Increase of acidification of synthetic brines by ultrasound-treated Lactiplantibacillus plantarum strains isolated from olives

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ABSTRACT

This paper focused on the evaluation of Ultrasound effect on the growth patterns (3-6% of salt and 45 °C), acidification (pH-decrease), interactions with microorganisms, and membrane permeability of nine strains of Lactiplantibacillus plantarum. Ultrasound treatment was applied at 20% of net power by modulating duration (2–10 min) and pulses (2–10 s).

Viable count (7.15−8.16 log CFU/mL) was never affected by Ultrasound, while the treatment increased the extent of pH decrease of at least three strains (109, 162 and c19). L. plantarum c19 was the best performer, as a low intensity treatment was able to increase its acidification, without affecting its growth. The effects could be attributed to an increased permeability of the cellular membrane, as suggested by the increase of released intracellular components. Other factors should be further assessed (e.g. possible changes in the metabolism) and the performances of Ultrasound-treated strains in real brines.

1. Introduction

One of the most recent advances in table olive processing is the use of autochthonous microorganisms with positive technological traits able not only to improve fermentation performances but also to produce typical food with unique and irreproducible characteristics [1,2]. This trend was moved by the initial consideration that autochthonous strains might be generally more adapted to the microflora naturally present on olives than allochthonous ones, ensuring a better domination of the microbiota, an effective competition against spoilage microflora, and a faster fermentation control from the earliest stages. Thus, several studies have been performed to isolate strains from natural fermentations, to test them for their technological abilities and to select the best performers as starters [2–5].

Some recent studies have demonstrated that low-intensity ultrasound (US) is able to stimulate bacterial metabolism by inactivating or enhancing the activity of different enzymes [6–8]. US is defined as a technology using sonic waves with frequencies from 20 kHz to 10 MHz applied for processing foods, sonochemistry and diagnostic. The application of US in the food industry uses power from 20 to 100 kHz and finds its reason in the numerous advantages associated. Some effects are related to the production of micro- and nano-emulsions [9] or to the extraction of bioactive compounds or essential oils from vegetable cells [10]; others rely upon the capacity to cause desirable changes in the physical and chemical structure of matrices thanks to processes like defoaming, deaeration, filtration, extrusion, etc. [10]. From a microbiological point of view, the effect is dual. The use of US is often aimed to inactive bacteria (lactobacilli and other gram-positive bacteria, pseudomonas, enterobacteria, and staphylococci) and fungi (Candida, Pichia, Rhodotorula, Saccharomyces, Zygosaccharomyces, Aspergillus, Fusarium, Penicillium, and Mucor species) in foods [11] or in biofilm [12]. On the other hand, a positive effect on bacteria has been recently suggested, as an increase in microbial biofilm formation by favouring the transport of oxygen and nutrients to deeper layers of the aggregate [6,12], a delay of acidification and post-acidification in active drinks containing probiotic microorganisms [13], an increase of fermentation efficiency [14] or a major cholesterol removal in the parent cells [15].

Thus, the aim of this study was to evaluate the effects of mild-US treatments on some technological properties of nine autochthonous Lactiplantibacillus plantarum strains isolated during the fermentation of table olives [16, Bevilacqua, unpublished results]. Specifically, the effect of US was evaluated on microbial growth patterns under stressing conditions (high temperature and presence of salt), acidification profiles, interactions with other
microorganisms; a final focus on membrane permeability (release of cell components) of US treated cells was made.

2. Materials and methods

2.1. Strains

Nine strains of L. plantarum, labelled with a numerical code (43, 78, 104, 109, 162, 172, G1, G3, Bevilacqua; unpublished results; c19, [16]) and isolated during the fermentation of table olives, were used in this research. All strains belong to the Culture Collection of the Laboratory of Predictive Microbiology, University of Foggia; they were stored at -20 °C in MRS broth (Oxoid, Milan, Italy) supplemented with 33% (V/V) of sterile glycerol (J.T. Baker, Milan, Italy). Before each assay, the microorganisms were grown twice in MRS broth incubated at 30 °C for 24 h, under anaerobiosis; then, the cultures were centrifuged at 3000 x g for 10 min at 4 °C, the supernatant was discarded, and the pellet re-suspended in distilled water. This operation was repeated two times to remove any residue of lab media and to obtain the bacterial solutions for inoculum.

2.2. Ultrasound treatment (US)

Aliquots of 20 mL of synthetic brine (6% NaCl w/V; 0.1% Glucose w/V; pH adjusted to 6.0 through NaOH 1.0 N) were individually inoculated to ca. 8 log CFU/mL with each tested strain and treated by a VC 130 Vibra Cell Ultrasound equipment (Soniics and Materials Inc., Newtown, CT, USA: net power, 130 W). The combinations used for US processing were listed in Table 1: the treatment was applied at 20% of net power by modulating the duration (2–10 min) and the pulses (2–10 s). Before each treatment, the ultrasonic probe (5 x 60 mm; diameter x the active component of horn) was cleaned with ethanol, washed with sterile distilled water, and put 2–3 cm below the surface of the samples. US treatment did not cause a temperature increase.

The viable count was measured before and after each treatment on MRS agar (37 °C for 48 h under anaerobic conditions). Synthetic brine inoculated with each strain but untreated through US was used as control (A1).

2.3. Growth assays

US-treated strains were inoculated in MRS broth to 5 log CFU/mL; the medium was supplemented with 3 or 6% NaCl or incubated at 45 °C. Growth was evaluated after 24 and 48 h as absorbance at 600 nm using a spectrophotometer UV–Vis DU 640 Beckman (Fullerton, CA, USA).

The results were modelled as Growth Index modified by Racioppo et al. [17]:
GI = Abs<sub>c</sub>/Abs<sub>c0</sub> x 100

where: Abs<sub>c</sub> is the absorbance of US-treated strain and Abs<sub>c0</sub> is the absorbance of control (untreated microorganism, combination A1).

Table 1

| Combinations | Power (%) | Time (min) | Pulse (s) |
|--------------|-----------|------------|-----------|
| A1           | 0         | 0          | 0         |
| B1           | 20        | 2          | 2         |
| B2           | 20        | 2          | 6         |
| B3           | 20        | 2          | 10        |
| C1           | 20        | 6          | 2         |
| C2           | 20        | 6          | 6         |
| C3           | 20        | 6          | 10        |
| D1           | 20        | 10         | 2         |
| D2           | 20        | 10         | 6         |
| D3           | 20        | 10         | 10        |

GI was analysed as follows:
GI < 25%: complete inhibition;
25% < GI < 75%: partial inhibition;
GI > 75%: no inhibition.
GI > 120%: growth enhancement.
Viable count was also evaluated after 24 and 48 h.

2.4. pH-decrease

Aliquots of synthetic brine (6% NaCl w/V; 0.1% Glucose w/V; pH 6.0) were individually inoculated with each US-treated strain to 5 log CFU/mL and incubated at 30 °C; the pH of the medium was evaluated after 24 and 48 h through a pH-meter Crison (Crison Instruments, Barcelona, Spain). Data were analyzed as pH decrease; untreated bacteria were used as controls.

2.5. Interactions with Staphylococcus aureus and Saccharomyces cerevisiae

L. plantarum c19 was studied in interaction with Staph. aureus (test A) and S. cerevisiae (test B); the pathogen was a wild strain isolated from table olives (Bevilacqua, unpublished results) belonging to the Culture Collection of the Laboratory of Predictive Microbiology (University of Foggia), whereas S. cerevisiae EC1118 (Lallemand Pty., Ontario, Canada) was a collection isolate.

Before each assay, Staph. aureus and S. cerevisiae were revitalised in Nutrient Broth (Oxoid) incubated at 37 °C for 48 h and Yeast-Peptone-Glucose broth (YPG; 10 g/L yeast extract; 20 g/L bacteriological peptone; 20 g/L glucose; Oxoid) incubated at 25 °C for 48 h, respectively.

Untreated and US-treated L. plantarum c19 were co-inoculated with Staph. aureus or S. cerevisiae in the synthetic brine at 5 log CFU/mL and incubated at 37 °C (Staph. aureus/L. plantarum) and 30 °C (S. cerevisiae/L. plantarum); after 24 and 48 h, microbiological analyses and measurements of pH were made.

Baird-Parker agar base, with egg yolk tellurite emulsion, incubated at 37 °C for 48 h, was used for Staph. aureus; MRS agar + 0.17 g/L of cycloheximide (Sigma-Aldrich, Milan, Italy), incubated at 37 °C for 48 h under anaerobic conditions, was used for L. plantarum c19; YPG agar, supplemented with 0.1% chloramphenicol (w/V) incubated at 25 °C for 48 h, was used for S. cerevisiae.

2.6. Release of intracellular components

Aliquots of sterile distilled water were inoculated with untreated and US-treated L. plantarum c19 (8 log CFU/mL) and incubated at 30 °C. After 2, 5 and 24 h the UV absorbance of each sample was measured at 260 nm and at 280 nm with a spectrophotometer UV–VIS DU 640 Beckman (Fullerton, CA).

The results were shown as increase of absorbance in US-treated samples as follows:

(ΔAbs<sub>c</sub> - ΔAbs<sub>c0</sub>) x 100

where: ΔAbs<sub>c</sub> is the absorbance of US-treated strain and ΔAbs<sub>c0</sub> is the absorbance of untreated one (A1).

2.7. Statistical analysis

The experiments were performed at least on two independent samples (that is batches prepared in different times and from different cell cultures); for each batch, the analyses were made twice. Significant differences were pointed out through t-test (paired comparison) or one-way ANOVA (Analysis of Variance) and Tukey’s test (multiple comparison); P-level was set to 0.05.

Statistic was done through the software Statistica for Windows, ver.
3. Results and discussion

The first step of this research was the assessment of the viability after US treatments; Table 2 shows the viable count of US-treated strains, compared to the control (untreated microorganism). None of the studied strains was affected by US-treatment, not recording a significant reduction of the viable count which was in the range 7.15–8.16 log CFU/mL (7.28–7.93 log CFU/mL for the combinations D1, D2, and D3, treated for 10 min).

US-treated bacteria were also studied in relation to their ability to grow at 45 °C (although this scenario is not encountered throughout olive production, it was used to test stress conditions, because preliminary experiments revealed that if a stress occurs some strains could experience a significant delay of growth at high temperatures) and with salt added (3 and 6% NaCl); LAB generally experience higher salt amounts in brine during olive fermentation, but in this preliminary step it was important to understand the role of US, without a confounding effect due to a possible interaction with other factors. The results were modelled with the equation of Growth Index (GI) (ratio treated vs untreated microorganisms) and modified to point out a positive effect (growth enhancement). A GI > 75% suggests that the treatment does not affect the growth kinetic of the target strains; a GI < 25% or in the range 25–75% highlights a strong or a partial inhibition, respectively. The importance of this assay is different from the viable count, because the latter measures if US affects viability, i.e. if the strains die or live. But after US treatment, the main question was how these surviving strains could grow. This information could be recovered from GI; in fact, it measures the ability of the survivors to grow: after a US treatment, a microorganism could not lose its viability, but the growth could be affected, delayed, inhibited (for a potential injury) or even enhanced (for a potential improved membrane permeability).

US-treatment significantly affected the growth of the strains 78, 109, 162 and 172 (GI < 25%, data not shown), while for L. plantarum 43, 104, G1, G3 and c19, the data shown in Table 3 suggested that the treatments did not affect the growth at 45 °C or in presence of 3–6% of salt (viable count 8.9–9.3 log CFU/mL in all combinations). A delay at 30 °C after 24 h was found for the strain 43, since GI was < 25% (viable count 6 log CFU/mL vs 8.7–9.1 log CFU/mL for the other strains); however, after 48 h GI was > 75% thus suggesting a not significant differences between the control and US-treated samples.

GI could be also used to highlight a growth enhancement (positive effect) of US-treated samples, that is a growth of the tested strains higher after US-treatment. This positive effect was evidenced by a GI > 120%; this condition was encountered for the strains 104 and G1 at 45 °C (combinations C1 and D3) and in the broth containing 6% salt (combinations B1, B2 and D1) (viable count 9.23–9.56 log CFU/mL).

The effect of US (inhibition or growth enhancement) was estimated on the mean value of GI, because it was optimized in the past as a qualitative tool for growth assessment [17]; however, the large standard deviation of some samples suggests a strong variability of the treatment, at least for some combinations.

Where and how US waves act is a matter of debate and the mode of action on the strains tested in this research should be confirmed at molecular levels and through microscopy; some preliminary results on other microorganisms suggest a modification in the charge of cell surface and an increase in the permeability of membrane, due to a transient formation of pores [6].

Literature reports several mechanisms on yeasts and bacteria and some of them could be useful to try to elucidate the mode of action of US towards the tested strains. Recently, the mechanism of sonoporation (the formation of transient cavities or pores on cell membranes from cavitation) has been postulated by Ojha, Mason, O’Donnell, Kerry, & Tiwari [18]; namely, the effect of US on microbial cells is the result of at least six different mechanisms (cavitation, push, pull, jetting, streaming and translation) which can act by one or together. The main effect of this mechanism is the formation of pores and the increase of permeability of membrane; if this phenomenon occurs only in several parts of membrane, it could be controlled and could also have a positive effect with an increased ratio of the exchange of nutrients and by-products with the outer environment.

Pore formation was found in the past for some strains of L. plantarum, as a consequence of US-treatment by Bezhadnia, Moosavi-Nasab, Tiwari & Setoodehd [7]; the analysis through TEM by the cited authors revealed pores and sub-lethal injury and a positive effect on the metabolism.

A stimulation of the metabolism was also reported in the past after US exposure for other microorganisms [19,20]. For example, Yang, Zhang, & Wang [21] reported that ultrasound treatment at 20 kHz followed by static incubation was found to increase the growth of Brevibacterium sp. cells.

However, an uncontrolled efflux of nutrients could cause a delay of cell metabolism and in turn cell death, above all if combined with the local increase of temperature due to US and mechanic injury of waves (growth delay in some combinations and biocidal effect of high-power US reported by the authors elsewhere).

The effect of US could rely on the size, the outer membrane and the shape of cell wall [11,22,23,24], but strains belonging to the same species could behave in a different way due to some little differences in the membrane.

The second experiment of this research was on acidification profile, evaluated as pH-decrease: Fig. 1 shows the pH decrease of US-treated L. plantarum 162 (a), 109 (b), and c19 (c) after 24 h in a synthetic brine (6% NaCl; 0.1% Glucose) incubated at 30 °C. After 24 h, for the strain 162, the pH decrease was 1.47 in the control (A1); but in all other combinations, a higher acidification was observed with values ranging from about 2.00 (B2) to 2.26 (C2); the differences amongst US-treated samples were not significant. For the strain 109, a different effect depending on the treatment time and the pulses was found, with pH

Table 2

| Combinations | Strains | 43 | 78 | 104 | 109 | 162 | 172 | G1 | G3 | c19 |
|--------------|---------|----|----|-----|-----|-----|-----|----|----|-----|
| A1 (control) |         | 7.89 ± 0.30 | 7.76 ± 0.32 | 7.53 ± 0.35 | 7.45 ± 0.31 | 7.57 ± 0.31 | 7.60 ± 0.30 | 7.42 ± 0.34 | 7.83 ± 0.30 | 7.53 ± 0.32 |
| B1           |         | 8.06 ± 0.15 | 7.80 ± 0.12 | 7.61 ± 0.38 | 7.28 ± 0.33 | 7.47 ± 0.33 | 7.75 ± 0.30 | 7.57 ± 0.35 | 7.70 ± 0.33 | 7.81 ± 0.34 |
| B2           |         | 8.16 ± 0.14 | 7.96 ± 0.30 | 7.77 ± 0.35 | 7.30 ± 0.32 | 7.80 ± 0.32 | 7.82 ± 0.31 | 7.62 ± 0.34 | 7.53 ± 0.31 | 7.15 ± 0.30 |
| B3           |         | 7.93 ± 0.24 | 7.63 ± 0.32 | 7.75 ± 0.31 | 7.28 ± 0.31 | 7.82 ± 0.31 | 7.85 ± 0.30 | 7.52 ± 0.30 | 7.70 ± 0.30 | 7.36 ± 0.32 |
| C1           |         | 8.04 ± 0.31 | 7.29 ± 0.33 | 7.97 ± 0.35 | 7.23 ± 0.33 | 7.85 ± 0.33 | 7.64 ± 0.30 | 7.58 ± 0.34 | 7.71 ± 0.35 | 7.48 ± 0.33 |
| C2           |         | 7.95 ± 0.21 | 7.81 ± 0.22 | 7.91 ± 0.35 | 7.20 ± 0.32 | 7.75 ± 0.33 | 7.73 ± 0.34 | 7.46 ± 0.35 | 7.60 ± 0.30 | 7.23 ± 0.31 |
| C3           |         | 7.98 ± 0.12 | 7.80 ± 0.31 | 7.89 ± 0.34 | 7.49 ± 0.32 | 7.60 ± 0.32 | 7.81 ± 0.30 | 7.57 ± 0.33 | 7.73 ± 0.34 | 7.15 ± 0.31 |
| D1           |         | 7.79 ± 0.30 | 7.29 ± 0.51 | 7.67 ± 0.33 | 7.38 ± 0.33 | 7.80 ± 0.33 | 7.61 ± 0.30 | 7.71 ± 0.34 | 7.81 ± 0.36 | 7.26 ± 0.32 |
| D2           |         | 7.93 ± 0.30 | 7.83 ± 0.31 | 7.75 ± 0.35 | 7.28 ± 0.35 | 7.87 ± 0.35 | 7.53 ± 0.31 | 7.60 ± 0.32 | 7.83 ± 0.35 | 7.28 ± 0.31 |
| D3           |         | 7.79 ± 0.31 | 7.84 ± 0.33 | 7.96 ± 0.35 | 7.40 ± 0.31 | 7.76 ± 0.31 | 7.67 ± 0.30 | 7.54 ± 0.30 | 7.62 ± 0.30 | 7.46 ± 0.33 |
were observed after US application. The last step of this research was focused only on real matrices, where the starters must interact with the natural microorganisms. In particular, A1 (co-inoculum of untreated starters) and A2 (co-inoculum of treated starters) showed a significantly lower acidification than the other treatments (Table 4). The viable count of L. plantarum c19 was co-inoculated with other microorganisms (Staph. aureus or S. cerevisiae) to simulate real matrices, where the starters must interact with the natural microbiota of brines and dressings. Staph. aureus was chosen as a potential pathogen target, since it is often isolated from brines, due to its halo-tolerance; on the other hand, S. cerevisiae was chosen as a representative of useful yeasts generally found in brines. Concerning the acidification expressed by L. plantarum c19 in presence of Staph. aureus after 48 h, the ANOVA analysis through the homogeneous group approach (Table 4) highlights a positive effect of US treatment on the strain performance. In particular, A1 (co-inoculum of untreated L. plantarum c19 and St. aureus) showed a significantly lower acidification than the other combinations containing the cocktail “US treated c19/pathogen”: the maximum acidification (pH decrease = 2.22) was observed for the combinations B2 (2 min/6 s) and C3 (6 min/10 s).

The viable count of Staph. aureus was 4.87 log CFU/mL at the beginning of the test (inoculum), while after 48 h significant differences were amongst the batch inoculated with the pathogen alone (4.95 log
Fig. 1. pH decrease caused by US-treated *Lactiplantibacillus plantarum* 162 (a), 109 (b), and c19 (c) after 24 h in synthetic brine (6% NaCl, 0.1% Glucose) incubated at 30 °C. Mean values ± standard deviation. Initial pH = 6.0. The symbol “*” denotes a significant difference compared to combination A1 (control) (*-test, P < 0.05).
Increase of Abs (%)

**Table 4**
ANOVA analysis through the homogeneous group approach on the acidification experienced by *Lactiplantibacillus plantarum* c19 in presence of *Staphylococcus aureus*. *S. a.*, pathogen alone.

| Samples | pH decrease after 24 h | homogenous group | Samples | pH decrease after 48 h | homogenous group |
|---------|----------------------|-----------------|---------|----------------------|-----------------|
|         | Group I | Group II | Group III | Group IV | Group I | Group II | Group III | Group IV | Group V |
| *S. a.* | 0.77    | **C1** | **B1** | **A1** | 1.74    | **C1** | **B1** | **A1** | **C1** | 2.17    |
| A1      | 1.30    |       |       |       | 1.75    |       |       |       |       | 1.75    |
| B1      | 1.62    | **C2** | **B2** | **A2** | 1.72    | **C2** | **B2** | **A2** | **C2** | 1.72    |
| B2      | 1.63    | **C3** | **B3** | **A3** | 1.69    | **C3** | **B3** | **A3** | **C3** | 1.69    |
| B3      | 1.74    |       |       |       | 1.99    |       |       |       |       | 1.99    |
| C1      | 1.77    | **C4** | **B4** | **A4** | 2.12    | **C4** | **B4** | **A4** | **C4** | 2.12    |
| C2      | 1.83    |       |       |       | 2.22    |       |       |       |       | 2.22    |
| C3      | 1.89    |       |       |       | 2.22    |       |       |       |       | 2.22    |

**Table 5**
Viable count of US-treated *Lactiplantibacillus plantarum* c19 after 48 h in synthetic brine (6% NaCl, 0.1% Glucose) incubated at 30 °C and co-inoculated with *Saccharomyces cerevisiae*. Mean values ± standard deviation.

| Treatment | *L. plantarum* (48 h) | *S. cerevisiae* (48 h) |
|-----------|-----------------------|------------------------|
| A1        | 3.67 ± 0.28A**        | 5.15 ± 0.10A           |
| B1        | 5.24 ± 0.08B          | 4.88 ± 0.31A           |
| B2        | 6.96 ± 0.17C          | 4.92 ± 0.01A           |
| B3        | 6.77 ± 0.05C          | 5.13 ± 0.26A           |
| C1        | 6.77 ± 0.15C          | 5.12 ± 0.16A           |
| C2        | 6.15 ± 0.24C          | 4.83 ± 0.10A           |
| C3        | 5.43 ± 0.10C          | 5.54 ± 0.17A           |
| D1        | 6.03 ± 0.08C          | 5.74 ± 0.22A           |
| D2        | 6.60 ± 0.13C          | 5.07 ± 0.18A           |
| D3        | 6.50 ± 0.12C          | 5.09 ± 0.12A           |
| *S. cerevisiae* | 5.17 ± 0.17A         |

A1, control (non-treated *L. plantarum* and *S. cerevisiae*). *S. cerevisiae*, yeast alone.

**, data within a column with different letters are significantly different (one-way ANOVA and Tukey’s test, *P* > 0.05).

CFU/mL), the combination A1 (not treated *L. plantarum* and *Staph. aureus*) (4.0 log CFU/mL) and the combinations B1-D3 (treated *L. plantarum* and *Staph. aureus*) (2.95–3.40 log CFU/mL).

The second co-inoculation test was carried out with *S. cerevisiae*: no significant differences were observed in term of acidification performances (data not shown), but an interesting result was found for cellular concentrations (Table 5); in fact, in all samples containing the US treated strain co-inoculated with the yeast, *L. plantarum* c19 appeared stimulated and showed viable counts higher (of about 1–2 log CFU/mL) than those observed in the control (A1). For *S. cerevisiae*, differences were not significant (*P* > 0.05), because it experienced a delay in the growth due probably to the low concentration of glucose in the brine and the lack of other nutrients.

In the last assay, the effect of US on the permeability of the membrane of *L. plantarum* c19 was studied by means of the release of intracellular components (absorbance reading at 260 and 280 nm). US-treatment increased Abs at 260 nm and 280 nm (Fig. 2) (from 43% to 80% at 260 nm and from 21% to 101% at 280 nm). Although preliminary, this assay suggests that US-treatment could cause a change in membrane permeability, as one could infer from Abs increase in the conditioning solution. Other experiments are required to elucidate the exact mode of action at molecular level, to assess if some changes occur in the composition of the membrane or if the treatment affects the production/release of osmotic regulators. The release of acids, nucleic acids, and proteins is probably the consequence of a kind of sub-lethal injury due to the formation of pores on the membranes caused by one or more of the six different mechanisms suggested for sonoporation (cavitation, push, pull, jetting, streaming and translation), as well explained in the review of Ojha et al. [18]. This effect is not a positive effect per se; however, it could positively affect cell metabolism for its meaning, because the increase in membrane permeability upon US exposure could allow and facilitate the transportation of molecules across the cell membrane [26] by accelerating the exchange of substances [27]. This higher diffusion of nutrients across the cell membrane, together with the improvement of mass transfers [28] and the pressure fluctuations generated during US treatments which can induce stress on cells and promote growth and metabolic changes, is often suggested as one of the main mechanisms indicated in literature for some of the beneficial effects recovered for US application [28]. Some positive examples regard the facilitation of cholesterol removal by lactobacilli [15], the production of riboflavin by *Eremothecium ashbyii* [27], an increase of nutrient transportation in the deeper layers of biofilm [18,36], or an improvement of fermentation efficiency [14,29]. Dahroudi et al. [14] studied the application of low intensity US to improve the lactic acid production of *Lactocaseibacillus casei* and recovered that sonication led to increasing cell membrane permeability; these authors also suggested that permeate and its products can be used in production of lactic acid with an advantageous decrease of production costs. Some examples of US to improve the production of useful metabolites (biosurfactants) as well as biomass were also reported for *L. plantarum* [7,8].

4. Conclusions

The most important findings of this research could be summarized as follows:

a) A low intensity US treatment was able to increase the acidification of *L. plantarum* c19, without affecting its growth, even under stressing conditions;
b) This positive effect (higher acidification) was also detectable in co-inoculation with a pathogen, such as *Staphylococcus aureus*. In addition, when co-inoculated with a positive yeast (*S. cerevisiae*), the growth of *L. plantarum* c19 seemed to be stimulated;
c) The observed effects could be potentially attributed to an increased permeability of the cellular membrane, as it can be inferred by a recovered increase of the release of intracellular components.

However, some open questions remain: 1. Do these results occur also in vivo tests on complex matrices? The research, in fact, was conducted under strict-controlled conditions, whereas in vivo other variables could play a significant role (for example phenolic compounds in brine); 2. A strain-dependence was found, but is it possible to design treatments at least at species level? Further investigations appear necessary, since the improvement of processing technologies is an essential step toward better, safer and more profitable table olive productions.

CRediT authorship contribution statement

Barbara Speranza: Investigation, Data curation, Writing - original draft, Writing - review & editing. Daniela Campaniello: Investigation, Writing - review & editing. Celia Altieri: Methodology, Writing - review & editing. Milena Sinigaglia: Methodology, Writing - review & editing, Funding acquisition. Antonio Bevilacqua: Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing. Maria Rosaria Corbo: Conceptualization, Methodology, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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