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Bacteriostatic or bactericidal? Impedometric measurements to test the antimicrobial activity of *Arthrospira platensis* extract

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1 **Bacteriostatic or bactericidal? Impedometric measurements to test the antimicrobial**  
2 **activity of *Arthrospira platensis* extract**

3  
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10  
11  
12 **Abstract**

13 In recent years, increasing consumer's demand for even cleaner label, functional, safe and  
14 high quality products has led to searching for new antimicrobial agents of natural origin that  
15 can improve quality and safety with reducing the impact on the product composition. In this  
16 scenario the use of algae extracts in food formulations as antimicrobial agents is taking more  
17 and more interest.

18 In particular, the antimicrobial potential of *Arthrospira platensis* has already been tested *in*  
19 *vitro*, using the agar well diffusion method or the broth dilution method, that can provide a  
20 quantitative estimation of minimum inhibitory concentration (MIC).

21 Even if several methods are already in use to test the MIC and minimum bactericidal  
22 concentration (MBC) of antimicrobials, the aim of the present research was to propose  
23 impedance measurement as a valid method for the *in vitro* evaluation of MIC and MBC of a  
24 natural antimicrobial extract of *A. platensis*. To this purpose, six different concentrations of *A.*  
25 *platensis* extract (0.1%, 0.15%, 0.2%, 0.25%, 0.3% and 0.5% v/v) were tested on

26 *Pseudomonas fluorescens* and *Serratia liquefaciens*, two species commonly involved in food  
27 spoilage, and *Listeria innocua*, as representative of the human food borne pathogenic species  
28 *Listeria monocytogenes*.

29 The results obtained confirmed the *in vitro* antimicrobial potential of *A. platensis* extract, but  
30 also highlighted how MIC and MBC could be different depending on both the concentration  
31 of antimicrobial and the tested strain. Furthermore, the proposed method allowed the  
32 identification of MIC and MBC values in a new way never used before for this purpose.

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34 **Keywords:** Impedometric measurement, *Arthrospira platensis* extract, natural antimicrobial,  
35 minimum inhibitory concentration, minimum bactericidal concentration.

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50 **1. Introduction**

51 Preservation techniques such as heat treatment, salting, acidification and drying are  
52 commonly used to prevent growth of spoilage and pathogenic bacteria in foods (Lucera et al.,  
53 2012). Those methods certainly lead to a modification of the natural composition of products,  
54 often affecting food quality standards such as nutritional, sensory, odor, color and texture  
55 (Lucera et al., 2012; Pisoschi et al., 2018). For these reasons, in a hurdle technology approach,  
56 antimicrobial compounds have been extensively used by industries to ensure food safety and  
57 protect products from spoilage along preparation, storage and distribution.

58 However, the addition of compounds of synthetic origin in food formulations fights with the  
59 increasing consumer's demand for even more clean label, functional, and high quality  
60 products. In recent years the interest in searching for new antimicrobial agents of natural  
61 origin that can improve quality and safety with reducing the impact on the product is growing  
62 (Ricci et al., 2019). Furthermore, natural preservatives could also constitute a valid alternative  
63 to address the problem of the growing microbial resistance to antibiotics (Lucera et al., 2012;  
64 Pisoschi et al., 2018).

65 To date, several natural antimicrobials exerting different roles are in use by food industries,  
66 and they are commonly grouped by the diverse origin: plants, animals, microbes and fungi  
67 (Pisoschi et al., 2018). Among the ingredients with high technological potential, that can be  
68 used as natural preservatives, seaweeds and microalgae are gaining a lot of attention (Martelli  
69 et al., 2020a; Pina-Pérez et al., 2017). Among them, the use of *Arthrospira platensis* in food  
70 formulation is taking more and more interest because of consumers request (Ásványi-Molnár  
71 et al., 2009; Barkallah et al., 2017; Batista et al., 2017; Beheshtipour et al., 2012; de Caire &  
72 Parada, 2000; Golmakani et al., 2019; Martelli et al., 2020b; Varga & Molnár-Ásványi, 2012;  
73 Zouari et al., 2011). *A. platensis*, commercially known as Spirulina, is a fresh-water  
74 cyanobacterium that has attracted a lot of attention due to its growing employment as human  
75 foodstuff and for its potential functional properties. This cyanobacterium is one of the most

76 important among microalgae showing antimicrobial activity towards many bacterial  
77 pathogens and fungi. It represents a novel source for antimicrobials because of its high level  
78 of amino acids and small peptides, phycocyanobilin, polyphenols, carotenoids, chlorophyll  
79 and other compounds that have proven an *in vitro* antimicrobial activity (Amaro et al., 2011;  
80 Elshouny et al., 2017; Kumar et al., 2013; Mala et al., 2009).

81 Until now, the antimicrobial potential of algae in general and *A.platensis* in particular, has  
82 already been tested *in vitro*, using the agar well diffusion method (Cakmak et al., 2014;  
83 Manivannan et al., 2011; Martelli et al., 2020a) or the broth dilution method (Gupta et al.,  
84 2010) that can provide a quantitative estimation of minimum inhibitory concentration (MIC).

85 In general, the methods for *in vitro* evaluation of antimicrobial activity has been widely  
86 reviewed by Balouiri et al. (2016) who listed and discussed in detail all the advantages and  
87 limitations of the methods actually in-use.

88 Nowadays, the disk-diffusion and broth or agar dilution methods are the most used, for ease  
89 of application, cost-effectiveness and immediacy of results interpretation. On the other hand,  
90 they are time-consuming and they could be subjected to manual undertaking and risk of errors  
91 by the users (Balouiri et al., 2016). The absorbance measurement of cell cultures is commonly  
92 associated to such mentioned methods, however it presents some limitation, such as the need  
93 for a calibration step, in order to correlate the results with viable counts, or the absence of cell  
94 physiological state consideration (Chorianopoulos et al., 2006).

95 Other techniques such as time-kill test or flow cytometry and bioluminescent methods are  
96 also used but, associated with the high performances, they have the disadvantages of needing  
97 specific equipment and user's training (Balouiri et al., 2016).

98 In addition, not all these methods enable the evaluation of MIC and minimum bactericidal  
99 concentration (MBC) with the same and unique approach.

100 Considering all these facts, the aim of the present research was to propose impedance  
101 measurement as a valid method for the *in vitro* evaluation of MIC and MBC of an *A. platensis*  
102 antimicrobial extract.

103 This method, initially used as growth index of lactic acid bacteria in milk (LAB) (Lanzanova  
104 et al., 1993) was recently reassessed by Bancalari et al. (2016) for the evaluation of the *in*  
105 *vitro* growth kinetics of LAB. Conversely, in the present research it was used to test the effect  
106 of six different concentrations of *A. platensis* extract (0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.5%  
107 v/v) on *Pseudomonas fluorescens* and *Serratia liquefaciens*, two species commonly involved  
108 in food spoilage, and on *Listeria innocua* as representative of the human food borne pathogen  
109 species *Listeria monocytogenes*.

110

## 111 **2. Material and methods**

### 112 *2.1. Arthrospira platensis extraction process*

113 An extraction process from dried *A. platensis*, kindly provided by S.a.Ba.r. (Novellara, RE,  
114 Italy), was carried out as described by Martelli et al. (2020a). Briefly, 100 mL of  
115 ethanol/water (70:30 v/v) acidified with 1% formic acid (CH<sub>2</sub>O<sub>2</sub>) solvent was used for  
116 extraction of 10 g of *A. platensis*. A double extraction was carried out, alternating twice a  
117 shaking cycle to a sonication one in Ultrasonic Cleaner sonicator (VWR, United States). The  
118 sample was then centrifuged (Eppendorf 5800 Centrifuge, Model 5810R, Hamburg,  
119 Germany) at 12,857 x g for 10 min at 10 °C. The solution was filtered with filter paper to  
120 recover the solid part so as to proceed to a second extraction. The two extracts obtained were  
121 combined and concentrated until fully dried with a rotary evaporator Strike 300 (Steroglass,  
122 Italy) at 4x g at a bath temperature of 40 °C. The concentrated extract was then suspended in  
123 sterile water to recover the soluble part, and then stored at -80 °C until use for antimicrobial

124 activity test. The obtained extract had a concentration of 235 mg/ml (23.5 % v/v). The final  
125 pH of the obtained extract was 4.0.

126

## 127 2.2. Bacterial strains and culture conditions

128 *P. fluorescens* 5026, *S. liquefaciens* 5006, and *L. innocua* Lin6 strains were used for the  
129 experiments. The strains, belonging to the Food Microbiology unit collection of the Food and  
130 Drug Department (University of Parma), have been previously isolated from different food  
131 matrixes and identified by 16S rRNA sequencing.

132 The strains, maintained as frozen stock cultures in Tryptic Soy Broth (TSB) (Oxoid, Ltd.,  
133 Basingstoke, United Kingdom) broth containing 20% (v/v) glycerol at  $-80^{\circ}\text{C}$ , were recovered  
134 in TSB by two overnight sub-culturing (2% v/v) at  $37^{\circ}\text{C}$  for *L. innocua* and  $30^{\circ}\text{C}$  for *P.*  
135 *fluorescens* and *S. liquefaciens*.

136

## 137 2.3. Experimental design and growth conditions

138 The activity of *A. platensis* extract on the tested strains was evaluated following the  
139 experimental design reported in Figure 1.

140 TSB (Oxoid) was reconstituted to 30 g/L (w/v) and sterilized at  $121^{\circ}\text{C}$  for 20 min. *A.*  
141 *platensis* extract was then added to reach a final concentration (v/v) in the medium of: a)  
142 0.50%; b) 0.30%; c) 0.25%; d) 0.20%; e) 0.15%; f) 0.10%; g) 0% (negative control sample  
143 without any addition of extract) (Fig.1).

144 The last sub-culturing step of each bacterial culture was ten-fold diluted in Ringer solution  
145 (Oxoid) and inoculated (2% v/v) in 18 mL of the growth media supplemented, respectively  
146 and separately, with all the different concentrations of *A. platensis* extract (Fig.1).

147 A 6 mL aliquot of each inoculated medium was then transferred into three sterilized BacTrac  
148 4300® vials (SY-LAB, Neupurkersdorf, Austria), and incubated at the optimal growth

149 temperature of each strain (37°C for *L. innocua* and 30°C for *P. fluorescens* and *S.*  
150 *liquefaciens*).  
151 After 30 hours of incubation, the content of the three vials for each strains was aseptically  
152 mixed and used to inoculate the fresh TSB medium in the flask. All the analysis were carried  
153 out in triplicate and monitored for 30 h by measuring the impedometric signal every 10 min.

#### 154 2.4. Impedometric measurement

155 Impedance measurements were performed by means of BacTrac 4300® Microbiological  
156 Analyzer system (SY-LAB, Neupurkersdorf, Austria). requiring the use of dedicate glass  
157 measuring cells (vials) with 4 electrodes.

158 The specific impedance E% value was measured and recorded every 10 min for 30 h  
159 (Bancalari et al., 2016; Bancalari et al., 2019). Each experiment was replicated twice and each  
160 analytical variable was measured in triplicate.

161 The results of the impedometric measurements were analyzed as previously reported by  
162 Bancalari et al. (2016) and the Lag and yEnd values, together with the observation of  
163 impedometric curves, were considered to evaluate the bacteriostatic or bactericidal activity of  
164 the antimicrobial extract on the tested strains, defining MIC and MBC.

165

#### 166 2.5. Evaluation of MIC and MBC

167 The MIC value was defined as the lowest concentration able to inhibit the growth of the tested  
168 strains. In our case, no growth means that no Lag values were recorded in 30 hours.

169 The MBC was determined by sub-culturing the cells exposed to different concentration of the  
170 antimicrobial extract, used to evaluate MIC, in fresh TSB medium and defined when no Lag  
171 values were recorded in 30 hours (Fig.1).

172

#### 173 2.6. Culture-independent viable counts



174 Fluorescence microscopy count was obtained by using the LIVE/DEAD<sup>®</sup> BacLight<sup>™</sup>  
175 Bacterial Viability kit (Molecular Probes, Oregon, USA) and Nikon Eclipse 80i  
176 epifluorescence microscope (Nikon, Tokyo, Japan) equipped with a C-SHG1 100 W mercury  
177 lamp. Nikon filter set B2A FITC was used for Lbh1 FITC labelled probe (excitation  
178 wavelength, 450–490 nm; emission wavelength, 500–520 nm). Nikon filter set G-2E/C was  
179 used for St4 Cy3-labelled probe (excitation wavelength, 540/25 nm; emission wavelength,  
180 605/55 nm). Pictures of each field were taken and then superimposed through the Nis  
181 Elements software (ver. 2.10 Nikon). (Bottari et al., 2010; Gatti et al., 2006; Santarelli et al.,  
182 2013). The analysis was performed on the sub-cultured cells after 30 h of incubation. One mL  
183 of ten-fold diluted sub-cultures was stained with LIVE/DEAD<sup>®</sup> and after 15 minutes filtered  
184 onto black polycarbonate filters (0.2 µm pore size) (Millipore Corp., Billerica, MA, USA).  
185 Then it was visualized by epifluorescence microscope (Nikon 80i, Tokyo, Japan) and cells  
186 were counted as previously described (Bottari et al., 2010; Gatti et al., 2006; Santarelli et al.,  
187 2013).

188 A minimum of five separate counts were performed for each sample. Results were reported as  
189 average values  $\pm$  standard deviation of total, viable and non-viable cells referred to one ml.

190

### 191 **3. Results and discussion**

#### 192 *3.1. Evaluation of A. platensis extract MIC by impedometric measurement*

193 The impedometric analysis was performed by means of BacTrac 4300<sup>®</sup> that enables the  
194 detection of bacteria activity in real time via the decrease of the impedance in an alternating  
195 current (AC) field. In fact, during duplication, bacteria viable cells break down sugars present  
196 in the medium into smallest molecules that make the medium more conductive, decreasing the  
197 overall resistance and total impedance. This variation is due only to the presence and

198 duplication of bacteria, and thus it is used as a measure of their metabolism (Bancalari et al.,  
199 2016).

200 The instrument is able to register, during the incubation time, two specific impedance values  
201 for each single measurement: i) the conventional conductance value (M-value) that  
202 corresponds to the overall medium impedance, and ii) the capacitance value (E-value) which  
203 is the measure of electrochemical double layer impedance in the vicinity of the electrodes.

204 Both these values, simultaneously recorded every 10 minutes, are shown as relative changes  
205 compared to a starting value and expressed as M% and E%. Furthermore, they are also  
206 visualized in real-time in a capacitance or conductance curve (Bancalari et al., 2019).

207 As the value of the double layer capacitance is more sensitive to any slight alteration in the  
208 nearby of the electrodes, it was more suitable for the measurement in TSB, and therefore E-  
209 value was chosen for this investigation (SY-LAB microbiology).

210 Differently from the method already proposed and available in literature (Chorianopoulos et  
211 al., 2006; Puttaswamy, 2013), at the end of the analysis all recorded capacitance data (E%)  
212 were used in two different ways: i) were fitted by the Gompertz equation, following the  
213 method previously reported by Bancalari et al. (2016) to obtain the kinetic parameter Lag  
214 and yEnd; ii) were used to build a graphical representation of the original capacitance curve  
215 (Fig. 2).

216 Lag value is an adjustment period measured in hours, and the greater the value, the bigger the  
217 time that the strains need to adapt before starting grow. yEnd is the highest variation of  
218 impedance recorded and it has been interpreted as the maximum acidifying capacity of the  
219 LAB strains (Fig. 2) (Bancalari et al., 2016). In this case, for the bacterial species considered  
220 in this study, it could be interpreted as metabolic capacity (Sauer et al., 2019; Silby et al.,  
221 2011; Yang & Bashir, 2008).

222 The calculated Lag and yEnd values, allowed to estimate and display the bacteriostatic  
223 activity, quantified as the Minimum Inhibitory Concentration (MIC) and bactericidal activity,  
224 as Minimum Bactericidal Concentration (MBC), of *A. platensis* extract on the tested strains.  
225 At first, MIC was evaluated by inoculating the strains in the medium in presence of different  
226 concentration of *A. platensis* extract and without any addition as a negative control (0%) as  
227 schematized in Figure 1.

228 By comparing the Lag values of the strains grown with different percentages of *A. platensis*  
229 extract to those obtained in the negative control, we were able to evaluate the presence or not  
230 of an inhibitory effect on strains growth. In particular, if the Lag values, in presence of the  
231 extract, were equal to the negative control ones, no effect was detected. On the other hand, if  
232 the Lag values increased along with the concentration of *A. platensis* extract used, an effect on  
233 the bacterial growth took place. When detected, Lag values were expressed in hours, while,  
234 when no growth was detected, “nd” was reported, which means that no variation of  
235 capacitance (E%) values were recorded in 30 hours (Table 1).

236 In the case of *L. innocua*, the Lag values were < 1 hour, both for the control and 0.10% of *A.*  
237 *platensis* extract, so in this case no effect on the growth was observed (Table 1). On the other  
238 hand, by increasing the concentration of antimicrobial extract, an initial, even if small, effect  
239 was detected when 0.15% was used. This concentration prolonged the Lag time until 1.21  
240 hours, but at the same time also affected the metabolic capacity of the strain, by lowering the  
241 yEnd value (Table 1). This means that the cells were in greatest difficulty as compared to the  
242 control, first of all at the beginning of their growth phase (>Lag), but also they were not able  
243 anymore to reach their maximum metabolic capacity. This effect is clearly visible also from  
244 the conductance curves (Fig. 3A1). Moving forward to the highest concentration of *A.*  
245 *platensis* extract, no growth was detected anymore, thus MIC value was established at 0.20%  
246 (Table 1). The same effect observed for *L. innocua* at 0.10% of extract in the medium was

247 found also for *P. fluorescens* and *S. liquefaciens*, even if these last showed a lower Yend  
248 value underling a lower metabolic capacity in TSB respect to *L. innocua* (Fig. 3B1 and 3C1,  
249 Table 1). Moreover, *P. fluorescens* growth was more affected by the addition of 0.15% of  
250 extract compared to *L. innocua*, as it showed a higher increase of the Lag phase, that reached  
251 27 hours, with a yEnd values of 3. This result means that a little amount of cells was able to  
252 grow, in fact they took a lot of time to leave the Lag phase. Consequently, they were not able  
253 to impact on the overall capacitance of the media and, therefore, also a lower value of yEnd  
254 was recorded (Table 1 and Fig. 3B1) (D’Incecco et al., 2020). However, as for *L. innocua*,  
255 MIC value of *P. fluorescens* was 0.20% (Table 1).

256 *S. liquefaciens*, showed a highest resistance to *A. platensis* extract, as compared to the other  
257 strains, in fact, its Lag phase was 19 hours at 0.20%, and it still maintained a good metabolic  
258 capacity, with a yEnd value of 18 (Table 1). MIC values for *S. liquefaciens* was 0.25% (Table  
259 1). This difference among species was already observed by Whiting & Buchanan (2007) who  
260 pointed out that the growth range and/or inactivation characteristics of bacteria may vary  
261 significantly between species and sometimes also within the same species. This underline the  
262 importance of using a fast, easy and reproducible method to test as much variables as possible  
263 to understand the behavior of the strains, ensuring the safety of food processes.

264

### 265 3.2. Evaluation of *A. platensis* extract MBC by impedometric measurement

266 To determine the MBC of the tested strains, the same method as for MIC was used. In  
267 addition, to better underline the bactericidal effect, the trend of capacitance curves obtained  
268 by the sub-cultured strains were also compared. After 30 hours of incubation, the three vials  
269 of each strains tested for MIC were mixed and sub-cultured in a fresh TSB medium and  
270 incubated at the same condition in BacTrac 4300® (Fig. 3A2, 3B2, 3C2). This procedure to  
271 sub-culturing the strains into a fresh medium without the antimicrobial addition is commonly

272 used to determine whether the cells are still alive or not, thus establishing if the effect on cells  
273 is bacteriostatic or bactericidal (Balouiri et al., 2016). As the 0.10% of *A. platensis* extract did  
274 not affect the growth of none of the bacteria tested (Table 1), it was not considered for the  
275 sub-culturing.

276 By determining the Lag and yEnd values of the sub-cultured strains, it was possible to  
277 observed a high variability within the tested species (Table 2). In particular, *L. innocua* and *P.*  
278 *fluoresces*, having both MIC values of 0.20%, when sub-cultured in TSB were able to grow  
279 even with 0.25% of *A. platensis* extract. This means that some alive cells were still present at  
280 the concentration of extract defined as MIC and gave rise to a higher MBC and corresponding  
281 to 0.30% of extract. This MBC value was stated also for *S. liquefaciens* even if it had higher  
282 MIC values compared to the other two strains (Table 2).

283 Going deeper in the results, we can observe that *L. innocua*, even if showed a longer Lag  
284 phase when a growing amount of *A. platensis* extract was used, still maintained a very high  
285 metabolic capacity as revealed from conductance curve observation (Fig. 3A2) and quantified  
286 from Yend value (Table 2). It is interesting to note that the activity of subcultures is at least  
287 equal, or higher than that of the control (Table 1). This means that, even if the number of alive  
288 cell decreased (>Lag), their metabolic activity, after 30 hours of contact with the *A. platensis*  
289 extract, has not been compromised or reduced at all.

290 *S. liquefaciens* showed the lowest Lag values and constant yEnd (Table 2, Fig. 3C2). This  
291 could mean that a greater number of alive cells were present when sub-culturing. In fact, as  
292 we can observe in Figure 3, the curves obtained using the first three concentrations (Fig. 3C2)  
293 were comparable to those obtained for the control (Fig. 3C1).

294 Although MBC referred to *P. fluorescens* is 0.30%, as for the other two strains, observing the  
295 Lag and Yend values (Table 2) and the curves (Fig. 3B2), the contact with *A. platensis* extract

296 for 30 hours, affected *Pseudomonas* cells on a higher extent compared to the other strains  
297 considered.

298 The *in vitro* antimicrobial effect of *A. platensis* extract has been already investigated on  
299 different spoilage and pathogenic bacteria (Kumar et al., 2013) and its activity was mainly  
300 attributed to the presence of lipids, tocopherols, C-phycoecyanin and extracellular  
301 polysaccharides. Considering that the above mentioned extraction method is comparable with  
302 our method, we can attribute to the same compounds the antimicrobial effect observed in the  
303 present study. In any case, further studies need to be carried out to confirm this hypothesis.  
304 More in detail, Sarada et al. (2011) reported that up to 40% of *A. platensis* total protein is  
305 represented by the protein-pigment complexes as C-phycoecyanin (C-PC) that was  
306 demonstrated to be able to control the growth of some Gram-negative bacteria. Despite few  
307 information are present in literature about the specific action of all tocopherols, lipids and  
308 extracellular polysaccharides in *A. platensis* extract, their antimicrobial effect has already  
309 been proved for other natural antimicrobial (Mariod et al., 2010; Ulusoy et al., 2009; Yue et  
310 al., 2017).

311

### 312 *3.3. Culture-independent viable counts*

313 The fluorescence microscopy count was used to estimate the cell number of the sub-cultured  
314 strains, to have a deeper view of the effect of *A. platensis* extract and to confirm the result  
315 obtained with the impedometric method.

316 After 30 hours of incubation of sub-cultured strains into the fresh TSB,  
317 the total (red+ green), viable (green) and dead (red) cells were counted (Bottari et al., 2010;  
318 Fakruddin et al., 2013).

319 In Table 3 the results of the cell counts are reported for each sub-cultured strain with 0.10%,  
320 0.20% and 0.30% of *A. platensis* extract.

321 The antimicrobial effect was variable within the species considered, but evident on both live  
322 and death cells (Table 3). In fact the number of live cells decreased along with the increasing  
323 concentration of extract and, on the other hand, dead cells increased with the increasing  
324 amount of antimicrobial in the medium.

325 In particular, regarding *L. innocua*, the number of live cells fell from 6.94 Log CFU/ml of the  
326 control to 2.47 Log CFU/ml of the sub-cultured cells that were grown with the 0.30% of *A.*  
327 *platensis* extract. This led to a 99.99% of inhibition corresponding to 4 decimal reductions,  
328 allowing to confirm that 0.30% was the MBC for *L. innocua* (Table 3).

329 The same results were obtained for *P. fluorescens* who showed a good reduction of viable  
330 cells, from 6.97 Log CFU/ml to 3.03 Log CFU/ml, with 99.99% of inhibition in the presence  
331 of 0.30% of *A. platensis* extract, confirming this value as the MBC for *P. fluorescens* (Table  
332 3).

333 Slight differences were observed for *S. liquefaciens*, who showed the highest viable cell count  
334 of 3.14 Log CFU/ml with 0.30% of *A. platensis* extract, highlighting a lower antimicrobial  
335 inhibition of 99.98%, but reasonably acceptable the MBC as 0.30% (Table 3).

336 It was therefore interesting to note that also the lowest concentrations of *A. platensis* extract  
337 showed different effect depending on the strains.

338 In fact, the % of inhibition calculated for *L. innocua* in presence of 0.10% of extract was the  
339 lowest if compared to the other two strains. This was in agreement with the impedometric  
340 results that showed how the sub-cultured cells from 0.10% of *A. platensis* extract had the best  
341 metabolic capacity, resulting less affected by the antimicrobial.

342 Conversely, the sub-cultured cells of *P. fluorescens* and *S. liquefaciens* were more affected by  
343 the extract, showing an inhibition of 91.17% and 60.46% respectively (Table 3).

344 To give a better view of the effect of *A. platensis* extract on cell viability, the fluorescence  
345 microscopy images are reported (Fig. 4).

346 For all the strains, the cells in contact with higher amount of *A. platensis* extract, despite their  
347 sub-culturing in a fresh medium, showed a high percentage of mortality (Fig. 4D). This  
348 further confirm the 0.3% of *A. platensis* extract as the MBC for all the tested strains.  
349 Conversely, the lowest concentration of *A. platensis* extract used (Fig. 4B) seems to have no  
350 effect on the cells viability, confirming the data obtained from the impedometric analysis.

351

#### 352 **4. Conclusions**

353 The results obtained from this research have a double impact: firstly, we confirmed the *in*  
354 *vitro* antimicrobial activity of *A. platensis* against two strains representative of food spoilage  
355 species, *P. fluorescens* and *S. liquefaciens*, and a *L. innocua* strain representative of the  
356 pathogenic species *L. monocytogenes*. In fact, even if we did not analyse the composition of  
357 the *A. platensis* extract, we can hypothesize that its activity could be related to the presence of  
358 small peptides, lipids, tocopherols, phycocyanin and extracellular polysaccharides.

359 MIC and MBC values could differ depending on the concentration of antimicrobial and the  
360 strain tested. Indeed, while MIC values varied for the three species considered, 0.20% for *L.*  
361 *innocua* and *P. fluorescens* and 0.25% for *Serratia*, MBC was 0.30% for all the species.

362 Secondly, with the proposed impedometric method we were able to assess MIC and MBC  
363 values in a new way never used before for this purpose. In fact, by evaluating the obtained  
364 parameters, Lag time (hours) and the metabolic capacity (yEnd), we were able to observed  
365 how each single strain differently replies to the variation of antimicrobial concentration.

366 Considering all that, the applications of this approach could be very interesting and impactful  
367 because by measuring the changing of capacitance values, even any small variation caused by  
368 microorganism metabolism are detected, making it a promising method

369 Furthermore, it would represent a good strategy to test other antimicrobial compounds than  
370 *A.platensis* as it allows the analysis of many variables or many samples at the same time and



371 in addition, it gives a fast, objective, reliable and easy-to-interpret result as compared to some  
372 of the conventional methods.

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### 376 **Author contribution**

377 **Elena Bancalari**: Conceptualization, Investigation , Methodology, Formal analysis, Data  
378 curation, Writing original draft- review & editing; **Francesco Martelli**: Conceptualization,  
379 Investigation, Formal analysis **Valentina Bernini**: Conceptualization, Review & editing,  
380 Supervision **Erasmus Neviani**: Review & editing **Monica Gatti**: Conceptualization,  
381 Methodology, Writing - review & editing, Supervision.

382

### 383 **Disclosure**

384 Authors declare that no conflict of interests exists. All authors have approved the final article.

385

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545 **Figure caption**

546 Figure 1. Schematic representation of the experimental design.

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548 Figure 2. Example of capacitance curves fitted to the Modified Gompertz equation (Gibson et  
549 al., 1988) using DMfit version 2.1 Excel add-in (<http://www.combase.cc/index.php/en/tools>).

550 Black circles symbols are the y values that DMfit uses to represent the E% data recorded by  
551 the BacTrac4300 each 10 minutes for 30 hours of incubation. Solid line is the fitted curve

552 described by Modified Gompertz equation. The two parameters used in this experiments can

553 be calculated by the ComBase too: i) lag time (Lag), and iii) maximum value of E% (Yend)

554 (Bancalari et al., 2019).

555

556 Figure 3. Capacitance curve of the three strains grown in presence of different concentrations  
557 of *A.platensis* extract (A1,B1,C1) and than sub-cultured in fresh culture medium (A2,B2,C2).

558 Concentration of *A.platensis* extract solution used for each analysis:

559 (—) 0%, (■) 0.1%, (▲) 0.15, (●)0.2%, (⋯) 0.25%, (◆) 0.3%, (- -) 0.5%

560

561 Figure 4. Microscopy fluorescence images of the sub-cultured cells in presence of the diverse  
562 antimicrobial concentrations, reported as: A) control 0%, B) 0.10%, C) 0.20% and D) 0.3%.

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