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1 **ANTIBACTERIAL ACTIVITY OF**
2 **PLASMA-TREATED POLYPROPYLENE MEMBRANE FUNCTIONALIZED**
3 **WITH LIVING *CARNOBACTERIUM DIVERGENS***
4 **IN COLD-SMOKED SALMON**

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22

23 ABSTRACT

24 In recent years, bacteriocins produced by lactic acid bacteria (LAB) have shown
25 great potential for food safety preservation, especially for ready-to-eat products. In
26 this study, bio-protective membrane was made from plasma-treated polypropylene
27 film and functionalized with *Carnobacterium divergens* V41 (bacteriocin-producing
28 strain) for the purpose of inhibiting *Listeria monocytogenes* growth in culture media
29 and cold-smoked salmon (CSS) at refrigerated temperatures. In semi solid Brain Heart
30 Infusion (BHI) agar, bio-protective plastic membrane led to a 3-Log reduction in *L.*
31 *monocytogenes* count compared to the control, after 14 days of aerobic incubation at
32 8°C. In vacuum-packed CSS, *L. monocytogenes* growth was inhibited by bio-
33 protective plastic membrane after 7 days of storage at 4°C and 21 days at 8°C.
34 Antilisterial activity of plastic membrane was even better than *C. divergens* cells
35 added in CSS by direct spraying. Stability test has shown that bio-protective plastic
36 membrane stored for 42 days at 4°C still exerted antimicrobial activity against *L.*
37 *monocytogenes* on BHI agar (2-Log reduction compared to the control). These
38 preliminary results demonstrate that bio-protective plastic membrane can be used to
39 control pathogenic bacteria in food products with potential industrial development.

40

41 Key-words: bacteriocin, polypropylene membrane, plasma, *Carnobacterium*
42 *divergens*, *Listeria monocytogenes*, cold-smoked salmon

43

44 **1- Introduction**

45 Contamination with *Listeria monocytogenes* is a major concern in food industry
46 because of its ubiquitous occurrence and its ability to survive and persist in
47 unfavorable environments. *L. monocytogenes* is responsible for listeriosis, a disease
48 lethal in 20-30% of the case especially for immune deficient people, elderly and
49 pregnant women. The number of case in Europe has been increasing steadily since
50 2006 to reach 5.6 cases of listeriosis/million inhabitants in 2019 (ANSES, 2020). This
51 microorganism is present in a wide range of raw and ready-to-eat food products
52 (Hartmann, et al., 2011; Løvdal, 2015) and cold-smoked salmon (CSS) is not an
53 exception. Indeed, salting and smoking steps contribute to minimize spoilage and
54 pathogenic bacterial contamination but do not totally eliminate *L. monocytogenes*. Its
55 prevalence in retail CSS is highly variable, from 0 to 61% giving an average value of

56 9.8% according to publications post year 2000 (Duffes, 1999; Løvdal, 2015).
57 Although generally present at low concentration just after processing, *L.*
58 *monocytogenes* can grow during the storage at refrigerated temperature under air or
59 vacuum-packaging and sometimes overpass the 100 CFU/g European regulatory limit
60 (Commission Regulation (EC) No 2073/2005). Therefore, inhibition of *L.*
61 *monocytogenes* growth contributes to minimize life-threatening health hazards and
62 extend the shelf life of CSS.

63 Many lactic acid bacteria (LAB) are widely used in food preservation (known as
64 biopreservation) owing to the capacity of production of a large variety of
65 antimicrobial substances (organic acids, bacteriocins, hydrogen peroxide...)
66 (Hartmann et al., 2011). *Carnobacterium* sp., frequently found in a large number of
67 food including fish, meat and some dairy products, is a promising genus as many
68 strains produce bacteriocins with antilisterial activity (Aymerich et al., 2019; Begrem
69 et al., 2021), do not acidify flesh and have few negative impact on the quality of food
70 (Joffraud et al., 2001; Stohr et al., 2001; Leisner et al., 2007; Leroi, 2010; Casaburi et
71 al., 2011). Among the twelve reported species, *Carnobacterium divergens*,
72 *Carnobacterium maltaromaticum* (formerly *Carnobacterium piscicola*) and
73 *Carnobacterium inhibens* have gained a lot of attention as protective cultures in order
74 to inhibit growth of *L. monocytogenes* in seafood products in last two decades (Duffes,
75 1999; Duffes et al., 1999a; Tahiri et al., 2009; Wiernasz et al., 2020; Passerini et al.,
76 2021). A wide range of studies have used the strain *C. divergens* V41 as a protective
77 culture in CSS and shrimp against *L. monocytogenes* (Duffes et al., 1999b, 2000;
78 Brillet et al., 2004; Saraoui et al., 2017). This strain has been isolated from salmon
79 viscera by Pilet et al. (1995). Its antilisterial activity is due to the production of the
80 extensively studied class IIa bacteriocin called divercin V41, which exerts its activity
81 by forming pore in target cell membrane (Connil et al., 2002; Metivier et al., 1998;
82 Richard et al., 2003, 2004; Rihakova et al., 2009). Previous study has shown that the
83 effect of the semi-purified divercin V41 had an immediate listericidal effect in CSS
84 which did not last during the 4 weeks of storage at chilled temperature. Introduction
85 of alive *C. divergens* V41 by spraying the pre-culture onto the surface inhibited
86 growth of *L. monocytogenes* till the end of shelf-life (usually 4 weeks) (Duffes et al.,
87 1999b). However, spraying bacteria tends to introduce water in the product, which
88 may favor growth of undesirable bacteria. Development of active packaging with
89 protective bacteria or their metabolites is a new trend to control the development of

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

96

97 **2- Materials and methods**

98 *2.1- Bacterial strains and culture media*

99 *Carnobacterium divergens* V41 and *Listeria monocytogenes* RF191 used in this
100 work were provided by EM³B laboratory (Ifremer, France). *C. divergens* V41 (co-
101 property Ifremer/Oniris, Nantes, France) was isolated from trout intestine and
102 characterized by Pilet et al. (1995). This strain produces divercin V41 which is a heat-
103 stable, 4.5 kDa class IIa bacteriocin (Metivier et al., 1998). Elliker and BHI broth with
104 20% sterile glycerol added (v/v) were used to prepare *C. divergens* and *L.*
105 *monocytogenes* aliquots which were stored at -20°C until use, respectively. Overnight
106 bacterial cultures were prepared by transferring aliquots to fresh Elliker or BHI broth
107 (1%, v/v) and incubated at 30°C or 37°C for *C. divergens* and *L. monocytogenes*,
108 respectively.

109 *2.2 - Preparation of bio-protective plastic membrane from alive Carnobacterium* 110 *divergens*

111 *2.2.1 - Improvement of adhesive property of polypropylene membrane*

112 Polypropylene films similar to those used in food industry packaging were
113 selected (purchased at a French smokehouse). Those membranes, in form of plastic
114 film, are commonly used to separate CSS slices for convenience. The membranes are
115 in form of rectangle (6 cm width and 12 cm length) and were used as a carrier for *C.*
116 *divergens* in production of bio-protective plastic films. In order to enhance adhesive
117 property of polypropylene membrane, cold plasma treatment was chosen to insert OH
118 groups on plastic surface and change its property from hydrophobic to hydrophilic.
119 Plasma treatment was performed at Centre de Transfert de Technologie (CTTM, Le
120 Mans University, France) using oxygen (O₂), argon (Ar) and nitrogen (N₂) gases
121 under the following parameters:

122 - O₂: 10 standard cm³/min (sccm) sweeping for 3 min at 0 W and then treating for
123 7 min at 200 W.

124 - Combination of Ar and O₂: Ar, 10 sccm sweeping for 3 min at 0 W and then
125 treating for 5 min at 200 W. Afterwards, O₂, 10 sccm sweeping for 3 min at 0 W and
126 then treating for 5 min at 200 W.

127 - Combination of Ar and N₂: Ar, 10 sccm sweeping for 3 min at 0 W and then
128 treating for 5 min at 200 W. Afterwards, N₂ 10 sccm sweeping for 3 min at 0 W and
129 then treating for 5 min at 200 W.

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

133

134 2.2.2 - Preparation of bio-protective polypropylene membrane

135 4200 mL of an overnight *C. divergens* culture in Elliker (approximately 10⁸
136 CFU/mL) was centrifuged at 8000 g at 4°C for 10 min in order to collect bacterial
137 cells. The pellet was re-suspended in 420 mL of fresh sterile Elliker broth to obtain a
138 suspension containing about 10⁹ CFU/mL. The suspension was transferred to 12
139 sterile square Petri dishes (120×120 mm) (35 mL/dish). The plasma-treated and
140 untreated plastic membranes were then soaked in *C. divergens* suspension for 3 h and
141 dried overnight under laminar flow hood at room temperature. Bio-protective
142 polypropylene membranes were placed in sterile square Petri dish sealed with
143 Parafilm[®] and stored at 4°C until use. Distribution of *C. divergens* cells on plastic
144 surface was visualized by using scanning electron microscope (SEM).

145 2.3- Evaluation of antibacterial activity of bio-protective polypropylene film against 146 *Listeria monocytogenes* in model conditions (semi solid Brain Heart Infusion agar)

147 Five mL of semi solid BHI agar (0.8% (w/v) agar) were poured in each well of five
148 sterile plastic plates used for cell culture (6 wells/plate). Solidified agar wells were
149 then surface-inoculated with 50 µL of a *L. monocytogenes* overnight culture diluted in
150 order to obtain a level of 10³ CFU/mL of BHI agar. The plates were then covered with
151 different plastic membranes as follow : First plate (Lot N-PM) : No plastic membrane ;
152 Second plate (Lot U-PM) : a piece of plastic membrane of 4 cm² (2 x 2 cm) deposited
153 onto the agar of each well ; Third plate (Lot P-PM) : a piece of plasma-treated plastic
154 membrane; Fourth plate (Lot U-PM + *Carno*) : a piece of plastic membrane treated

155 with *C. divergens* ; Fifth plate (Lot P-PM + *Carno*) : a piece of plasma-treated plastic
156 membrane treated with *C. divergens*. Plastic plates were sealed by parafilm and then
157 incubated at 8°C for 14 days. Bacterial enumeration was carried out at day 0, 7 and 14.
158 Plastic membranes were removed from the wells and BHI agar from each well was
159 transferred to stomacher plastic bag with filter. Sterile physiological saline solution
160 (40 mL, 0.1% (w/v) tryptone (Biokar) and 0.85% (w/v) NaCl) was added. The sample
161 was homogenized for 2 minutes in a stomacher (Lab Blender, London, UK) at room
162 temperature and serial dilutions in physiological saline solution were prepared for
163 bacterial enumeration. *C. divergens* was enumerated by surface plating onto Elliker
164 agar (Biokar) containing two antibiotics (gentamycin and streptomycin, 5 µg/mL for
165 each) and incubated anaerobically at 30°C for 48 h. *L. monocytogenes* growth was
166 completely inhibited by addition of gentamycin and streptomycin. *L. monocytogenes*
167 was determined by surface plating onto Palcam agar (BK145, Biokar) containing a
168 selective supplement (BS00408, Biokar) under aerobic incubation at 37°C for 24 h.
169 All the experiments were done in sextuple.

170

171 2.4- Evaluation of antilisterial activity of bio-protective plastic membrane in cold- 172 smoked salmon

173 Fresh vacuum-packed CSS slices (approximately 33-35 g/slice) used in this work
174 were purchased from a French smokehouse company, transported to laboratory one
175 day before experiment and stored at 4°C. Composition of CSS was analyzed in
176 triplicate, including water 59.58%, protein 17.64%, lipid 16.10%, salt 2.57% in water
177 phase, phenols 0.46% and ash 3.58%. First *Listeria monocytogenes* overnight culture
178 diluted to reach 10⁴ CFU/mL was evenly sprayed on all salmon slices (1% v/w) in
179 order to obtain a level of approximately 10² CFU/g of salmon. After 1 h conservation
180 at 4°C for absorption, bio-protective plastic membranes (plasma-treated membrane
181 treated with *C. divergens*) were placed on salmon slices. In order to make a
182 comparison with a method commonly used in Ifremer, *Carnobacterium divergens* was
183 also incorporated on another batch of CSS by spraying (1% v/w) with a level of about
184 10⁶ CFU/g of salmon. The level was proven to be necessary for significant inhibition
185 of *L. monocytogenes* (Duffes et al., 1999b). The control was slices of CSS covered
186 with plasma-treated membrane without the addition of *C. divergens*. The salmon
187 slices were then vacuum-packed and incubated for 28 days under the following

188 conditions: 7 days at 4°C which corresponds to the storage temperature in distributors
189 and 21 days at 8°C which is considered as the usual storage temperature in consumer
190 refrigerators. Bacterial enumeration was performed at day 0, 7, 14, 21 and 28 of
191 incubation. Salmon flesh (23-25 g) was aseptically cut into small pieces and then
192 transferred to a stomacher plastic bag with filter. Physiological saline solution was
193 added in a 4:1 ratio (v/w). The sample was homogenized for 2 min in a stomacher and
194 left at room temperature for 30 min for resuscitation. The homogenate was serially
195 diluted in physiological saline solution for bacterial enumeration. Total LAB,
196 including *C. divergens* V41, and *L. monocytogenes* were enumerated as previously
197 described. Cell counts of *L. monocytogenes* below 10² CFU/g of salmon were
198 determined by gently mixing 1 mL of the mother suspension with 20 mL of molten
199 Palcam agar cooled down at 50°C. The experiments were performed in triplicate.

200

201 2.5- Evaluation of stability of bio-protective plastic membrane against *Listeria* 202 *monocytogenes*

203 After preparation as described above, bio-protective plastic membranes were
204 placed in empty square Petri dish sealed with Parafilm[®] and stored at 4°C to evaluate
205 their stability over time (day 0, 21 and 42). Stability was assessed by testing
206 antibacterial activity against *L. monocytogenes* in semi solid BHI agar as described in
207 2.3 and by enumeration of *C. divergens* viable cell count. The experiment was carried
208 out in triplicate.

209

210 2.6- Statistical analysis

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

215

216 3- Results

217 3.1- Change of plastic membrane property after plasma treatment

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

221 and hydrophobic. Contact angle of untreated membrane is 96, 63, 68° with water,
222 diiodomethane and ethylene glycol, respectively. After treatment with O₂, Ar/ O₂ and
223 Ar/N₂, a sharp decrease in contact angle of plastic membrane was observed with all
224 liquids tested. This change indicated that plastic surface became hydrophilic after
225 treatment. Plasma treatment using Ar/N₂ gave the best change in contact angle and
226 then was chosen to improve adhesive property of plastic membrane used in all
227 experiments below.

228

229 3.2- Adsorption of *Carnobacterium divergens* cells on polypropylene surface

230 Scanning electron microscopy (SEM) images of adsorption of *C. divergens* cells
231 on untreated and Ar/N₂ plasma-treated polypropylene membrane are shown in Fig. 1.
232 It seems likely that distribution of bacterial cells was more homogeneous on treated
233 plastic surface than the other. Hence plasma-treated polypropylene membrane (P-PM)
234 can be used as a carrier in order to add bio-protective bacterial strains to food
235 products.

236

237 3.3- Antibacterial activity of bio-protective plastic membrane against *Listeria* 238 *monocytogenes* in semi solid BHI agar

239 The result of *L. monocytogenes* enumeration in semi solid BHI agar is presented
240 in Fig. 2. As expected, the initial levels of *L. monocytogenes* in all wells were almost
241 similar, about 10³ CFU/mL of BHI agar. *L. monocytogenes*, as a mono-culture,
242 rapidly reached 10⁹ CFU/mL in lots with no plastic membrane (N-PM), untreated
243 plastic membrane (U-PM) and plasma-treated plastic membrane (P-PM) after 14 days
244 of incubation at 8°C, indicating that neither of plastic membranes affected the growth
245 of this food-borne pathogen. The presence of *Carnobacterium divergens* led to a
246 significant reduction in number of *L. monocytogenes* that reached 1.4×10⁸ and
247 8.4×10⁶ CFU/mL of BHI agar in wells covered with a piece of plastic membrane
248 treated with *C. divergens* (U-PM + *Carno*) and in wells covered with a piece of
249 plasma-treated plastic membrane treated with *C. divergens* (P-PM + *Carno*),
250 respectively, at the end of the experiment (p<0.05). Concerning the protective LAB
251 (data not shown), the initial concentration of *C. divergens* was four times higher in
252 sample covered with the P-PM + *Carno* than in U-PM + *Carno* although the
253 difference was not statistically different (4.4×10⁷ and 1.1×10⁷ CFU/mL of BHI agar
254 respectively). The growth of *C. divergens* did not clearly differ at the end of the

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

257

258 3.4- Antibacterial activity of bio-protective plastic membrane against *Listeria* 259 *monocytogenes* in cold-smoked salmon

260 The addition of *Carnobacterium divergens* through Ar/N₂ plasma-treated plastic
261 membrane was chosen to evaluate antibacterial activity against *L. monocytogenes* in
262 CSS. Growth rates of *L. monocytogenes* with or without *C. divergens* in vacuum-
263 packed CSS are presented in Fig. 3. The initial level of *L. monocytogenes* was similar
264 in all lots, approximately 10^2 CFU/g of salmon. *L. monocytogenes* population did not
265 distinctly change after 7 days of storage at 4°C for all lots. When the temperature was
266 shifted to 8°C, *L. monocytogenes* grew rapidly from 2.8×10^2 to 5.5×10^5 CFU/g of
267 salmon after 28 days of storage. *L. monocytogenes* growth was strongly inhibited with
268 the two *C. divergens* incorporation techniques all over storage ($p < 0.05$). Enumeration
269 of the pathogenic bacteria was 2.4×10^2 CFU/g of salmon at the end of storage with *C.*
270 *divergens* adsorbed on plasma-treated plastic membrane and 5.5×10^2 CFU/g with *C.*
271 *divergens* added as a spray. Endogenous LAB cell counts increased rapidly from
272 3.2×10^3 to 4.7×10^7 CFU/g of salmon in sample with *L. monocytogenes* alone (the
273 control). The initial concentration of LAB (supposed to be *C. divergens*) in CSS
274 covered with bio-protective plastic membrane was 6.4×10^7 CFU/g of salmon and
275 remained at the same level all over storage (5.8×10^7 CFU/g of salmon), whereas it
276 grew from 8.2×10^5 to 2.3×10^8 CFU/g of salmon in case of *C. divergens* added by
277 spraying (data not shown).

278

279 3.5- Stability of bio-protective plastic membrane against *Listeria monocytogenes*

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

288 activity was noticed. The difference in *L. monocytogenes* cell count between the
289 control and P-PM + *Carno* after 14 days of incubation was approximately 2 Log
290 (4.3×10^9 compared to 9.2×10^7 CFU/mL of BHI agar at day 21 of storage; 5.9×10^9
291 compared to 1.5×10^8 CFU/mL at day 42 of storage) (Figure 4). However, the *C.*
292 *divergens* viable cells of bio-protective P-PM were almost constant with membranes
293 stored for 0, 21 and 42 at 4°C, increasing from 10^7 at the beginning to approximately
294 10^9 CFU/mL of BHI agar at the end of the experiment (data not shown).

295

296 **4- Discussion**

297 The results of this work demonstrated potential production and application of bio-
298 protective plastic membrane made from bacteriocin-producing strains for inhibition of
299 *Listeria monocytogenes* growth in CSS at refrigerated temperatures. Various studies
300 have investigated antibacterial activity of LAB and bacteriocins in food products
301 (Leroi, 2010; Yap, et al., 2021). *Carnobacterium divergens* V41 strain used in the
302 study exerts a bacteriostatic effect against *L. monocytogenes* growth on both model
303 conditions (culture medium) and CSS at chilled temperatures (Brillet, et al., 2004).
304 Using a *C. divergens* V41 mutant depleted in bacteriocin production, Richard et al.
305 (2004) have shown that the activity was due to the production of divercin V41.
306 Although addition of bacteriocins can inhibit the growth of spoilage and pathogenic
307 bacteria in food, the anti-microbial effect reduces over time due to a rapid decrease in
308 bacteriocin concentration (Duffes et al, 1999a; Degli Esposti et al., 2018). An
309 alternative way to overcome this problem is the incorporation of viable bacterial cells
310 onto the film matrix for bacteriocin production during the storage of food at
311 refrigerated temperatures (Concha-Meyer et al., 2011; Degli Esposti et al., 2018).
312 Plastics have been commonly used for food packaging thanks to low cost, long shelf-
313 life, convenience, easy handling and storage (Allahvaisi, 2012). In this work
314 polypropylene membrane was chosen as a carrier for *C. divergens* cells in the
315 production of bio-protective plastic film against *L. monocytogenes*. Adhesive property
316 of plastic membrane was considerably improved by cold treatment plasma
317 (Hegemann et al., 2003). The treatment results were confirmed by dramatic decreases
318 in contact angle with water, diiodomethane and ethylene glycol. However, the
319 hydrophobic recovery was observed with time due to oxidation of the plastic surface
320 (Bormashenko et al., 2021). In the study, the hydrophobic recovery was also noticed

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

337 In vacuum-packed CSS, *L. monocytogenes* was completely inhibited by both *C.*
338 *divergens* added through plasma-treated plastic membrane and by spraying. However,
339 bio-protective plasma-treated membrane resulted in a 2-times stronger inhibition of *L.*
340 *monocytogenes* growth in comparison with spraying method (2.4×10^2 CFU/g and
341 5.5×10^2 CFU/g of salmon at the end of shelf-life, respectively). This may be related to
342 the initial concentration of *C. divergens* in CSS which was higher in slices covered by
343 bio-protective plastic membrane (about 10^7 CFU/g salmon) than in sprayed slices (10^6
344 CFU/g). Subsequent growth in sprayed CSS slices led to higher concentration after 28
345 days of storage (10^8 vs 10^7 CFU/g with plastic membrane), but this had few incidence
346 on the anti-listerial activity. Therefore, the initial concentration of *C. divergens* seems
347 crucial to observe a good inhibition, confirming work in our laboratory (confidential
348 data). Concha-Meyer et al. (2011) have successfully developed a new way of
349 incorporating LAB strain in CSS, or a combination of both strains and nisin, by
350 mixing them in alginate. This led to a bacteriostatic effect on *L. monocytogenes* in
351 CSS over a period of 28 days at 4°C. This method, as well as the addition of *C.*
352 *divergens* by spraying, might cause an increase in humidity of food products, which

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

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

361

362 4- Conclusions

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

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377

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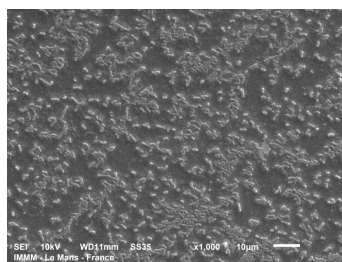
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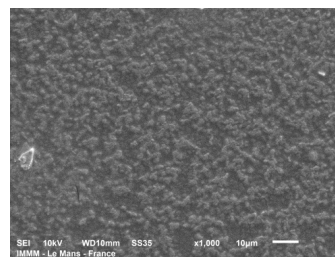
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Figures

Fig. 1. SEM images of untreated (A) and Ar/N₂ 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/N₂ plasma-treated plastic membrane; U-PM + *Carno*: *L. monocytogenes* with *Carnobacterium divergens* adsorbed on untreated plastic membrane; P-PM + *Carno*: *L. monocytogenes* with *C. divergens* adsorbed on Ar/N₂ plasma-treated plastic membrane. The error bars indicate \pm standard deviation of the means.

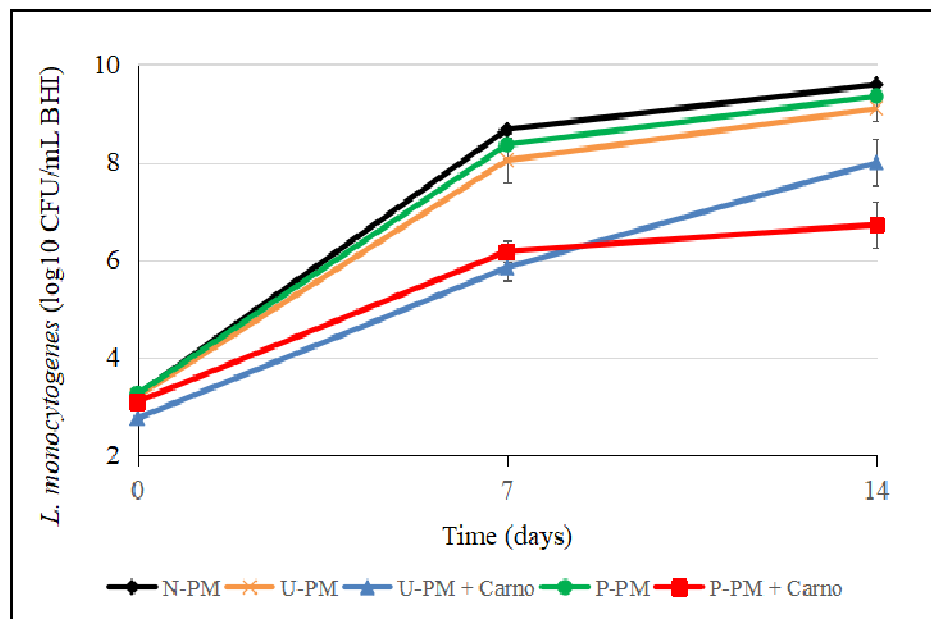


Fig.3. Growth of *Listeria monocytogenes* in cold-smoked salmon at 4°C for 7 days and at 8°C for 21 days (log₁₀ CFU/g salmon). P-PM: *L. monocytogenes* alone and plasma-treated plastic membrane; P-PM + *Carno*: *L. monocytogenes* with *Carnobacterium divergens* adsorbed on plasma-treated plastic membrane;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

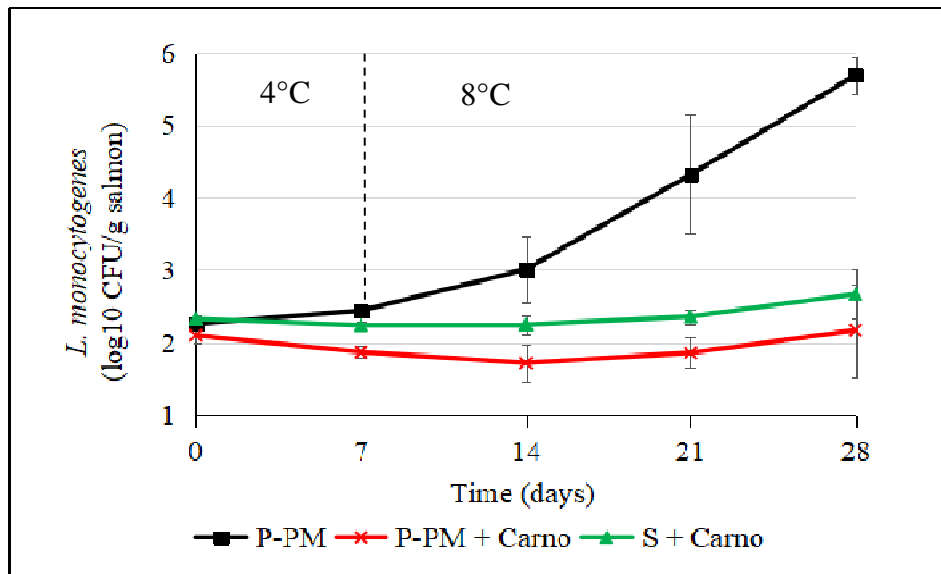
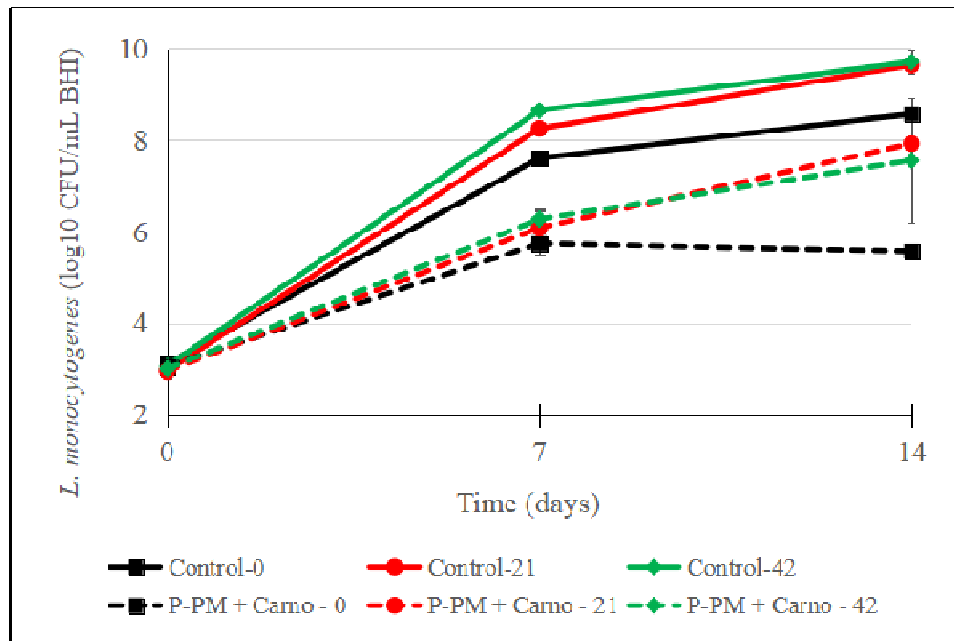


Fig. 4. Stability of bio-protective plasma-treated plastic membrane stored for 0, 21 and 42 at 4°C, estimated by antibacterial activity against *Listeria monocytogenes* on semi solid BHI agar as mono-culture (control) and co-culture with *Carnobacterium divergens* adsorbed on P-PM (P-PM + *Carno*).



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