Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 13, Issue 1, 2024, 2
https://doi.org/10.33263/LIANBS131.002

Decontamination of Fresh Cut Produce Using *Lactobacillus rhamnosus* GG Spent Medium

Umesh U. Jadhav [1,*], Rushikesh Pol [1], Vitthal T. Barvkar [2], Ankita Dabade [3], Gauri Babar [3], Isha Athale [4], Sunil Bhapkar [1], Deeksha Patil [1], Surekha Satpute [1], Karishma Pardesi [1], Mansingraj Nimbalkar [5], Sandeep Pai [6]

[1] Department of Microbiology, Savitribai Phule Pune University, Pune, Maharashtra India
[2] Department of Botany, Savitribai Phule Pune University, Pune, Maharashtra India
[3] Department of Microbiology, Yashwantrao Mohite College, Bharati Vidyapeeth, Pune, Maharashtra India
[4] Institute for Chemistry and Biology of the Marine Environment, Carl von Ossietzky Universität Oldenburg, Germany
[5] Department of Botany, Shivaji University, Kolhapur, Maharashtra India
[6] Rayat Shikshan Sanstha's Dada Patil Mahavidyalaya, Karjat, Dist. Ahmednagar, Maharashtra India
* Correspondence: ujadhav@unipune.ac.in (U.U.J), umeshjadhav02@gmail.com (U.U.J);
Scopus Author ID 23968915500

Received: 17.09.2022; Accepted: 30.10.2022; Published: 25.02.2023

Abstract: The consumption of fresh and minimally processed fruits and vegetables may cause the spread of food-borne diseases. The use of traditional chemical sanitizers is not suitable for the decontamination of such food. Therefore, there is a need to explore alternative processes. The use of probiotic cultures can be a suitable alternative. Hence, this study was conducted to evaluate the effect of *Lactobacillus rhamnosus* GG (*L. rhamnosus* GG) spent medium on *Escherichia coli* (*E. coli*) in suspension and on cucumber pieces. The growth parameters of *L. rhamnosus* GG were optimized. The LC-QTOF-MS-based quantitative analysis showed 18 mM lactic acid in the *L. rhamnosus* GG spent medium. The spent medium was collected and tested for the inactivation of *E. coli*. Results showed that 72 mM of lactic acid was required for *E. coli* growth inhibition. It has been observed that the antimicrobial activity remained unchanged even after treating the spent medium with catalase and trypsin, while the loss of antimicrobial activity was noticed after altering the pH of the spent medium. It required 10 minutes to kill all the population of *E. coli* on the cucumber surface. The sensory qualities of cucumber remain unchanged after spending medium treatment. Hence, our results showed that the *L. rhamnosus* GG spent medium can be used as a potential surface disinfectant for fresh-cut produce.

Keywords: fresh cut produce; *L. rhamnosus* GG; spent medium; decontamination.

1. Introduction

The trend to use fresh-cut fruits and vegetables in the regular diet is increasing in India and worldwide due to their ability to provide vitamins, dietary fiber, and minerals [1,2]. When fruits or vegetables are processed to alter their physical appearance along with maintaining their fresh form are referred to as fresh-cut produce (FCP) [3]. It comprises cut fruits, salad mixes, and many more forms. The use of FCP in the diet helps to prevent many health disorders [1-4]. The presence of moisture, favorable pH conditions, and damaged surfaces during processing make the FCP vulnerable to microbial attack [5,6]. Numerous sources, such as wastewater irrigation, farming tools, and workers, will contribute to the pathogen contamination of FCP [7,8]. Many recent incidences of disease spread happened because of consuming contaminated FCP [9,10]. The most commonly linked pathogens to FCP include

bacteria, parasites, and viruses, with *E. coli* and *Salmonella* being the most prominent pathogens in outbreaks related to FCP [11,12]. Therefore, FCP should be decontaminated to make it safe for consumption. However, conventional heating treatments usually cannot decontaminate them since it alters their properties [13-15]. Some physical methods are employed to decontaminate FCP [16-18]. However, all these methods are associated with certain limitations [19-21]. Also, various chemical sanitizers are commonly used in high concentrations to deal with contaminations [7,22,23]. Some of these sanitizers have been shown to produce carcinogenic and mutagenic by-products [24]. This is a serious concern regarding the use of chemical sanitizers. Hence, there is a demand for novel, environmentally friendly antimicrobials, particularly natural or biologically active compounds [25]. Protective microorganisms fit in well with this new tendency, and several bacteria and yeasts have been identified as bioprotective agents [26,27]. However, these bioprotective agents should be target specific and fulfill biosafety criteria [12]. Lactic acid bacteria (LAB) can fulfill these requirements. For centuries, people have been using fermented food products. In most of these processes, LAB played a crucial role. Lactic acid is one of the ancient antibacterial substances to be harnessed by humankind. This phenomenon has been used to preserve vegetables, sausages, cheese, and animal silage feed production. LAB is considered food-grade microorganisms, non-pathogenic, and generally recognized as safe (GRAS). LAB is able to tolerate the acidic condition of the environment, NaCl concentration, and bile salts [28,29]. Hence, the use of LAB for the decontamination of FCP is a suitable alternative. Considering these benefits, the present study was undertaken to study the efficacy of *L. rhamnosus* GG for the decontamination of FCP.

2. Materials and Methods

2.1. Microorganism used.

L. rhamnosus GG (ATCC 53103) was isolated from the commercial product SuperFlora^{TM}GG (Sun Pharma Ltd.). The SuperFlora^{TM}GG is normally sold as a probiotic supplement. It was purchased from the local medical store. Rogosa and Sharpe (MRS) were used for the cultivation of *L. rhamnosus* GG. The composition of MRS was (in g/L) proteose peptone 10, beef extract 10, yeast extract 05, dextrose 20, polysorbate (80) 01, ammonium citrate 02, sodium acetate 05, magnesium sulfate 0.1, manganese sulfate 0.05, dipotassium sulfate 02, agar 12 and pH 6.5± 0.2. Both the media were sterilized at 121 ºC, 15 lbs pressure for 20 minutes using an autoclave. The pathogenic microorganisms *E. coli* NCIM1665, *Acinetobacter baumannii* (*A. baumannii*), *Klebsiella pneumonia* (*K. pneumoniae* NCIM2098), *Staphylococcus aureus* (*S. aureus* NCIM2079), *Salmonella typhimurium* (*S. typhimurium* NCIM2501), *Enterobacter aerogenes* (*E. aerogenes* NCIM2340) were procured from NCIM Pune, India. Lysogeny broth (LB) medium was used for the growth of these pathogens. The composition of LB was (in g/L) tryptone 10, sodium chloride (NaCl) 10, Yeast extract 5, and pH 7.0 ± 0.2.

2.2. Determination of growth curve, pH profile, optimization of L. rhamnosus GG growth conditions, and collection of the spent medium.

The 100 mg powder of *L. rhamnosus* GG was taken aseptically from the SuperFlora^{TM}GG sachet. It was added aseptically into the 100 mL sterile MRS broth and incubated overnight at 37 ˚C. After incubation, a loopful culture was inoculated on sterile MRS

agar by streak plate method. The plate was incubated at 37 ˚C. A single isolated colony was picked from this MRS agar plate and added to 100 mL sterile MRS broth. The flask was incubated overnight at 37 ˚C with continuous shaking at 120 rpm. The growth of *L. rhamnosus* GG was monitored by observing optical density (OD) every 3 h at 600 nm using a spectrophotometer up to the stationary phase. A similar experiment was conducted to study the change in pH of MRS broth during *L. rhamnosus* GG growth. The culture was grown as described above, and the pH of the broth was monitored every 15, 30, 45, and 60 h. An inoculum of *L. rhamnosus* GG was obtained, as described in the above section. A fixed cell concentration ($1x10^5$ CFU/ml) was inoculated in MRS broth with varying pH (ranging from 2.5-7.0). The flasks were incubated for 18 h at 37 ˚C. The OD was measured at 600 nm. The *L. rhamnosus* GG was grown as described in the above section, and after sufficient growth, the broth was centrifuged to collect the spent medium. The collected supernatant was filter sterilized by a 0.22 µm pore size nitrocellulose membrane filter.

2.3. Quantitative analysis of Lactic acid using LC-QTOF-MS.

The metabolites were extracted using the following method. The *L. rhamnosus* GG spent medium was mixed with MS-grade methanol in equal volume and vortexed. It was then sonicated in a bath sonicator for 20 minutes. The solution was then centrifuged at 14000 rpm for 15 minutes. The supernatant was collected, and it was further used for LC-QTOF-MS analysis. The standard lactic acid sample was prepared (1 mg/ml) and used for comparison. The presence of lactic acid in *L. rhamnosus* GG spent medium was determined using liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS) using a method described by Vasav et al. [30] with slight modifications. The LC-QTOF-MS analysis was carried out on Agilent 6530 Q-TOF (Agilent, USA) mass spectrometer connected to HPLC Prime Infinity II 1260 system (800 bar) and dual electrospray ionization (ESI) source was used for ionization. For LC-based separation, the Infinity Lab Poroshell 120 EC-C18 (2.1×150 mm, 1.9 µm particle size, Agilent, USA) column was used at 40 °C with a flow rate of 0.3 ml/min. The solvents used were 100% MS-grade water (Solvent A) and 100% MS-grade acetonitrile (Solvent B), containing 0.1% formic acid. A 20 min gradient was used for separation. It started with 2% B for the first 0.3 min and increased to 30% in the next 2 min. The B percentage was increased from 30% to 45% till 7 min and further increased to 98% till 12 min, at which it was held for the next 3 min. The column was equilibrated to the initial ratio of solvents (98% A: 2% B) in the last 5 min. The MS parameters were tuned as; the gas temperature at 325 °C, drying gas at 10 L/min, nebulizer at 35 psi, and fragment or at 120 volts. The peak area of lactic acid was calculated using Agilent MassHunter Qualitative Navigator B.08.00 and Qualitative Workflow B.08.00 and calculated relative concentration.

2.4. Antimicrobial activity of L. rhamnosus GG spent medium.

The agar well diffusion method was used to test the antimicrobial activity of *L. rhamnosus* GG spent medium. The LB agar plate containing *E. coli, A. baumannii, K. pneumoniae, S. aureus, S. typhimurium, E. aerogenes* was prepared. The 100 µl filter sterilized *L. rhamnosus* GG spent medium was added into each well. Plates were kept in the refrigerator for 20 minutes to allow diffusion of the spent medium. Plates were incubated at 37^{o}C for 24 h. The antimicrobial activity was determined by observing the diameter (in mm) of the zone of inhibition.

2.5. Determination of minimum inhibitory concentration of lactic acid in L. rhamnosus GG spent medium required for inhibition of microbial growth.

A 100 µl of OD-adjusted culture of *E. coli* (Cell density $1x10^5$ CFU/ml) was plated on Luria agar. Various lactic acid concentrations (36-180 mM) were added to each well, respectively. Plates were kept in the refrigerator for 20 minutes to allow diffusion of the spent medium. Plates were incubated at 37 ºC for 24 h and further examined to observe antimicrobial activity measured as inhibition zone diameter in mm. This activity was also determined by incubating the *E. coli* cells with various lactic acid concentrations (36-180 mM) for a fixed time interval. The cells were then serially diluted, and the colony-forming units (CFU) were determined.

2.5. Identification of antimicrobial substance in L. rhamnosus GG spent medium.

The mechanism behind the antimicrobial activity of *L. rhamnosus* GG spent medium was studied using the agar well diffusion technique described by Toure et al. [31] with some modifications. The antimicrobial activity of Lactobacillus is mostly due to the production of bacteriocin, hydrogen peroxide, and organic acids. The *L. rhamnosus* GG was grown overnight in 100 ml MRS broth at 37 ˚C. The broth was centrifuged at 6000 rpm for 20 min at 4 ˚C. The *L. rhamnosus* GG spent medium was distributed into equal portions and used in different assays.

2.6. Bacteriocin assay.

The 5 ml of *L. rhamnosus* GG spent medium was taken and treated with 1 mg/ml trypsin at room temperature for 2 to 4 h. Then the treated spent medium was filter sterilized through a 0.22 µm pore size filter. The 100 µl treated spent medium was added into a well. The plates were kept at 4˚C for 20 min to allow diffusion of treated spent medium and then incubated for 24 h at 37˚C. The diameter (in mm) of the zone of inhibition was recorded.

2.7. Hydrogen peroxide assay.

The 5 ml of *L. rhamnosus* GG spent medium was taken and treated with 0.5 mg/ml catalase enzyme. Then the treated spent medium was filter sterilized through a 0.22 µm pore size filter. The 100µl treated spent medium was added into a well. The plates were kept at 4˚C for 20 min to allow diffusion of treated spent medium and then incubated for 24 h at 37˚C. The diameter of the zone of inhibition was recorded.

2.8. Organic acid assay.

The 5 ml of *L. rhamnosus* GG spent medium was taken. The spent medium pH was adjusted to pH 6.5 using 1N NaOH. Then the spent medium was filter sterilized through a 0.22 µm pore size filter. The 100 µl treated spent medium was added into a well. The plates were kept at 4 ˚C for 20 min to allow diffusion of treated spent medium and then incubated for 24 h at 37˚C. The diameter of the zone of inhibition was recorded.

2.9. Decontamination of fresh-cut produce.

The fresh cucumber was purchased from the local vegetable market. It was surface sterilized with 90% ethanol. The cucumber was cut into circular pieces, which were surface

sterilized into 70% ethanol. They were washed with distilled water. The *E. coli* suspension ($1x10^5$ CFU/ml) was poured on cucumber pieces and left for 30 min at room temperature for cell attachment. Then the cucumber pieces were dried. The pieces were then treated with *L. rhamnosus* GG spent medium for 5 and 10 min. Then cucumber pieces were washed with sterile saline. The saline was collected and serially diluted. An appropriate dilution was selected to determine the CFU on the LB agar plate.

3. Results and Discussion

3.1. Isolation and growth of L. rhamnosus GG.

The *L. rhamnosus* GG was isolated from the commercial probiotic product SuperFloraTMGG. The growth pattern of *L. rhamnosus* GG was studied in MRS broth. The stationary phase was achieved after 30 h of incubation (Figure 2).

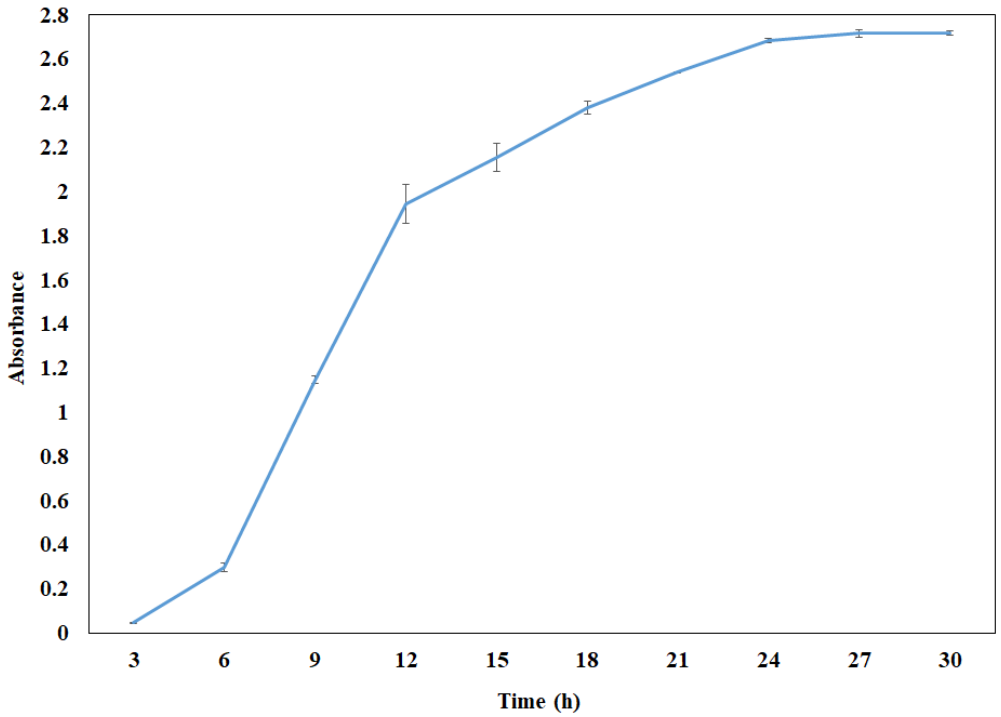
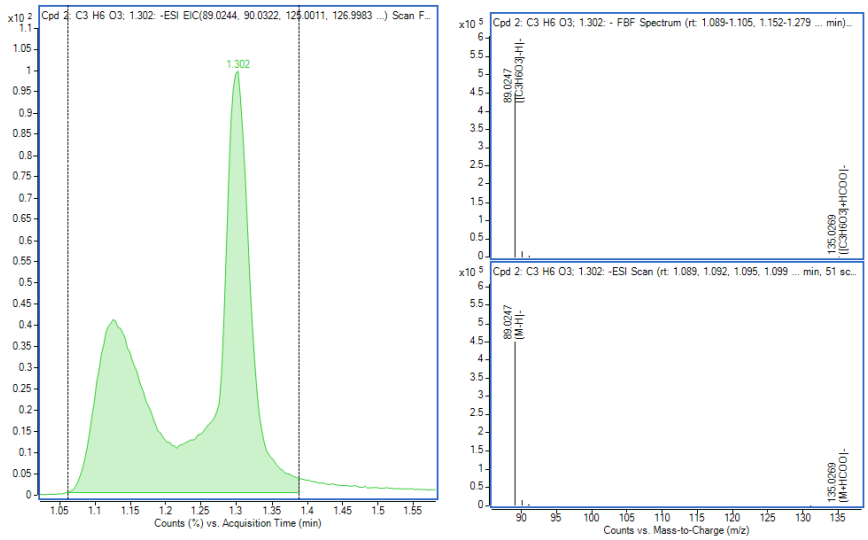
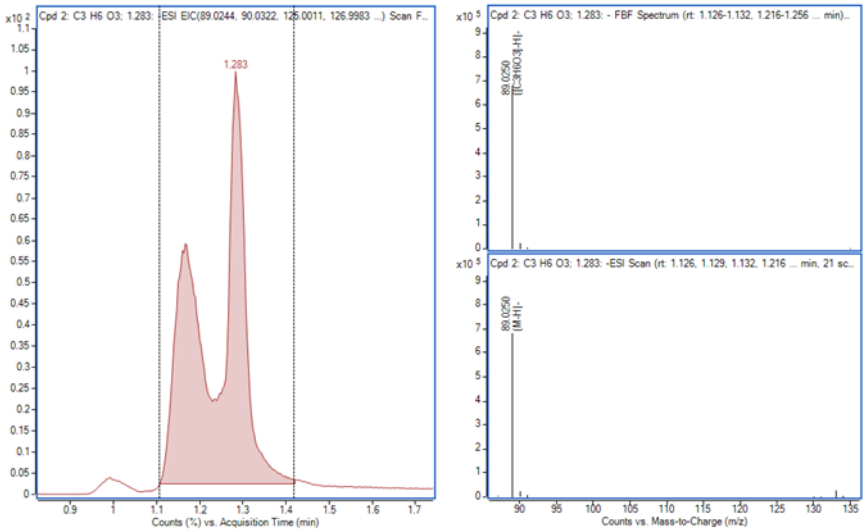


Figure 2. Growth curve of *L. rhamnosus* GG.

The optimum growth pH for *L. rhamnosus* GG was 7.0 (±0.2) (data not shown). The change in pH of the culture broth was studied during the growth of *L. rhamnosus* GG. The initial pH of the MRS culture broth was 7.0 (±0.2). The *L. rhamnosus* GG was known to produce organic acid. This production of organic acid resulted in a decrease in the pH of MRS broth. The pH dropped significantly from 7.0 (±0.2) to 3.51 (±0.2) after 45 h incubation. No further change in pH was observed (data not shown). The principal organic acid produced by LAB is lactic acid. Therefore, *L. rhamnosus* GG spent medium was subjected to LC-QTOF-MS analysis. The analysis showed the presence of lactic acid in *L. rhamnosus* GG spent medium. The amount of lactic acid produced by *L. rhamnosus* GG was quantified to around 20 mM (Figure 3). Considering these results, the *L. rhamnosus* GG spent medium was collected after 45 h incubation to study its antimicrobial effect.



(a)



(b)

Formula	m/z	Mass	RT	Area	Ions	Sample	Name	Diff (ppm)	Score
C3 H6 O3	89.025	90.0323	1.282	16859866	4	SN.d	L-(+)-lactic acid	6.83	97.17
C3 H6 O3	89.0247	90.0319	1.302	12105943	4	LA.d	L-(+)-lactic acid	2.62	99.51
C3 H6 O3	89.0252	90.0325	1.293	822077	4	MRS.d	L-(+)-lactic acid	9.3	95.07

(c)

Figure 3. LCMS analysis **(a)** standard; **(b)** spent medium; **(c)** compound identification.

3.2. Antimicrobial activity of L. rhamnosus GG spent medium against various pathogens.

The *L. rhamnosus* GG spent medium was tested against various pathogens (Figure 4) (*E. coli, A. baumanii, K. pneumoniae, S. aureus, S. Typhimurium, E. aerogenes*). It showed good antimicrobial activity against all the tested pathogens with the inhibition (14-18 mm) of the growth of test pathogens. Among these pathogens, around 18.0 (±0.89) mm zone of inhibition was observed for *E. coli* (Table 1).

All further experiments were carried out using *E. coli*. The probiotic potential of lactobacilli with antagonistic activity was also reported by Prabhurajeshwar and Chandrakanth [29]. They isolated thirty lactic acid bacterial strains. They found that, out of these 30 isolates, 16 were potential probiotics in nature with noticeable antagonistic activity against all tested indicator microorganisms [29].

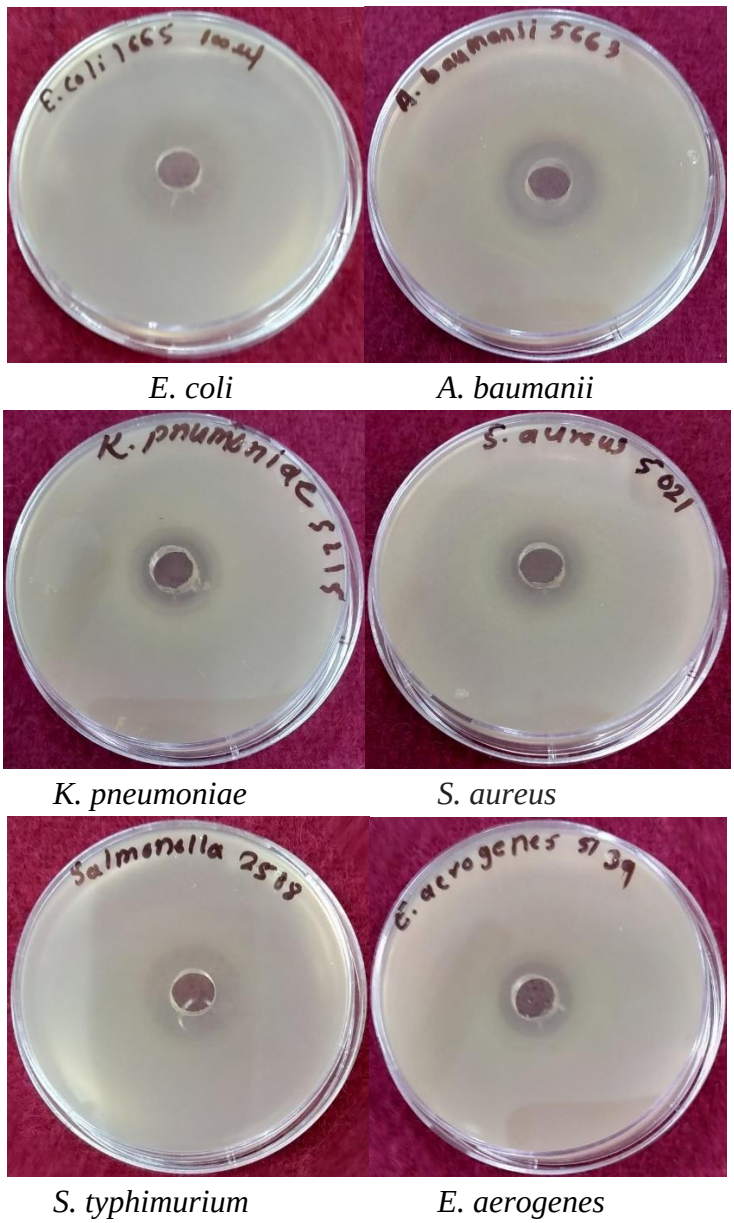
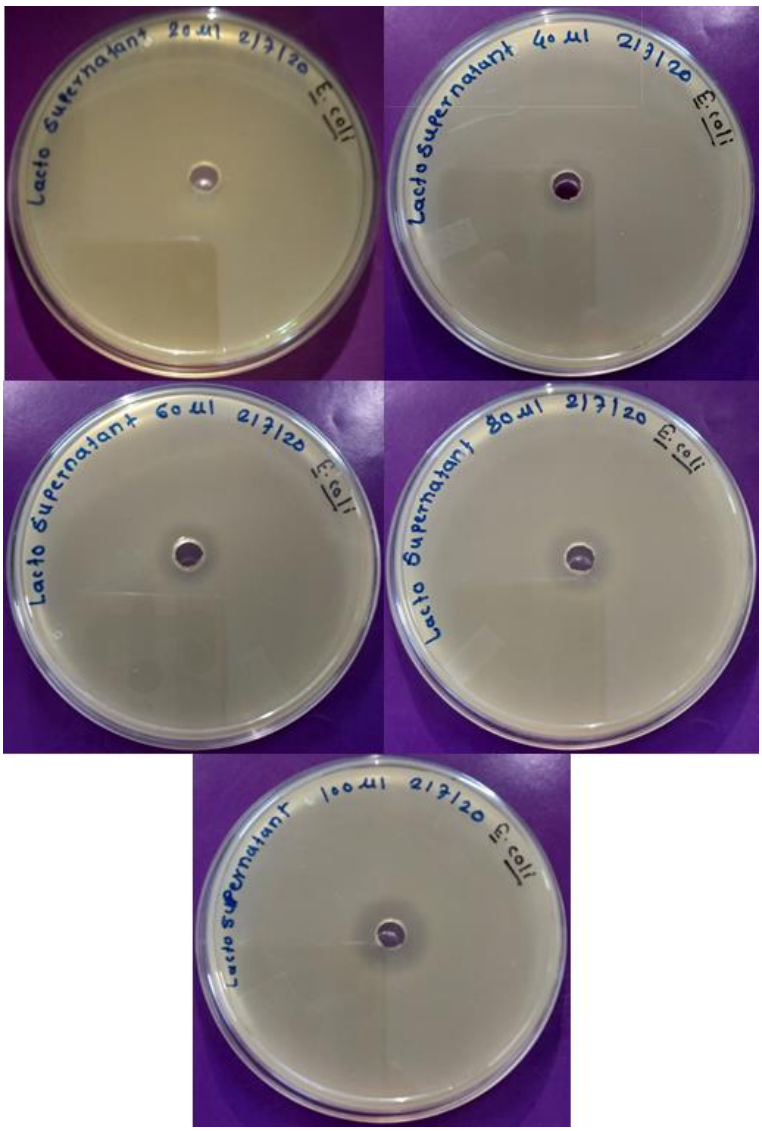


Figure 4. Antimicrobial activity tested by well diffusion method against various pathogens.

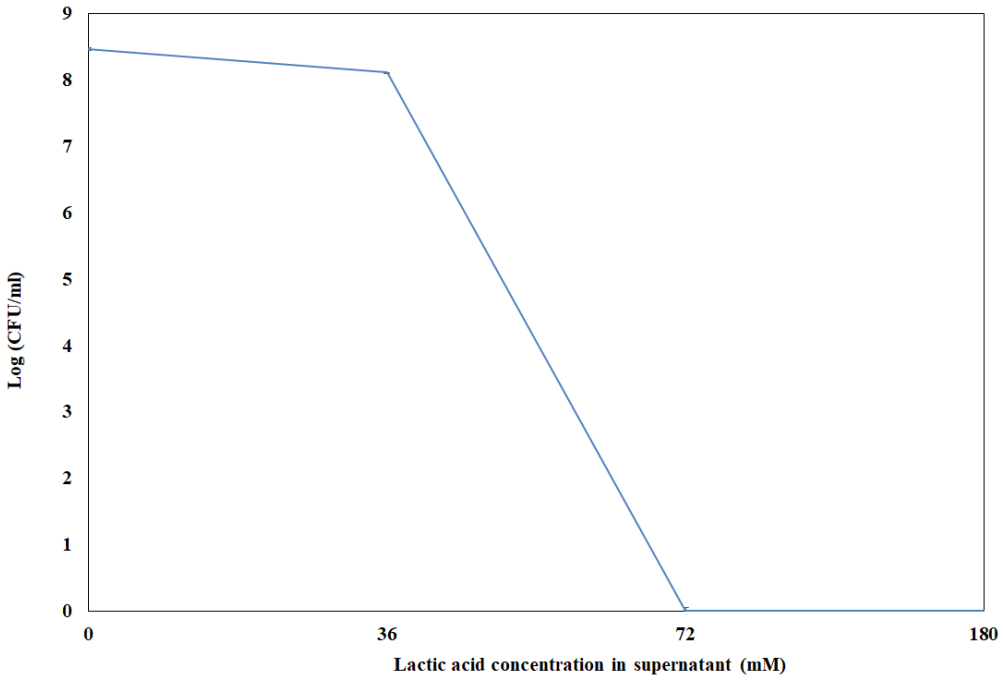
Table 1. The antimicrobial activity of *L. rhamnosus* GG spent medium against various pathogens.

Sr. No.	Microorganism	Gram nature	Zone of inhibition (mm)
1	*E. coli*	Gram-negative	18.0 (±0.89)
2	*A. baumanii*	Gram-negative	17.66 (±1.03)
3	*K. pneumoniae*	Gram-negative	15.66 (±0.51)
4	*S. aureus*	Gram-positive	15.0 (±0.89)
5	*S. typhimurium*	Gram-negative	15.0 (±1.54)
6	*E. aerogenes*	Gram-negative	14.66 (±0.51)

The minimum inhibitory concentration required for *E. coli* growth inhibition was determined. It was evaluated by the well-diffusion method and direct contact method. The well-diffusion method showed that 72 mM of lactic acid present in *L. rhamnosus* GG spent medium was sufficient for *E. coli* growth inhibition (Figure 5a). It was further confirmed by the direct contact method. The bacterial count decreased with increased lactic acid concentration in the supernatant. The 100% reduction in bacterial load was achieved when cells were incubated with 72 mM of lactic acid (Figure 5b).



(a)

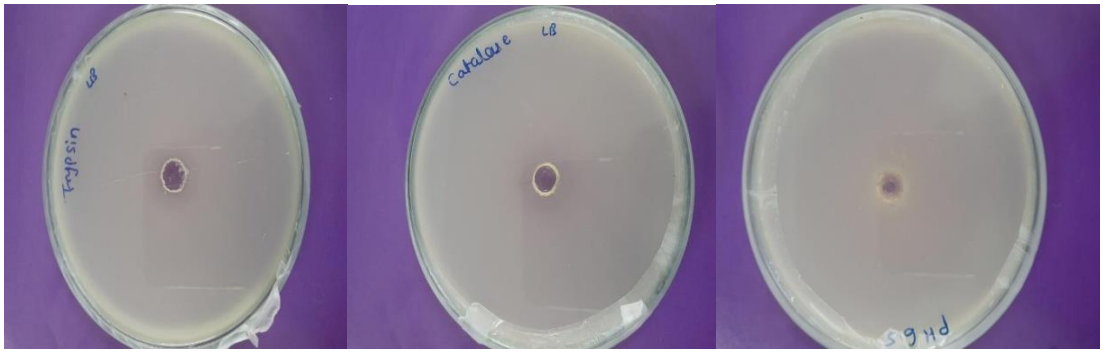


(b)

Figure 5. Determination of minimum inhibitory concentration **(a)** well diffusion method; **(b)** direct contact method.

3.3. Mechanism of antimicrobial activity.

The LAB exerts a variety of mechanisms to prevent the growth and activity of food-borne pathogens and spoilage microorganisms. The production of organic acids, bacteriocins, and other low molecular mass compounds, such as hydrogen peroxide by LAB, are responsible for such antimicrobial activity [32–34]. Hence, a study was conducted to identify the antimicrobial mechanism of *L. rhamnosus* GG. The *L. rhamnosus* GG spent medium was treated with trypsin and tested for antimicrobial activity. This trypsin-treated supernatant showed antimicrobial activity (Figure 6a). Similarly, the spent medium was treated with catalase enzyme. This treatment also did not affect the antimicrobial activity of the *L. rhamnosus* GG spent medium against the test pathogen (Figure 6b). These results suggest that the pathogen inhibition was not due to bacteriocin or hydrogen peroxide. However, the neutralized *L. rhamnosus* GG spent medium (pH 6.5) showed no pathogen inhibition. This indicated that the inhibitory effect of the *L. rhamnosus* GG spent medium was due to organic acid production (Figure 6c). *L. rhamnosus* GG is known to produce lactic acid. The undissociated form of lactic acid can cross the cell membrane barrier of the pathogen. Inside the microbial cell, it dissociates to its ionic form, reducing intracellular pH, and thereby disrupting the cell's transmembrane proton motive force. The pathogen needs to spend energy to maintain pH. This is done by pumping out the acid and by producing alkaline metabolites. Also, this acid stress generates free radicals, which further damage all cellular mechanisms and inhibit the pathogen [35–37]. The use *L. rhamnosus* GG spent medium as a sanitizer is advantageous compared to other sanitizers. A very short exposure time is required for decontamination. This is a very difficult situation for bacteria. They cannot generate resistance by adapting their structure or their metabolism. The sudden severe acid stress leads to an unmitigated shock of oxidative stress [38]. Hence, *L. rhamnosus* GG spent medium can be used as a sanitizer for longer periods without having the risk of generation of resistance mechanism by pathogens.



(a) (b) (c)

Figure 6. Study the mechanism behind antimicrobial activity **(a)** bacteriocin assay; **(b)** hydrogen peroxide assay; **(c)** organic acid assay.

3.4. Decontamination of fresh-cut produce.

There is a need for novel processing technologies for treating fresh foods since outbreaks are associated with fresh food consumption [39]. Various processes have been described for the decontamination of FCP. However, probiotic culture use for such applications has been reported recently [40,41]. The present study explored the possibility of using the probiotic *L. rhamnosus* GG to control the pathogen growth on fresh-cut cucumber. The

cucumber pieces were first inoculated with *E. coli*. It was observed that when the cucumber piece was dipped in the *L. rhamnosus* GG supernatant and left for 5 mins at room temperature only 2 x 10^4 CFU/ml were observed, whereas after 10 mins there was no cfu. The 15 x 10^4 CFU/ml were obtained from the untreated cucumber surface (Figure 7). Hence the *L. rhamnosus* GG spent medium completely inhibited the growth of *E. coli* on the cucumber surface in 10 minutes. Trias et al. [27] used lactic acid bacteria to inhibit the growth of food-borne human pathogens. They used selected strains to treat wounded Golden Delicious apples and Iceberg lettuce leaf cuts. They observed a 1 to 2 log CFU/wound reduction for *S. typhimurium* and *E. coli* and complete inhibition for *L. monocytogenes*. One study reported that a combination of peracetic acid with lactic acid controlled the pathogenic effect on leafy vegetables and iceberg lettuce [41]. Another study used LAB as biocontrol agent to preserve minimally processed fruits and vegetables [42]. Iglesias et al. [43] co-inoculated the pathogens with *L. rhamnosus* GG on pears. They observed a 2 and 3-log units reduction for *Salmonella* and *L. monocytogenes*, respectively. Trias et al. [27] and Iglesias et al. [43] grew the lactobacilli cells on FCP, which inhibited pathogen growth. The advantage of the present process is the use of *L. rhamnosus* GG spent medium. It is not necessary that *L. rhamnosus* GG cells should remain present on FCP. A simple spraying of *L. rhamnosus* GG spent medium on the FCP surface inhibited pathogen growth. This type of application strategy will help to make the decontamination process easy. The bacterium can be grown separately, and the spent medium can be used for decontamination. It will avoid the incubation period of *L. rhamnosus* GG. This will not only make the process effective but also cost-effective.

Figure 7. Decontamination of cucumber piece.

4. Conclusions

The present study showed that the spent medium of probiotic culture L. rhamnosus GG was useful for inactivating several pathogens. This property of the spent medium was further tested for decontamination of cucumber. The results showed that the antimicrobial activity of

L. rhamnosus GG spent medium was mainly because of lactic acid present in it. Hence, the *L. rhamnosus* GG spent medium can be used as an alternative surface disinfectant for FCP.

Funding

No funding was received for carrying out this work.

Acknowledgments

Sunil Bhapkar acknowledges the Council of Scientific and Industrial Research (CSIR), India, for the research fellowship. Rushikesh Pol acknowledges the Indian Council of Medical Research (ICMR), India, for the research fellowship. Deeksha Patil acknowledges the Department of Biotechnology (DBT), India, for the research fellowship.

Conflicts of Interest

The authors declare that they have no competing interests.

References

1. Yousuf, O.; Titikshya, S.; Singh, A. Fresh-cut fruits and vegetables: scope in developing countries and approaches to improve quality and safety. *Int. j. chem. Stud.* **2018,** *6*, 2226-2229, https://doi.org/10.22271/chemi.
2. Raffo A.; Paoletti F. Fresh-cut vegetables processing: environmental sustainability and food safety issues in a comprehensive perspective. *Front. Sustain. Food Syst.* **2022,** *5*, 681459, https://doi.org/10.3389/fsufs.2021.681459.
3. Qadri, O.; Yousuf, B.; Srivastava, A. Fresh-cut fruits and vegetables critical factors influencing microbiology & novel approaches to prevent microbial risks-A review. *Cogent. Food. Agric.* **2015,** *1*, 1121606, https://doi.org/10.1080/23311932.2015.1121606.
4. Maringgal, B.; Hashim, N.; Mohamed, I.S.; Mohamed, M.T.M.; Hamzah, M.H.; Mohd Ali, M. Effect of kelulut honey nanoparticles coating on the changes of respiration rate, ascorbic acid, and total phenolic content of papaya (Carica papaya L.) during Cold Storage. *Foods* **2021,** *10*, 432, https://doi.org/10.3390/foods10020432. .
5. Allende, A.; Martínez, B.; Selma, V.; Gil, M.I.; Suárez, J.E.; Rodríguez, A. Growth and bacteriocin production by lactic acid bacteria in vegetable broth and their effectiveness at reducing *Listeria monocytogenes* in vitro and in fresh-cut lettuce. *Food Microbiol.* **2007,** *24*, 759-766, https://doi.org/10.1016/j.fm.2007.03.002.
6. Pinto, L.; Cefola, M.; Bonifacio, M.A.; Cometa, S.; Bocchino, C.; Pace, B.; De Giglio, E.; Palumbo, M.; Sada, A.; Logrieco, A.F. et al. effect of red thyme oil (Thymus vulgaris L.) vapours on fungal decay, quality parameters and shelf-life of oranges during cold storage. *Food Chem.* **2021,** *336*, 127590, https://doi.org/10.1016/j.foodchem.2020.127590.
7. Pachepsky, Y.; Shelton, R.; McLain, J.; Patel, J.; Mandrell, R. Irrigation waters as a source of pathogenic microorganisms in produce: A review. *Adv. Agron.* **2011,** *113*, 73-138, https://doi.org/10.1016/B978-0-12-386473-4.00007-5.
8. Fufa, D.D. Novel approach to enhance the shelf life of fresh cut fruits and vegetables: A Review. *J Food Process Technol*. **2021,** *12*, 891. https://doi.org/10.1025/897-0-12-386473.
9. Callejón, R.; Rodríguez-Naranjo, M.; Ubeda, C.; Hornedo-Ortega, R.; Garcia-Parrilla, M.; Troncoso A. Reported food-borne outbreaks due to fresh produce in the United States and European Union: trends and causes. *Food-borne Pathog. Dis.* **2015,** *12*, 32-38, https://doi.org/10.1089/fpd.2014.1821.
10. CDC. Reports of selected *E. coli* outbreak investigations. Centers for Disease Control and Prevention. **2021** https://www.cdc.gov/ecoli/outbreaks.html.
11. Mukherjee, A.; Speh, D.; Dyck, E.; Diez-Gonzalez, F. Preharvest evaluation of coliforms, *Escherichia coli*, Salmonella, and *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. *J. Food Prot.* **2004,** *67*, 894-900, https://doi.org/10.4315/0362-028x-67.5.894.

12. Soni, A.; Choi, J.; Brightwell, G. Plasma-Activated Water (PAW) as a disinfection technology for bacterial inactivation with a focus on fruit and vegetables. *Foods* **2021,** *10*, 166, https://doi.org/10.3390/foods10010166.
13. Badr, A.N.; Gromadzka, K.; Shehata, M.G.; Stuper-Szablewska, K.; Drzewiecka, K.; Abdel-Razek, A.G.; Youssef, M.M. Encapsulated bioactive ingredients of grape by-products applicate in fresh-cut fruit and juices diminished the ochratoxins. *J. Food Process Preservat.* **2021,** *45*, 15112, https://doi.org/10.1111/jfpp.15112.
14. Zou, Y.; Yu, Y.; Cheng, L.; Li, L.; Zou, B.; Wu, J.; Zhou, W.; Li, J.; Xu, Y. Effects of curcumin-based photodynamic treatment on quality attributes of fresh-cut pineapple. *LWT Food Sci. Technol.* **2021,** *141*, 110902, https://doi.org/10.1016/j.foodchem.2018.08.042.
15. Giannakourou, M.C.; Tsironi, T.N. Application of processing and packaging hurdles for fresh-cut fruits and vegetables preservation. *Food*s **2021,** *10*, 830, https://doi.org/10.3390/foods10040830.
16. Schnabel, U.; Handorf, O.; Stachowiak, J.; Boehm, D.; Weit, C.; Weihe, T.; Schäfer, J.; Below, H.; Bourke, P.; Ehlbeck, J. Plasma functionalized water: From bench to prototype for fresh-cut lettuce. *Food Eng. Rev.* **2021,** *13*, 115-135, https://doi.org/10.1007/s12393-020-09238-9.
17. Wang, H.; Fang, X.-M.; Sutar, P.P.; Meng, J.-S.; Wang, J.; Yu, X.-L.; Xiao, H.-W. Effects of vacuum-steam pulsed blanching on drying kinetics, colour, phytochemical contents, antioxidant capacity of carrot and the mechanism of carrot quality changes revealed by texture, microstructure and ultrastructure. *Food Chem.* **2021,** *338*, 127799, https://doi.org/10.1016/j.foodchem.2020.127799.
18. Ben-Fadhel, Y.; Cingolani, M.C.; Li, L.; Chazot, G.; Salmieri, S.; Horak, C.; Lacroix, M. Effect of γ-irradiation and the use of combined treatments with edible bioactive coating on carrot preservation. *Food Packag. Shelf Life* **2021,** *28*, 100635, https://doi.org/10.1016/J.FPSL.2021.100635.
19. Botondi, R.; Barone, M.; Grasso, C. A Review into the effectiveness of ozone technology for improving the safety and preserving the quality of fresh-cut fruits and vegetables. *Foods* **2021,** *10*, 748, https://doi.org/10.3390/foods10040748.
20. Zhao, H.; Fan, Z.; Wu, J.; Zhu, S. Effects of pre-treatment with S-nitrosoglutathione-chitosan nanoparticles on quality and antioxidant systems of fresh-cut apple slices. *LWT Food Sci. Technol.* **2021,** *139*, 110565, https://doi.org/10.1016/j.lwt.2020.110565.
21. Rodríguez-Arzuaga, M.; Salsi, M.S.; Piagentini, A.M. Storage quality of fresh-cut apples treated with yerba mate (Ilex paraguariensis). *J. Food Sci. Technol.* **2021,** *58*, 186-196, https://doi.org/10.1007/s13197-020-04528-w.
22. Artés F.; Allende A. Processing lines and alternative preservation techniques to prolong the shelf-life of minimally fresh processed leafy vegetables. *Eur. J. Hortic. Sci.* **2005,** *70*, 231-245, https://eurekamag.com/research/004/283/004283301.php.
23. Hassenberg, K.; Geyer, M.; Mauerer, M.; Praeger, U. Influence of temperature and organic matter load on chlorine dioxide efficacy on *Escherichia coli* inactivation. *Food Sci. Technol.* **2017,** *79*, 349-354, https://doi.org/10.1016/j.lwt.2017.01.030.
24. Gil, M.; Selma, M.; López-Gálvez, F.; Allende, A. Fresh-cut product sanitation and wash water disinfection: problems and solutions. *Int. J. Food Microbiol.* **2009,** *134*, 37-45, https://doi.org/10.1016/j.ijfoodmicro.2009.05.021.
25. Zhang, X.; Fan, X.; Solaiman, D.; Ashby, R.; & Liu, Z; Mukhopadhyay, S.; Yan, R. Inactivation of *Escherichia coli* O157:H7 in vitro and on the surface of spinach leaves by biobased antimicrobial surfactants. *Food Control* **2016,** *60*, 158-165, https://doi.org/10.1007/s10068-017-0240-x.
26. Vermeiren, L.; Devlieghere, F.; Debevere, J. Evaluation of meat bornlactic acid bacteria as protective cultures for the biopreservation. *Int. J. Food Microbiol.* **2004,** *96*, 149-164, https://doi.org/10.1016/j.ijfoodmicro.2004.03.016.
27. Trias, R.; Bañeras, L.; Montesinos, E. Bioprotection of golden delicious apples and iceberg lettuce against food borne bacterial pathogens by lactic acid bacteria. *Int. J. Food Microbiol.* **2008,** *123*, 50-60, https://doi.org/10.1016/j.ijfoodmicro.2007.11.065.
28. Stiles, M.; Holzapfel, W. Lactic acid bacteria of foods and their current taxonomy. *Int. J. Food Microbiol.* **1997,** *36*, 1-29, https://doi.org/10.1016/s0168-1605(96)01233-0.
29. Prabhurajeshwar, C.; Chandrakanth, R. Probiotic potential of Lactobacilli with antagonistic activity against pathogenic strains: An in vitro validation for the production of inhibitory substances. *Biomed. J.* **2017,** *40*, 270-283, https://doi.org/10.1016/j.bj.2017.06.008.

30. Vasav, A.; Pable, A.; Barvkar, V. Differential transcriptome and metabolome analysis of *Plumbago zeylanica* L. reveal putative genes involved in plumbagin biosynthesis. *Fitoterapia* **2020,** *147*, 104761, https://doi.org/10.1016/j.fitote.2020.104761.
31. Toure, R.; Kheadr, E.; Lacroix, C.; Moroni, O.; Fliss, I. Production of antibacterial substances by Bifidobacterial isolates from infant stool active against *Listeria monocytogenes*. *J. Appl. Microbiol.* **2003,** *95*, 1058-1069, https://doi.org/10.1046/j.1365-2672.2003.02085.x.
32. Batish, V.; Roy, U.; Lal, R.; Grover, S. Antifungal attributes of lactic acid bacteria-a review. *Crit. Rev. Biotechnol.* **1997,** *17*, 209-225, https://doi.org/10.3109/07388559709146614.
33. Alakomi, H.; Skyttä, E.; Saarela, M.; Mattila-Sandholm, T.; Latva-Kala, K.; Helander, I. Lactic acid permeabilizes Gram-negative bacteria by disrupting the outer membrane. *Appl. Environ. Microbiol.* **2000,** *66*, 2001-2005, https://doi.org/10.1128/AEM.66.5.2001-2005.2000.
34. Cleveland, J.; Montville, T.; Nes, I.; Chikindas, M. Bacteriocins: safe, natural antimicrobials for food preservation. *Int. J. Food Microbiol.* **2001,** *71*, 1-20, https://doi.org/10.1016/s0168-1605(01)00560-8.
35. Ricke, S. Perspectives on the use of organic acids and shortchain fatty acids as antimicrobials. *Poult. Sci.* **2003,** *82*, 632-639, https://doi.org/10.1093/ps/82.4.632.
36. Raybaudi-Massilia, R.; Mosqueda-Melgar, J.; Soliva-Fortuny, R.; Martin-Belloso, O. Control of pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials. *Compr. Rev. Food Sci. Food Saf.* ***2009,*** 8, 157-180, https://doi.org/10.1111/j.1541-4337.2009.00076.x.
37. Desriac, N.; Broussolle, V.; Postollec, F.; Mathot, A.; Sohier, D.; Coroller, L.; Leguerinel, I. *Bacillus cereus* cell response upon exposure to acid environment: toward the identification of potential biomarkers. *Front. Microbiol* **2013,** *4*, 1-13, https://doi.org/10.3389/fmicb.2013.00284.
38. Boomsma, B.; Bikker, E.; Lansdaal, E.; Stuut, P. L-Lactic Acid-A safe antimicrobial for home- and personal care formulations. *SOFW Journal* **2015,** *141*, 1-5, https://doi.org/10.14202/vetworld.2019.397-401.
39. Ramos-Villarroel, A.; Martín-Belloso, O.; Soliva-Fortuny, R. Combined effects of malic acid dip and pulsed light treatment son the inactivation of *Listeria innocua* and *Escherichia coli* on fresh-cut produce. *Food Control* **2015,** *52*, 112-118, https://doi.org/10.1016/j.foodcont.2014.12.020.
40. Abadias, M.; Alegre, I.; Usall, J.; Torres, R. Evaluation of alternative sanitizers to chlorine disinfection for reducing food borne pathogens in fresh-cut apple. *Postharvest Biol. Technol.* **2011,** *59*, 289-297, https://doi.org/10.1016/j.postharvbio.2010.09.014.
41. Haute, S.; López-Gálvez, F.; Gómez-López, V.; Devlieghere, E.; Allende, A.; Sampers, I. Methodology for modeling the disinfection efficiency of fresh-cut leafy vegetables wash water applied on peracetic acid combined with lactic acid. *Int. J. Food Microbiol.* **2015,** *208*, 102-113, https://doi.org/10.1016/j.ijfoodmicro.2015.05.020.
42. Siroli, L.; Patrignani, F.; Serrazanetti, D.; Tabanelli, G.; Montanari, C.; Gardini, F.; Lanciotti, R. Lactic acid bacteria and natural antimicrobials to improve the safety and shelf-life of minimally processed sliced apple & lamb's lettuce. *Food Microbiol.* **2015,** *47*, 78-84, https://doi.org/10.1016/j.fm.2014.11.008.
43. Iglesias, M.; Abadias, M.; Anguera, M.; Sabata, J.; Vinas, I. Antagonistic effect of probiotic bacteria against food borne pathogens on fresh-cut pear. *LWT Food Science and Technol.* **2017,** *81*, 243-249, https://doi.org/10.1016/j.lwt.2017.03.057.