dish). The plasma-treated and untreated plastic membranes were then soaked in *C. divergens* suspension for 3 h and dried overnight under laminar flow hood at room temperature. Bio-protective polypropylene membranes were placed in sterile square Petri dish sealed with Parafilm® and stored at 4°C until use. Distribution of *C. divergens* cells on plastic surface was visualized by using scanning electron microscope (SEM).

2.3- Evaluation of antibacterial activity of bio-protective polypropylene film against Listeria monocytogenes in model conditions (semi solid Brain Heart Infusion agar)

Five mL of semi solid BHI agar (0.8% (*w/v*) agar) were poured in each well of five sterile plastic plates used for cell culture (6 wells/plate). Solidified agar wells were then surface-inoculated with 50 µL of a *L. monocytogenes* overnight culture diluted in order to obtain a level of 10^3 CFU/mL of BHI agar. The plates were then covered with different plastic membranes as follow : First plate (Lot N-PM) : No plastic membrane ; Second plate (Lot U-PM) : a piece of plastic membrane of 4 cm^2 (2 x 2 cm) deposited onto the agar of each well ; Third plate (Lot P-PM) : a piece of plasma-treated plastic membrane; Fourth plate (Lot U-PM + *Carno)* : a piece of plastic membrane treated

with *C. divergens* ; Fifth plate (Lot P-PM + *Carno)* : a piece of plasma-treated plastic membrane treated with *C. divergens*. Plastic plates were sealed by parafilm and then incubated at 8°C for 14 days. Bacterial enumeration was carried out at day 0, 7 and 14. Plastic membranes were removed from the wells and BHI agar from each well was transferred to stomacher plastic bag with filter. Sterile physiological saline solution (40 mL, 0.1% (*w/v*) tryptone (Biokar) and 0.85% (*w/v*) NaCl) was added. The sample was homogenized for 2 minutes in a stomacher (Lab Blender, London, UK) at room temperature and serial dilutions in physiological saline solution were prepared for bacterial enumeration. *C. divergens* was enumerated by surface plating onto Elliker agar (Biokar) containing two antibiotics (gentamycin and streptomycin, 5 µg/mL for each) and incubated anaerobically at 30°C for 48 h. *L. monocytogenes* growth was completely inhibited by addition of gentamycin and streptomycin. *L. monocytogenes* was determined by surface plating onto Palcam agar (BK145, Biokar) containing a selective supplement (BS00408, Biokar) under aerobic incubation at 37°C for 24 h. All the experiments were done in sextuple.

2.4- Evaluation of antilisterial activity of bio-protective plastic membrane in cold-smoked salmon

Fresh vacuum-packed CSS slices (approximately 33-35 g/slice) used in this work were purchased from a French smokehouse company, transported to laboratory one day before experiment and stored at 4°C. Composition of CSS was analyzed in triplicate, including water 59.58%, protein 17.64%, lipid 16.10%, salt 2.57% in water phase, phenols 0.46% and ash 3.58%. First *Listeria monocytogenes* overnight culture diluted to reach 10^4 CFU/mL was evenly sprayed on all salmon slices (1% *v/w*) in order to obtain a level of approximately 10^2 CFU/g of salmon. After 1 h conservation at 4°C for absorption, bio-protective plastic membranes (plasma-treated membrane treated with *C. divergens*) were placed on salmon slices. In order to make a comparison with a method commonly used in Ifremer, *Carnobacterium divergens* was also incorporated on another batch of CSS by spraying (1% *v/w*) with a level of about 10^6 CFU/g of salmon. The level was proven to be necessary for significant inhibition of *L. monocytogenes* (Duffes et al., 1999b). The control was slices of CSS covered with plasma-treated membrane without the addition of *C. divergens*. The salmon slices were then vacuum-packed and incubated for 28 days under the following

conditions: 7 days at 4°C which corresponds to the storage temperature in distributors and 21 days at 8°C which is considered as the usual storage temperature in consumer refrigerators. Bacterial enumeration was performed at day 0, 7, 14, 21 and 28 of incubation. Salmon flesh (23-25 g) was aseptically cut into small pieces and then transferred to a stomacher plastic bag with filter. Physiological saline solution was added in a 4:1 ratio (*v/w*). The sample was homogenized for 2 min in a stomacher and left at room temperature for 30 min for resuscitation. The homogenate was serially diluted in physiological saline solution for bacterial enumeration. Total LAB, including *C. divergens V41,* and *L. monocytogenes* were enumerated as previously described. Cell counts of *L. monocytogenes* below 10^2 CFU/g of salmon were determined by gently mixing 1 mL of the mother suspension with 20 mL of molten Palcam agar cooled down at 50°C. The experiments were performed in triplicate.

2.5- Evaluation of stability of bio-protective plastic membrane against Listeria monocytogenes

After preparation as described above, bio-protective plastic membranes were placed in empty square Petri dish sealed with Parafilm® and stored at 4°C to evaluate their stability over time (day 0, 21 and 42). Stability was assessed by testing antibacterial activity against *L. monocytogenes* in semi solid BHI agar as described in 2.3 and by enumeration of *C. divergens* viable cell count. The experiment was carried out in triplicate.

2.6- Statistical analysis

Experimental data related to enumeration of *C. divergens* and *L. monocytogenes* were statistically analysed by one-way ANOVA and the Tukey test with a level of significance of 95%. All statistical analysis were done with the aid of Minitab software, release 13 (Pennylvania, USA).

3- Results

3.1- Change of plastic membrane property after plasma treatment

In order to evaluate effectiveness of cold plasma treatment in change of membrane property contact angle of plastic surface was measured. The results were summarized in Table 1. Before plasma treatment polypropylene surface is highly inert

and hydrophobic. Contact angle of untreated membrane is 96, 63, 68° with water, diiodomethane and ethylene glycol, respectively. After treatment with O_2, Ar/ O_2 and Ar/N_2, a sharp decrease in contact angle of plastic membrane was observed with all liquids tested. This change indicated that plastic surface became hydrophilic after treatment. Plasma treatment using Ar/N_2 gave the best change in contact angle and then was chosen to improve adhesive property of plastic membrane used in all experiments below.

3.2- Adsorption of Carnobacterium divergens cells on polypropylene surface

Scanning electron microscopy (SEM) images of adsorption of *C. divergens* cells on untreated and Ar/N_2 plasma-treated polypropylene membrane are shown in Fig. 1. It seems likely that distribution of bacterial cells was more homogeneous on treated plastic surface than the other. Hence plasma-treated polypropylene membrane (P-PM) can be used as a carrier in order to add bio-protective bacterial strains to food products.

3.3- Antibacterial activity of bio-protective plastic membrane against Listeria monocytogenes in semi solid BHI agar

The result of *L. monocytogenes* enumeration in semi solid BHI agar is presented in Fig. 2. As expected, the initial levels of *L. monocytogenes* in all wells were almost similar, about 10^3 CFU/mL of BHI agar. *L. monocytogenes,* as a mono-culture, rapidly reached 10^9 CFU/mL in lots with no plastic membrane (N-PM), untreated plastic membrane (U-PM) and plasma-treated plastic membrane (P-PM) after 14 days of incubation at 8°C, indicating that neither of plastic membranes affected the growth of this food-borne pathogen. The presence of *Carnobacterium divergens* led to a significant reduction in number of *L. monocytogenes* that reached 1.4×10^8 and 8.4×10^6 CFU/mL of BHI agar in wells covered with a piece of plastic membrane treated with *C. divergens* (U-PM + *Carno*) and in wells covered with a piece of plasma-treated plastic membrane treated with *C. divergens* (P-PM + *Carno),* respectively, at the end of the experiment ($p<0.05$). Concerning the protective LAB (data not shown), the initial concentration of *C. divergens* was four times higher in sample covered with the P-PM + *Carno* than in U-PM + *Carno* although the difference was not statistically different (4.4×10^7 and 1.1×10^7 CFU/mL of BHI agar respectively). The growth of *C. divergens* did not clearly differ at the end of the

experiment and the final concentrations were 3.0×10^8 and 4.9×10^8 CFU/mL of BHI agar in lots P-PM + *Carno* and U-PM + *Carno*, respectively.

3.4- Antibacterial activity of bio-protective plastic membrane against Listeria monocytogenes in cold-smoked salmon

The addition of *Carnobacterium divergens* through Ar/N_2 plasma-treated plastic membrane was chosen to evaluate antibacterial activity against *L. monocytogenes* in CSS. Growth rates of *L. monocytogenes* with or without *C. divergens* in vacuum-packed CSS are presented in Fig. 3. The initial level of *L. monocytogenes* was similar in all lots, approximately 10^2 CFU/g of salmon. *L. monocytogenes* population did not distinctly change after 7 days of storage at 4°C for all lots. When the temperature was shifted to 8°C, *L. monocytogenes* grew rapidly from 2.8×10^2 to 5.5×10^5 CFU/g of salmon after 28 days of storage. *L. monocytogenes* growth was strongly inhibited with the two *C. divergens* incorporation techniques all over storage ($p<0.05$). Enumeration of the pathogenic bacteria was 2.4×10^2 CFU/g of salmon at the end of storage with *C. divergens* adsorbed on plasma-treated plastic membrane and 5.5×10^2 CFU/g with *C. divergens* added as a spray. Endogenous LAB cell counts increased rapidly from 3.2×10^3 to 4.7×10^7 CFU/g of salmon in sample with *L. monocytogenes* alone (the control). The initial concentration of LAB (supposed to be *C. divergens*) in CSS covered with bio-protective plastic membrane was 6.4×10^7 CFU/g of salmon and remained at the same level all over storage (5.8×107 CFU/g of salmon), whereas it grew from 8.2×10^5 to 2.3×10^8 CFU/g of salmon in case of *C. divergens* added by spraying (data not shown).

3.5- Stability of bio-protective plastic membrane against Listeria monocytogenes

After production (day 0 of storage) bio-protective plasma-treated plastic membrane (P-PM + *Carno*) caused a 3-Log reduction of *L. monocytogenes* counts compared to the control on semi solid BHI agar after 14 days of incubation. Indeed, *L. monocytogenes* in the control, as mono-culture, grew rapidly from 1.3×10^3 to 3.8×10^8 CFU/mL of BHI agar at day 14, whereas bio-protective polypropylene membrane led to a considerable inhibition of *L. monocytogenes* that grew from 1.1×10^3 to 3.8×10^5 CFU/mL. The membranes were still active after 21 and 42 days of storage at 4°C in the empty Petri dish sealed by Parafilm® although a slight decrease in anti-listerial

activity was noticed. The difference in *L. monocytogenes* cell count between the control and P-PM + *Carno* after 14 days of incubation was approximately 2 Log (4.3×10^9 compared to 9.2×10^7 CFU/mL of BHI agar at day 21 of storage; 5.9×10^9 compared to 1.5×10^8 CFU/mL at day 42 of storage) (Figure 4). However, the *C. divergens* viable cells of bio-protective P-PM were almost constant with membranes stored for 0, 21 and 42 at 4°C, increasing from 10^7 at the beginning to approximately 10^9 CFU/mL of BHI agar at the end of the experiment (data not shown).

4- Discussion

The results of this work demonstrated potential production and application of bio-protective plastic membrane made from bacteriocin-producing strains for inhibition of *Listeria monocytogenes* growth in CSS at refrigerated temperatures. Various studies have investigated antibacterial activity of LAB and bacteriocins in food products (Leroi, 2010; Yap, et al., 2021). *Carnobacterium divergens* V41 strain used in the study exerts a bacteriostatic effect against *L. monocytogenes* growth on both model conditions (culture medium) and CSS at chilled temperatures (Brillet, et al., 2004). Using a *C. divergens* V41 mutant depleted in bacteriocin production, Richard et al. (2004) have shown that the activity was due to the production of divercin V41. Although addition of bacteriocins can inhibit the growth of spoilage and pathogenic bacteria in food, the anti-microbial effect reduces over time due to a rapid decrease in bacteriocin concentration (Duffes et al, 1999a; Degli Esposti et al., 2018). An alternative way to overcome this problem is the incorporation of viable bacterial cells onto the film matrix for bacteriocin production during the storage of food at refrigerated temperatures (Concha-Meyer et al., 2011; Degli Esposti et al., 2018). Plastics have been commonly used for food packaging thanks to low cost, long shelf-life, convenience, easy handling and storage (Allahvaisi, 2012). In this work polypropylene membrane was chosen as a carrier for *C. divergens* cells in the production of bio-protective plastic film against *L. monocytogenes*. Adhesive property of plastic membrane was considerably improved by cold treatment plasma (Hegemann et al., 2003). The treatment results were confirmed by dramatic decreases in contact angle with water, diiodomethane and ethylene glycol. However, the hydrophobic recovery was observed with time due to oxidation of the plastic surface (Bormashenko et al., 2021). In the study, the hydrophobic recovery was also noticed

due to evaluation of the kinetics of hydrophobic recovery following plasma treatment by measuring angle contact of plastic membrane with water over 27 days of storage at room temperature (data not shown). Hence, in the study plastic membrane was used within 7 days after the plasma treatment. In order to produce bio-protective plastic membrane, *C. divergens* V41 cells were adsorbed on the polypropylene surface (untreated and plasma-treated membrane) and dried under laminar flow hood overnight at room temperature. One or 2 hours after placing the membrane onto the surface of BHI agar, *C. divergens* migrated into agar. Although not statistically different, *C. divergens* count was 4 times higher with plasma-treated membrane that with untreated one, in relation with the higher adherence visualized by SEM. This could explain why *C. divergens* adsorbed on plasma-treated membrane were more efficient on reduction of *L. monocytogenes* than *C. divergens* adsorbed on untreated membrane. (+ 1.2 Log CFU/mL of BHI agar of reduction at the end of storage). Although *C. divergens* culture was washed before used, it is also possible that divercin was produced and adhered to the membrane during the three hours of membrane soaking followed by the drying period for one night at room temperature.

In vacuum-packed CSS, *L. monocytogenes* was completely inhibited by both *C. divergens* added through plasma-treated plastic membrane and by spraying. However, bio-protective plasma-treated membrane resulted in a 2-times stronger inhibition of *L. monocytogenes* growth in comparison with spraying method (2.4×10^2 CFU/g and 5.5×10^2 CFU/g of salmon at the end of shelf-life, respectively). This may be related to the initial concentration of *C. divergens* in CSS which was higher in slices covered by bio-protective plastic membrane (about 10^7 CFU/g salmon) than in sprayed slices (10^6 CFU/g). Subsequent growth in sprayed CSS slices led to higher concentration after 28 days of storage (10^8 *vs* 10^7 CFU/g with plastic membrane), but this had few incidence on the anti-listerial activity. Therefore, the initial concentration of *C. divergens* seems crucial to observe a good inhibition, confirming work in our laboratory (confidential data). Concha-Meyer et al. (2011) have successfully developed a new way of incorporating LAB strain in CSS, or a combination of both strains and nisin, by mixing them in alginate. This led to a bacteriostatic effect on *L. monocytogenes* in CSS over a period of 28 days at 4°C. This method, as well as the addition of *C. divergens* by spraying, might cause an increase in humidity of food products, which

favors endogenous bacterial growth and speed up the spoilage process. Bio-protective plastic membrane may be a good alternative to avoid this problem.

In this study, viability of *C. divergens* cells on bio-protective plastic membrane stored at 4°C remained fairly constant over a period of 42 days. However, slight decrease in anti-listerial activity of membrane was noticed (3-Log reduction of *L. monocytogenes* at day 0 and 2 Log at day 21 and 42). Bacteriocin production by *C. divergens* cells which may occur on the membrane may be negatively affected by low temperature and long-time storage (Jung, et al., 1992; Schillinger et al., 1996).

4- **Conclusions**

The results obtained from this work indicated that bio-protective plasma-treated polypropylene membrane was highly effective against *Listeria monocytogenes* growth in both medium model and CSS during refrigerated storage. These membranes are commonly used in industry to separate CSS slices and this approach can be interesting for potential application in food preservation with an industrial development as new packaging. However antibacterial activity of plastic membrane should be evaluated on other spoilage and pathogenic bacteria as well as on sensory characteristics of CSS. In addition, production of free-dryed *Carnobacterium divergens* cells could be studied in the future in order to facilitate mass fabrication of bio-protective plastic membrane at industrial level.

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References

Allahvaisi, S. (2012). Polypropylene in the industry of food packaging. *Polypropylene*. Faith Dogan (Ed.)

ANSES (2002). Fiche de description de danger biologique transmissible par les aliments: *Listeria monocytogenes*. Saisine n°2016-SA-0081. Mise à jour: Avril 2020. https://www.anses.fr/fr/system/files/BIORISK2016SA0081Fi.pdf.

Aymerich, T., Rodriguez, M., Garriga, M., & Bover-Cid, S. (2019). Assessment of the bioprotective potential of lactic acid bacteria against *Listeria monocytogenes* on vacuum-packed cold-smoked salmon stored at 8 degrees C. *Food Microbiology*, *83*, 64-70. https://doi.org/10.1016/j.fm.2019.04.011

Begren, S., Ivaniuk, F., Gigout-Chevalier, F., Kolypczuk, K., Bonnetot, B., Leroi, F., Grovel, O., Delbarre-Ladrat, D., & Passerini, D. (2020). New insight into antimicrobial compounds from food and marine-sourced *Carnobacterium* species through phenotype and genome analyses.

Microorganisms, *8*(7), 1093. https://doi.org/10.3390/microorganisms8071093. Open Access version : https://archimer.ifremer.fr/doc/00641/75335/

Bormashenko, E., Legchenkova, I., Navon-Venezia, S., Frenkel, M., & Bormashenko, Y. (2021). Investigation of the Impact of Cold Plasma Treatment on the Chemical Composition and Wettability of Medical Grade Polyvinylchloride. *Applied Sciences*, *11*(1), 300. https://doi.org/10.3390/app11010300

Brillet, A., Pilet, M. F., Prevost, H., Bouttefroy, A., & Leroi, F. (2004). Biodiversity of *Listeria monocytogenes* sensitivity to bacteriocin-producing *Carnobacterium* strains and application in sterile cold-smoked salmon. *Journal of Applied Microbiology*, *97*(5), 1029-1037. https://doi.org/10.1111/j.1365-2672.2004.02383.x

Casaburi, A., Nasi, A., Ferrocino, I., Monaco, R.D., Mauriello, G., Villani, F., & Ercolini, D. (2011). Spoilage-related activity of *Carnobacterium maltaromaticum* strains in air-stored and vacuum-packed meat. *Applied and Environmental Microbiology*, *77*(20), 7382-7393. https://doi.org/10.1128/AEM.05304-11

Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *OJL 338, 22-12-2005*, 1-26.

Concha-Meyer, A., Schöbitz, R., Brito, C., & Fuentes, R. (2011). Lactic acid bacteria in an alginate film inhibit *Listeria monocytogenes* growth on smoked salmon. *Food Control*, *22*(3-4), 485-489. https://doi.org/10.1016/j.foodcont.2010.09.032

Connil, N., Plissoneau, L., Onno, B., Pilet, M.-F., Prévost, H., & Dousset, X. (2002). Growth of *Carnobacterium divergens* and production of biogenic amines and divercin V41 in sterile cold-smoked salmon extract at varying temperatures, NaCl levels, and glucose concentraions. Journal of Food Production, *65*(2), 333-338. https://doi.org/10.4315/0362-028X-65.2.333

Degli Esposti, M., Toselli, M., Sabia, C., Messi, P., de Niederhäusern, S., Bondi, M., & Iseppi, R. (2018). Effectiveness of polymeric coated films containing bacteriocin-producer living bacteria for *Listeria monocytogenes* control under simulated cold chain break. *Food microbiology*, *76*, 173-179. https://doi.org/10.1016/j.fm.2018.05.005

Duffes, F. (1999). Improving the control of *Listeria monocytogenes* in cold smoked salmon. *Trends in Food Science & Technology*, *10*(6-7), 211-216. https://doi.org/10.1016/S0924-2244(99)00051-5

Duffes, F., Corre, C., Leroi, F., Dousset, X., & Boyaval, P. (1999a). Inhibition of *Listeria monocytogenes* by in situ produced and semipurified bacteriocins of *Carnobacterium* spp. on vacuum-packed, refrigerated cold-smoked salmon. *Journal of food protection*, *62*(12), 1394-1403. https://doi.org/10.4315/0362-028X-62.12.1394

Duffes, F., Leroi, F., Boyaval, P., & Dousset, X. (1999b). Inhibition of *Listeria monocytogenes* by *Carnobacterium* spp. strains in a simulated cold smoked fish system stored at 4°C. *International journal of food microbiology*, *47*(1-2), 33-42. https://doi.org/10.1016/S0168-1605(98)00206-2

Duffes, F., Leroi, F., Dousset, X., & Boyaval, P. (2000). Use of a bacteriocin producing *Carnobacterium piscicola* strain, isolated from fish, to control *Listeria monocytogenes* development in vacuum-packed clod-smoked salmon stored at 4 °C. *Science des Aliments*, *20*, 153-158.

Hartmann, H. A., Wilke, T., & Erdmann, R. (2011). Efficacy of bacteriocin-containing cell-free culture supernatants from lactic acid bacteria to control *Listeria monocytogenes* in food. *International Journal of Food Microbiology*, *146*(2), 192-199. https://doi.org/10.1016/j.ijfoodmicro.2011.02.031

Hegemann, D., Brunner, H., & Oehr, C. (2003). Plasma treatment of polymers for surface and adhesion improvement. *Nuclear instruments and methods in physics research section B: Beam interactions with materials and atoms*, *208*, 281-286. https://doi.org/10.1016/S0168-583X(03)00644-X

Joffraud, J.J., Leroi, F., Roy, C., & Berdagué, J.L. (2001). Characterisation of volatile compounds produced by bacteria isolated from the cold-smoked salmon flora. *International Journal of Food Microbiology*, *66*, 175-184. https://doi.org/10.1016/S0168-1605(00)00532-8

Jung, D.-S., Bodyfelt, F. W., & Daeschel, M. A. (1992). Influence of fat and emulsifiers on the efficacy of nisin in inhibiting *Listeria monocytogenes* in fluid milk. *Journal of dairy science*, *75*(2), 387-393. https://doi.org/10.3168/jds.S0022-0302(92)77773-X

Leisner, J. J., Laursen, B. G., Prévost, H., Drider, D., & Dalgaard, P. (2007). *Carnobacterium*: positive and negative effects in the environnment and in foods. *FEMS Microbiology Reviews*, *31*(5), 592-613. https://doi.org/10.1111/j.1574-6976.2007.00080.x

Leroi, F. (2010). Occurrence and role of lactic acid bacteria in seafood products. *Food microbiology*, *27*(6), 698-709. https://doi.org/10.1016/j.fm.2010.05.016

Løvdal, T. (2015). The microbiology of cold smoked salmon. *Food Control*, *54*, 360-373. https://doi.org/10.1016/j.foodcont.2015.02.025

Maillet, A., Denojean, P., Bouju-Albert, A., Scaon, E., Leuillet, S., Dousset, X., Jaffrès, E., Combrisson, J., & Prévost, H. (2021). Characterization of bacterial communities of cold-smoked salmon during storage. *Foods*, *10*(2), 362. https://doi.org/10.3390/foods10020362

Mauriello, G., De Luca, E., La Storia, A., Villani, F., & Ercolini, D. (2005). Antimicrobial activity of a nisin-activated plastic film for food packaging. *Letters in Applied Microbiology*, *41*(6), 464-469. https://doi.org/10.1111/j.1472-765X.2005.01796.x

Metivier, A., Pilet, M.-F., Dousset, X., Sorokine, O., Anglade, P., Zagorec, M., Picard, J.-C., Marion, D., Cenatiempo, Y., & Fremaux, C. (1998). Divercin V41, a new bacteriocin with two disulphide bonds produced by *Carnobacterium divergens* V41: primary structure and genomic organization. *Microbiology*, *144*(10), 2837-2844. https://doi.org/10.1099/00221287-144-10-2837

Pilet, M.-F., Dousset, X., Barré, R., Novel, G., Desmazeaud, M., & Piard, J.-C. (1995). Evidence for two bacteriocins produced by *Carnobacterium piscicola* and *Carnobacterium divergens* isolated from fish and active against *Listeria monocytogenes*. *Journal of Food Protection*, *58*(3), 256-262. https://doi.org/10.4315/0362-028X-58.3.256

Richard, C., Brilet, M.F., Pilet, H., Prévost, H., & Drider, D. (2003). Evidence on inhibition of *Listeria monocytogenes* by divercin V41 action. *Letters in Applied Microbiology*, *36*(5), 288-292. https://doi.org/10.1046/j.1472-765X.2003.01310.x

Richard, C., Drider, D., Elmorjani, K., Marion, D. & Prévost, H. (2004). Heterologous expression and purification of active divercin V41, a class IIa bacteriocin encoded by a synthetic gene in *Escherichia coli*. *Journal of Bacteriology*, *186*(13), 4276-4284. https://doi.org/10.1128/JB.186.13.4276-4284.2004

Rihakova, J., Belguesmia, Y., Petit, V.W., Pilet, M.F., Prévost, H., Dousset, X., & Drider, D. (2009). Divercin V41 from gene characterization to food applications: 1998-2008, a decade of solved and unsolved questions. *Letters in Applied Microbiology*, *48*(1), 1-7. https://doi.org/10.1111/j.1472-765X.2008.02490.x

Rodrigues, D., Sousa, S., Rocha-Santos, T., Silva, J., Lobo, J. S., Costa, P., Amaral, M. H., Pintado, M. M., Gomes, A. M., Malcata, F. X., Malcata, F. X., & Freitas, A. C. (2011). Influence of L-cysteine, oxygen and relative humidity upon survival throughout storage of probiotic bacteria in whey protein-based microcapsules. *International Dairy Journal*, *21*(11), 869-876. https://doi.org/10.1016/j.idairyj.2011.05.005

Saraoui, T., Cornet, J., Guillouet, E., Pilet, M.F., Chevalier, F., Joffraud, J.J., & Leroi, F. (2017). Improving simultaneously the quality and safety of cooked and peeled shrimp using a cocktail of bioprotective lactic acid bacteria. *International Journal of Food Microbiology*, *241*, 69-77. http://dx.doi.org/10.1016/j.ijfoodmicro.2016.09.024

Schillinger, U., Geisen, R., & Holzapfel, W. (1996). Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods. *Trends in Food Science & Technology*, *7*(5), 158-164. https://doi.org/10.1016/0924-2244(96)81256-8

Stohr, V., Joffraud, J.J., Cardinal, M., & Leroi, F. (2001). Spoilage potential and sensory profile associated with bacteria isolated from cold-smoked salmon. *Food Research International*, 34, 797-806. https://doi.org/10.1016/S0963-9969(01)00101-6

Tahiri, I., Desbiens, M., Kheadr, E., Lacroix, C., & Fliss, I. (2009). Comparison of different application strategies of divergicin M35 for inactivation of *Listeria monocytogenes* in cold-smoked wild salmon. *Food Microbiology*, *26*, 783-793. https://doi.org/10.1016/j.fm.2009.05.003

Wiernasz, N., Leroi, F., Chevalier, F., Comet, J., Cardinal, M., Rohloff, J., Passerini, D., Skírnisdóttir, S., & Pilet, M.F. (2020). Salmon gravlax biopreservation with six lactic acid bacteria: A polyphasic approach to assessing the impact on organoleptic properties, microbial ecosystem and volatilome composition. *Frontiers in microbiology*, *10*(3103). https://doi.org/10.3389/fmicb.2019.03103

Yap, P.-C., MatRahim, N.-A., AbuBakar, S., & Lee, H. Y. (2021). Antilisterial Potential of Lactic Acid Bacteria in Eliminating *Listeria monocytogenes* in Host and Ready-To-Eat Food Application. *Microbiology Research*, *12*(1), 234-257. https://doi.org/10.3390/microbiolres12010017

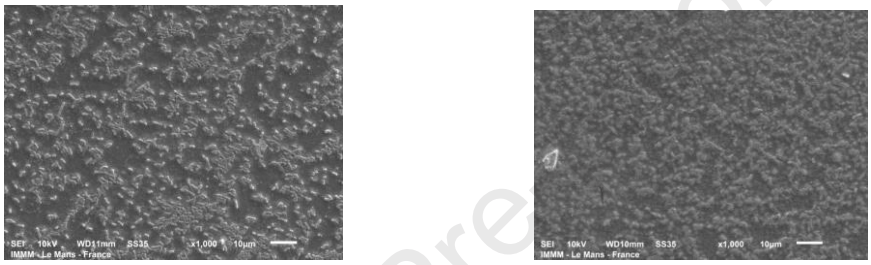
Tables

Table 1. Contact angle results of polypropylene membrane before and after plasma treatment

Treatment	Contact angle measurement (°) ± SD		
	Water	Diiodomethane	Ethylene glycol
Untreated membrane	96 ± 3	63 ± 1	68 ± 1
O_2	27 ± 1	36 ± 1	7 ± 1
Ar and O_2	24 ± 1	35 ± 1	8 ± 2
Ar and N_2	11 ± 2	27 ± 3	8 ± 1

Figures

Fig. 1. SEM images of untreated (A) and Ar/N_2 plasma-treated polypropylene membrane (B) adsorbed with *Carnobacterium divergens* (magnification ×1000)



A B

Fig. 2. Growth of *Listeria monocytogenes* in semi solid BHI agar at 8°C (Log10 CFU/mL BHI agar). Lot N-PM: *L. monocytogenes* alone, without plastic membrane; U-PM: *L. monocytogenes* and a piece of untreated plastic membrane; P-PM: *L. monocytogenes* and a piece of Ar/N_2 plasma-treated plastic membrane; U-PM + *Carno*: *L. monocyogenes* with *Carnobacterium divergens* adsorbed on untreated plastic membrane; P-PM + *Carno*: *L. monocytogenes* with *C. divergens* adsorbed on Ar/N_2 plasma-treated plastic membrane. The error bars indicate ± standard deviation of the means.

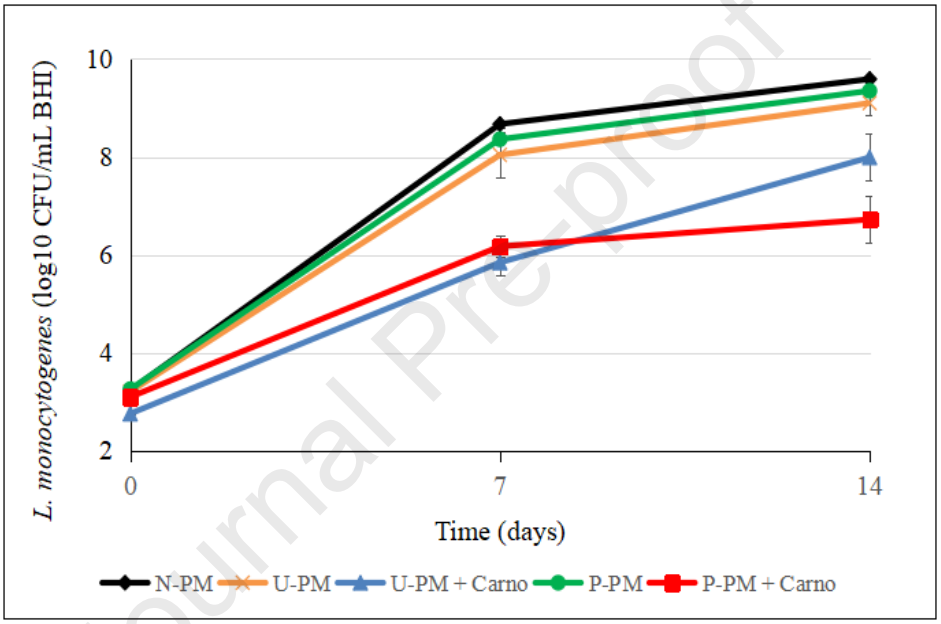


Fig.3. Growth of *Listeria monocytogenes* in cold-smoked salmon at 4°C for 7 days and at 8°C for 21 days (log10 CFU/g salmon). P-PM: *L. monocytogenes* alone and plasma-treated plastic membrane; P-PM + *Carno*: *L. monocytogenes* with *Carnobacterium divergens* adsorbed on plasma-treated plastic membrane; S - *Carno*: *L. monocytogenes* with *C. divergens* sprayed on cold-smoked salmon. The error bars indicate ± standard deviation of the means.

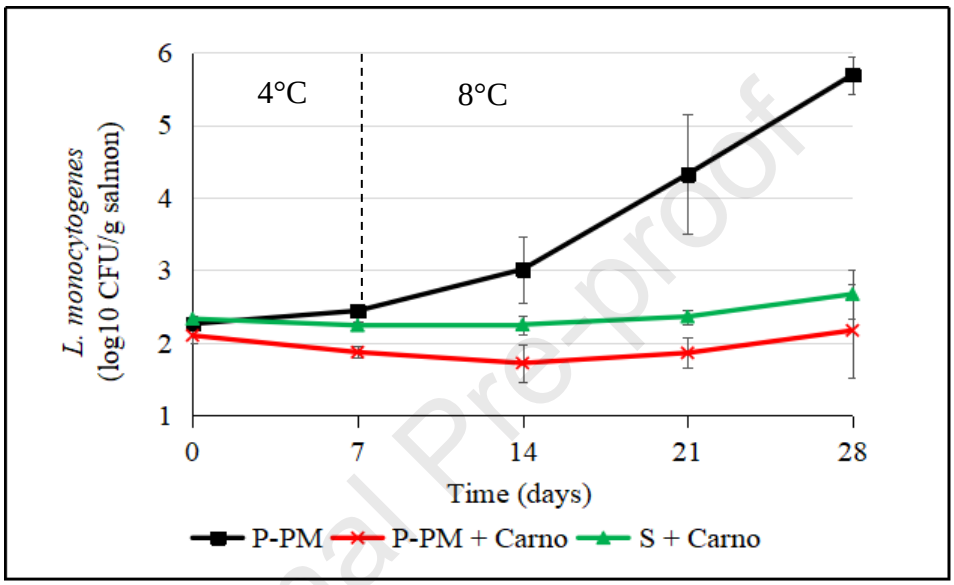
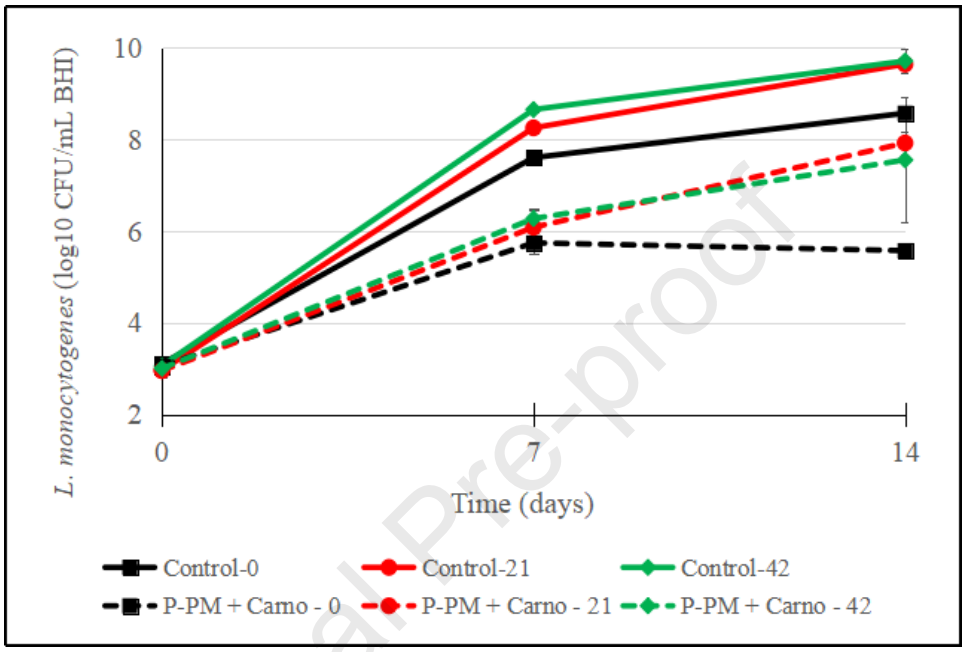


Fig. 4. Stability of bio-protective plasma-treated plastic membrane stored for 0, 21 and 42 at 4°C, estimated by antibacterial activity against *Listeria monocytogenes* on semi solid BHI agar as mono-culture (control) and co-culture with *Carnobacterium divergens* adsorbed on P-PM (P-PM + *Carno*).



Highlights

- Modification of polypropylene surface by plasma treatment in order to fix bioprotective *Carnobacterium divergens* bacteria.
- Antilisterial activity of bio-protective plastic film tested in BHI and smoked salmon.
- Assessment of stability of bio-protective polypropylene film in BHI over storage time.
- Potentiality of development of a new active food packaging

Conflict of interest form

Manuscript title : Antilisterial Activity of Polypropylene Functionalized with Living *Carnobacterium Divergens* in Cold-Smoked Salmon

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