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

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

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Abstract

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

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

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

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

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

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

1. Introduction

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

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

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

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

Until now, the antimicrobial potential of algae in general and *A.platensis* in particular, has already been tested *in vitro*, using the agar well diffusion method (Cakmak et al., 2014; Manivannan et al., 2011; Martelli et al., 2020a) or the broth dilution method (Gupta et al., 2010) that can provide a quantitative estimation of minimum inhibitory concentration (MIC). In general, the methods for *in vitro* evaluation of antimicrobial activity has been widely reviewed by Balouiri et al. (2016) who listed and discussed in detail all the advantages and limitations of the methods actually in-use.

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

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

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

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

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

2. Material and methods

2.1. Arthrospira platensis extraction process

An extraction process from dried *A. platensis*, kindly provided by S.a.Ba.r. (Novellara, RE, Italy), was carried out as described by Martelli et al. (2020a). Briefly, 100 mL of ethanol/water (70:30 v/v) acidified with 1% formic acid (CH_2O_2) solvent was used for extraction of 10 g of *A. platensis*. A double extraction was carried out, alternating twice a shaking cycle to a sonication one in Ultrasonic Cleaner sonicator (VWR, United States). The sample was then centrifuged (Eppendorf 5800 Centrifuge, Model 5810R, Hamburg, Germany) at 12,857 x g for 10 min at 10 °C. The solution was filtered with filter paper to recover the solid part so as to proceed to a second extraction. The two extracts obtained were combined and concentrated until fully dried with a rotary evaporator Strike 300 (Steroglass, Italy) at 4x g at a bath temperature of 40 °C. The concentrated extract was then suspended in sterile water to recover the soluble part, and then stored at -80 °C until use for antimicrobial

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

2.2. Bacterial strains and culture conditions

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

The strains, maintained as frozen stock cultures in Tryptic Soy Broth (TSB) (Oxoid, Ltd., Basingstoke, United Kingdom) broth containing 20% (v/v) glycerol at −80°C, were recovered in TSB by two overnight sub-culturing (2% v/v) at 37°C for *L. innocua* and 30°C for *P. fluorescens* and *S. liquefaciens*.

2.3. Experimental design and growth conditions

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

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

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

A 6 mL aliquot of each inoculated medium was then transferred into three sterilized BacTrac 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*

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

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

3. Results and discussion

3.1. Evaluation of A. platensis extract MIC by impedometric measurement

The impedometric analysis was performed by means of BacTrac 4300® that enables the detection of bacteria activity in real time via the decrease of the impedance in an alternating current (AC) field. In fact, during duplication, bacteria viable cells break down sugars present in the medium into smallest molecules that make the medium more conductive, decreasing the 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 el. (2016) to obtained 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).

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

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

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

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

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

3.2. Evaluation of A. platensis extract MBC by impedometric measurement

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

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

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

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

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

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

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

The *in vitro* antimicrobial effect of *A. platensis* extract has been already investigated on different spoilage and pathogenic bacteria (Kumar et al., 2013) and its activity was mainly attributed to the presence of lipids, tocopherols, C-phycocianin and extracellular polysaccharides. Considering that the above mentioned extraction method is comparable with our method, we can attribute to the same compounds the antimicrobial effect observed in the present study. In any case, further studies need to be carried out to confirm this hypothesis.

More in detail, Sarada et al. (2011) reported that up to 40% of *A. platensis* total protein is represented by the protein-pigment complexes as C-phycocyanin (C-PC) that was demonstrated to be able to control the growth of some Gram-negative bacteria. Despite few information are present in literature about the specific action of all tocopherols, lipids and extracellular polysaccharides in *A. platensis* extract, their antimicrobial effect has already been proved for other natural antimicrobial (Mariod et al., 2010; Ulusoy et al., 2009; Yue et al., 2017).

3.3. Culture-independent viable counts

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

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

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

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

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

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

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

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

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

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

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

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

4. Conclusions

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

MIC and MBC values could differ depending on the concentration of antimicrobial and the strain tested. Indeed, while MIC values varied for the three species considered, 0.20% for *L. innocua* and *P. fluorescens* and 0.25% for *Serratia*, MBC was 0.30% for all the species. Secondly, with the proposed impedometric method we were able to assess MIC and MBC values in a new way never used before for this purpose. In fact, by evaluating the obtained parameters, Lag time (hours) and the metabolic capacity (yEnd), we were able to observed how each single strain differently replies to the variation of antimicrobial concentration. Considering all that, the applications of this approach could be very interesting and impactful because by measuring the changing of capacitance values, even any small variation caused by microorganism metabolism are detected, making it a promising method

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

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

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Author contribution

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

Disclosure

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

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Figure caption

Figure 1. Schematic representation of the experimental design.

Figure 2. Example of capacitance curves fitted to the Modified Gompertz equation (Gibson et al., 1988) using DMfit version 2.1 Excel add-in (http://www.combase.cc/index.php/en/tools). Black circles symbols are the y values that DMfit uses to represent the E% data recorded by the BacTrac4300 each 10 minutes for 30 hours of incubation. Solid line is the fitted curve described by Modified Gompertz equation. The two parameters used in this experiments can be calculated by the ComBase too: i) lag time (Lag), and iii) maximum value of E% (Yend) (Bancalari et al., 2019).

Figure 3. Capacitance curve of the three strains grown in presence of different concentrations of *A.platensis* extract (A1,B1,C1) and than sub-cultured in fresh culture medium (A2,B2,C2). Concentration of *A.platensis* extract solution used for each analysis:

(—) 0%, (■) 0.1%, (▲) 0.15, (●)0.2%, (···) 0.25%, (◆) 0.3%, (– –) 0.5%

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