https://doi.org/10.17113/ftb.63.01.25.8771 original scientific paper

Antibacterial Effect of Phenylboronic Acid on *Escherichia coli* and Its Potential Role as a Decontaminant of Fresh Tomato Fruits

Running title: PBA as *E. coli* decontaminant

Branka Bedenić[1], Katarina Martinko[2], Edyta Đermić[2], Lovorka Vujić[3], Siniša Ivanković[4], Mladen Miloš[5], Isidoro Feliciello[6] and Damir Đermić[7*]

[1]Biomedical Research Center Šalata-BIMIS University of Zagreb School of Medicine, Department for Clinical Microbiology and Infection prevention and Control, University Hospital Centre Zagreb, Croatia
[2]University of Zagreb Faculty of Agriculture, Division of Phytomedicine, Department of Plant Pathology, Zagreb, Croatia
[3]University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb, Croatia
[4]Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia
[5]Faculty of Chemistry and Technology, University of Split, Split, Croatia
[6]Department of Clinical Medicine and Surgery, University of Naples Federico II, Napoli, Italy
[7]Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia

Received: 11 July 2024
Accepted: 13 February 2025

SUMMARY

Research background. Food safety is threatened by the contamination of fresh fruits and vegetables by pathogenic bacteria, among which the particularly widespread ones are coliform bacteria. Due to the continuous increase in the incidence of severe diseases caused by the consumption of fresh (tomato) fruits contaminated with *Escherichia coli*, antimicrobial postharvest measures are needed. The problem is that many active antimicrobial compounds have a weak and short-lasting effect and/or are not environmentally friendly. Recently, the antibacterial and antifungal

*Corresponding author:
E-mail: dermic@irb.hr

activity of environmentally friendly agent phenylboronic acid (PBA), including on a couple of tomato pathogens, was reported.

Experimental approach. This study aimed to determine the antibacterial effect of PBA on *E. coli* and three enteropathogenic Enterobacterales, and to check its ability to serve as a bacterial decontaminant of fresh tomato fruits.

Results and conclusions. The minimal inhibitory concentration (MIC) of PBA against *E. coli*, as well as *Shigella sonnei*, *Salmonella enteritidis,* and *Yersinia enterocolitica* were 1.0, 1.2, 1.0 and 0.8 mg/mL, respectively. Moreover, we have shown that PBA has a bacteriostatic effect on *E. coli* at lower concentrations and a bactericidal effect at higher (>3.0 mg/mL) concentrations. Importantly, the study found that an *E. coli* strain resistant to seven commonly used antibiotics, as well as strains producing extended-spectrum beta-lactamases (ESBL), is as sensitive to PBA as the wild-type strain lacking any resistance, suggesting that PBA's mechanism of action differs from that of all these antibiotics. Finally, we have shown that washing and incubating contaminated tomato fruits in PBA solution reduces the growth of *E. coli* washed from fresh tomato fruits in a dose- (0.5–3.0 mg/mL) and time-dependent manner, while having no adverse effect on the tomato fruits.

Novelty and scientific contribution. This is the first report of PBA's antibacterial effect on medically important bacteria *E. coli, S. enteritidis, S. sonnei and Y. enterocolitica*. Moreover, we show that PBA kills multiple-antibiotic resistant *E. coli,* including those producing ESBL*,* revealing it as a promising agent against such bacteria*.* Finally, PBA is shown to be an effective decontaminant of *E. coli* on fresh tomato fruits.

Keywords: *Shigella sonnei*; *Salmonella enteritidis*; *Yersinia enterocolitica;* multiple-antibiotic resistant *Escherichia coli*; ESBL

INTRODUCTION

Contamination of fresh fruits and vegetables by pathogenic bacteria constitutes a public health risk, which makes it a permanent challenge for the modern food industry (*1,2*). Among the most common contaminants of fresh food is *Escherichia coli* (*3*), the consumption of which leads to various gastrointestinal infections, as it is a dangerous human pathogen (*1,4*). For instance, a large outbreak of *E. coli* O104:H4 occurred in Germany in 2011, affecting 3842 people and resulting in 53 deaths, which was attributed to the consummation of contaminated fresh bean sprouts (*5*). The bacterium is a Gram-negative and flat rod-shaped (*6*). Being a facultative anaerobe, *E. coli* can survive in the absence of oxygen (*7*), which increases the risk of food contamination (*1*). *E. coli* is an indicator of

fecal contamination and water pollution (*8,9*). Thus, irrigation water contaminated with *E. coli* supplied to crops is a potential source of contamination of fresh fruits and vegetables (*9,10*), by coming in contact with the plant foliage or wounds (*11*) through bioaerosols generated by sprinkler irrigation (*12*). Solomon *et al.* (*13*) state that such a mode of spread increases the population of *E. coli* on fresh fruits the most. Among the cultivated plants, tomatoes have one of the highest risks of contamination by *E. coli* (*10*).

Fresh tomato fruits are occasionally contaminated with pathogens, which results in food-borne diseases and epidemics. Guo *et al.* (*14*) reported that in 1990, 176 cases in humans, caused by the consumption of raw tomato fruits, were reported in Illinois, Michigan, Minnesota, and Wisconsin, USA. Due to the continuous increase in diseases transmitted by consumption of contaminated raw vegetables such as tomatoes, effective antimicrobial methods are needed during processing of harvested fruits (*15*).

A recent report (*2*) describes the decontamination of the *E. coli* population on the surface of fruits using various compounds with antimicrobial activity. Although effective in reducing cross-contamination of fruits with *E. coli*, some decontaminating agents are of limited utility because their effectiveness decreases rapidly (*16*) and some are explosive or irritant (*17*). An alternative approach to decontamination of *E. coli* from tomatoes is the use of environmentally friendly and highly efficient compounds at low concentrations (*2*). We have recently reported the antibacterial and antifungal activity of phenylboronic acid (PBA) on tomato pathogens at the concentrations that are not toxic to the plant (*18-20*), which could make PBA a suitable candidate for the decontamination of fresh fruits, especially since PBA is well tolerated by mammals (*21,22*) and is considered environmentally friendly (*20,23,24*). PBA is a derivative of the medically important boric acid (*25*), which in certain concentrations has a significant antimicrobial effect on some medically important bacteria (*26*), whereas its activity on *E. coli* and its relatives from the Enterobacteriaceae family, such as *Salmonella enteritidis, Shigella sonnei and Yersinia enterocolitica* has not been reported yet. Therefore, in this study we have determined the PBA MIC for these common causative agents of foodborne illnesses, as well as the *in vitro* effect of PBA on *E. coli* growth and viability, including on multidrug resistant strains. Finally, we determined the PBA inactivation of *E. coli* washed from the surface of fresh tomato fruits.

MATERIALS AND METHODS

Bacterial strains

We used a wild-type K-12 strain MG1655, which is a commonly used laboratory strain close to the archetypal *E. coli* K-12 strain (*27*). It has no antibiotic resistance and is not pathogenic. We constructed an MG1655 derivative DE728 resistant to seven commonly used antibiotics: tetracycline, ampicillin, chloramphenicol, kanamycin, rifampicin, streptomycin, and nalidixic acid. The antibiotic resistance was produced either by selecting forward mutations of the *E. coli* genes *rpsL*, *gyrB* and *rpoB* for streptomycin, nalidixic acid and rifampicin resistance, respectively; or by the introduction of transposon-marked alleles by P1 phage transduction (*28*): *thr*::Tn*10*, *zoi*::Tn*3*, *malB*::Tn*9* and *ΔproA*::Km, which bring resistance to tetracycline, ampicillin, chloramphenicol and kanamycin, respectively. *Salmonella enteritidis, Shigella sonnei Yersinia enterocolitica,* as well as *E. coli* strains producing extended-spectrum beta-lactamases, are from the collection of the Department for Microbiology and Parasitology of the Medical School, University of Zagreb.

The *E. coli* strains that produce ESBL were clinical isolates isolated from urine (Table S1) as described (*29*).

Determination of minimum inhibitory concentration of PBA

The minimum inhibitory concentration (MIC) of PBA for *E. coli*, *S. enteritidis, S. sonnei and Y. enterocolitica* was determined by agar dilution according to CLSI standards (*30*). An inoculum of 10^4 CFU per spot was applied on an LB agar (Gibco, Waltham, USA) plate containing a certain concentration of PBA, which were then incubated for 24 h at 37 °C. MIC was defined as the lowest concentration inhibiting the growth of the colonies on agar. As a control, plates were used that lacked PBA, and the normal growth and bacterial viable cells titer was determined.

Preparation of PBA concentration range

Based on the determined MIC for *E. coli*, PBA (Merck, New Jersey, USA) was prepared in a certain range of concentrations (1/2 MIC, 1 MIC, 2 MIC and 3 MIC). A stock solution of PBA at a concentration of 10 mg/mL was prepared in sterile water or LB, which was then diluted to the final concentrations from 0.4 to 4.0 mg/mL.

Determination of growth kinetics and viability of E. coli treated with PBA

In accordance with a previously described procedure (*18*), *E. coli* was grown in a liquid LB medium at 37 °C with aeration. The bacterial culture in the exponential growth phase was diluted 10-

fold into the fresh LB medium containing PBA. Incubation of the bacteria in the PBA-enriched medium was done at 37 °C with aeration, during which the samples were periodically taken, and their optical density (A_{600}) was determined by a colorimeter (Novaspec II, Amersham Pharmacia Biotech, USA) as well as their viable bacteria count. The titer of viable bacteria (either wild-type or its derivative resistant to: tetracycline, ampicillin, chloramphenicol, kanamycin, rifampicin, streptomycin, and nalidixic acid) was determined by a serial dilution of bacterial cultures in 67 mmol/L phosphate buffer and plating them on LB agar plates lacking PBA, which were incubated at 37 °C for 48 h. The optical density and viable cells' titer at the start of incubation were used as a reference for expressing their changes during the incubation.

PBA treatment of tomato fruits contaminated with E. coli

Considering the determined MIC, *E. coli* was tested against a range of MIC concentrations according to the modified method of inactivation of *E. coli* by washing from fresh tomato fruits according to Zhang *et al*. (*2*). Thirty-six cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) fruits were immersed in a suspension of *E. coli* ($7.9 \cdot 10^8$ CFU/mL) and soaked for 30 min. After soaking, the fruits were dried on a paper towel to facilitate bacterial adherence to the fruit surface. Each tomato fruit was then placed in a sterile plastic box (8 cm×6 cm×7 cm), and the prepared PBA solution was added. A sealed box containing the fruit immersed in a certain concentration of PBA was placed on a shaker and secured using adhesive tape. By running the shaker (1000 rpm/2 min), the fruit was washed with the PBA solution. The procedure was repeated with control solutions of sterile distilled water and ethanol (EtOH) (1.0 %). After rinsing, the solution in which the tomato fruits were washed, was pipetted in a volume of 100 µl onto an LB medium in sterile Petri dishes (9 cm) and evenly dispersed with a glass plate spreader. The inoculated Petri dishes were incubated in an air chamber at 37 °C in the dark.

The experiment was repeated with the incubation of tomato fruits in PBA solutions for 120 minutes. The duration of exposure was determined by a preliminary experiment (data not shown) which determined that there was no cracking or discoloration of the tomato fruits after 120 minutes of immersion in PBA.

Readings of the results were performed 72 h after setting up the experiment by photographing the grown colonies in petri dishes. Measurement of the total area (cm^2) of *E. coli* colonies was

performed using the software ImageJ according to Guzmán *et al*. (*31*) to determine the degree of pathogen inactivation.

Statistical data analysis was performed by one-way analysis of variance (One Way ANOVA), and differences between treatments were evaluated by the Tukey test ($p \leq 0.05$) (*32*) using SPSS v. 27 (*33*).

RESULTS AND DISCUSSION

Determination of PBA minimum inhibitory concentration

The growth of bacterial colonies was followed on the plates with concentrations ranging from 0.7 to 2.5 mg/mL PBA. We analyzed the common human pathogenic Gram-negative bacteria that are the most prevalent causative agents of food-borne infections, such as *E. coli* and its relatives: *S. enteritidis, S. sonnei and Y. enterocolitica*. As shown in Table 1, the minimal concentrations of PBA that blocked the growth of *E. coli*, *S. enteritidis, S. sonnei and Y. enterocolitica* were 1.0, 1.0, 1.2 and 0.8 mg/mL, respectively. Hence, we infer these concentrations to be PBA MICs for these bacteria. The observed PBA MICs are similar to the one of their plant pathogenic relative *Erwinia amylovora* (0.8 mg/mL), while being about twice as high as the PBA MIC for another plant pathogenic bacterium *Pseudomonas syringae* pv. *tomato* (0.5 mg/mL) (*18*).

The effect of PBA on growth and viability kinetics of E. coli

Since the PBA MICs for *E. coli* and its bacterial relatives were similar, we used the former in further research, mostly due to its facultative pathogenic character and the consequent convenience of working with it, as well as our ample experience in working with that organism. To better characterize the effect of PBA on *E. coli* physiology, we measured growth and survival kinetics of the bacterium in LB medium containing various concentrations of PBA. As shown in Fig. 1a) PBA slowed the bacterial growth in a dose-dependent manner. The *E. coli* mass-doubling time was ~26 min in medium lacking PBA, which increased greatly in medium containing 2.0 or 3.0 mg/mL PBA, where the bacteria stopped growing. There was a decrease in bacterial optical density when the medium contained 4.0 mg/mL PBA (Fig. 1a). These results thus indicate that PBA in concentrations of 2.0 to 3.0 mg/mL has a bacteriostatic effect whereas a concentration of 4.0 mg/mL shows a bactericidal effect on *E. coli*.

We directly measured the effect of PBA on bacterial viability by determining the viable cell count in cultures containing PBA. In medium lacking PBA, the bacterial viable count, expressed as the titer of colony forming units (CFU/mL), grew exponentially (Fig. 1b). On the other hand, the viable cells' titer grew only marginally in medium containing 2.0 mg/mL PBA and then somewhat fell after prolonged incubation (overnight). The survival of *E. coli* was even more reduced upon incubation in the medium with 3.0 mg/mL PBA, which was even more pronounced when the PBA concentration was 4.0 mg/mL, in which case it was reduced about 100-fold after overnight incubation (Fig. 1b). These results show that 2.0 mg/mL of PBA in LB medium acts as bacteriostatic, whereas PBA at 3.0 mg/mL has a mild bactericidal effect, which greatly rises when the PBA concentration increases to 4.0 mg/mL. The higher PBA concentrations required to inhibit *E. coli* growth and viability in this assay compared to those in the MIC assay are likely due to the much shorter exposure period in the former. Moreover, the MIC assay does not differentiate between bacteriostatic and bactericidal effects of the assayed agent (the effects are added), whereas the growth and viability kinetics assays are able to differentiate between the two.

Resistance to multiple antibiotics does not affect E. coli sensitivity to PBA

We determined how resistance to multiple commonly used antibiotics (*e.g.* tetracycline, ampicillin, chloramphenicol, kanamycin, rifampicin, streptomycin, and nalidixic acid) affects *E. coli* survival upon exposure to PBA. As shown in Fig. 2, the DE728 strain, resistant to seven antibiotics, showed a drop in viable cell titer that is comparable to the survival of its wild-type progenitor (MG1655) when exposed to the similar concentration of PBA (compare Fig. 2 and Fig. 1b). This result indicates that the mechanism of PBA toxicity to the bacterium differs from those of the seven tested antibiotics, with the corollary that PBA can be used against *E. coli* irrespective of its antibiotic resistance, hence qualifying it as a valuable alternative for treating infections associated with multiple-antibiotic resistant *E. coli* strains. Moreover, since the mechanisms of antibiotic activity, as well as the mechanisms of bacterial resistance to antibiotics, are conserved among different bacteria, the unaffected killing of multiple-antibiotic resistant *E. coli* by PBA should represent a general trait among bacterial species.

In light of the growing problem of the increasing incidence of antibiotic resistance of medicinally important pathogenic bacteria (such as Enterobacterales), we determined the PBA MIC against *E. coli* resistant to newer antibiotics, namely strains producing CTX-M beta-lactamases belonging to extended spectrum (ESBL), which are resistant to new-generation penicillin and cephalosporins

(Table 2). The problem with ESBL-producing bacteria is that ESBLs are mainly encoded by plasmids, which often also carry genes encoding resistance to other classes of antimicrobials (for example, aminoglycosides, quinolones, tetracyclines, *etc.*) (Table S1) (*29*). This multiple resistance to antimicrobial agents limits the treatment options of ESBL-producing bacteria and poses a risk to successful treatment. As shown in Table 2, the PBA MIC for all 9 strains producing ESBL was similar to the MIC of the control *E. coli* strain that is devoid of any resistance (1.2 mg/mL). Their MICs varied from 0.8 to 1.3 mg/mL. Our results indicate that PBA can be applied against *E. coli* producing ESBL, therefore alleviating the problem of multiple resistance in those bacteria. Certainly, further research is required to better optimize the use of PBA in human medicine.

The drop of A_{600} (cell density) in cultures treated with higher concentrations of PBA (>4.0 mg/mL) (Fig. 2) indicates that the mechanism of killing *E. coli* by PBA involves disintegration of the cells. The same effect was observed upon exposure of *E. amylovora* and *P. syringae* pv. *tomato* to PBA, albeit at lower concentrations (2.0 and 3.0 mg/mL, respectively) compared to *E. coli* (*18*). However, the common trait of PBA action on all three bacteria is that the cell density drop is observed at about 4 MIC.

Antibacterial effect of PBA on E. coli washed from tomato fruits

After washing the tomato fruits with PBA of ½ MIC (0.5 mg/mL), 1 MIC (1.0 mg/mL), 2 MIC (2.0 mg/mL) and 3 MIC (3.0 mg/mL), the growth of *E. coli* colonies was recorded. The growth area of *E. coli* after washing with ½ MIC, 1 MIC, 2 MIC and 3 MIC PBA from tomato fruits was reduced by 41, 59, 53 and 85 %, respectively, compared to the control wash with dH_2O (Table 3).

Washing of *E. coli* cells with ½ MIC, 1 MIC, 2 MIC and 3 MIC PBA from tomato fruits in the indicated concentrations, resulted in a decrease in the growth area of bacterial colonies by 20, 44, 36 and 80 %, respectively, compared to the control washing with EtOH (1.0 %) (Table 3).

Mean values of *E. coli* colony growth were statistically significant in all tested variants (½ MIC, 1 MIC, 2 MIC and 3 MIC PBA) compared to control washings with dH_2O and EtOH (1.0 %) according to the Tukey test (Table 3). Our results thus show that PBA retards the growth of *E. coli* washed from tomato fruits.

Antibacterial effect of prolonged incubation with PBA on the growth of E. coli washed from tomato fruits

The growth of *E. coli* colonies washed from tomato fruits was recorded after incubation for 120 min with PBA in concentrations ½ MIC (0.5 mg/mL), 1 MIC (1.0 mg/mL), 2 MIC (2.0 mg/mL) and 3 MIC (3.0 mg/mL).

As shown in Table 3, the growth area of *E. coli* colonies after washing with ½ MIC, 1 MIC, 2 MIC and 3 MIC PBA from tomato fruits and after immersion of fruits in the specified concentration range for 120 min, was inhibited by 97, 68, 94 and 97 %, respectively, compared to washing with dH_2O. Washing of *E. coli* and exposure of fruits at the indicated concentrations, resulted in 96, 56, 92 and 96 % respective reduction in bacterial colony growth area compared to the control washing with EtOH (1.0 %).

Mean colony growth values of *E. coli* were statistically significant in all tested variants (½ MIC, 1 MIC, 2 MIC and 3 MIC PBA) compared to mean colony growth values in control variants with dH_2O and EtOH (1.0 %) according to the Tukey test (Table 3).

We can therefore conclude that PBA reduces the growth of *E. coli* washed from tomato fruits in a dose- and time-dependent manner. Earlier, Shen *et al*. (*34*), reported stronger inhibition of *E. coli* in an aqueous chlorine solution with increased incubation time. They showed that inactivation of the *E. coli* depends on the efficacy and concentration of the compound and the time of exposure of the bacterium, which is in accord with our results.

Moreover, the results show higher efficacy of PBA compared to the ethanol (EtOH), which is a commonly used disinfectant. Namely, 1.0 % solution of ethanol showed less effect on *E. coli* than PBA at 0.5 mg/mL, (*i.e.* 0.05 %). Surely, one can increase the EtOH concentration, but that would have potentially negative side effects since ethanol is a strong oxidant and can therefore negatively impact the quality of tomato fruits. On the other hand, PBA is a weak acid, which would therefore weakly affect the tomato fruit even at 1.0 % concentration (we did not observe any adverse effect of PBA in concentrations that we used on tomato fruits, not shown), while we noted quite a strong effect on *E. coli* washed from the tomato fruit already with 0.3 % PBA, meaning that there is a possibility of using higher PBA concentrations than we used here. This is especially relevant since PBA is environmentally friendly (*20,23,24*) and is well tolerated by mammals (*21,22*). For instance, the LD_{50} (oral) for a rat is 0.74 g/kg of PBA, as compared to the LD_{50} of NaCl, which is 3.0 g/kg (*35*).

By comparing the results of our *in vitro* experiments, a difference can be observed between the inhibitory effect of PBA dissolved in distilled water at prolonged exposure, where an antibacterial effect on *E. coli* was achieved at 0.5 mg/mL PBA (Table 3), and the inhibitory effect of PBA dissolved

in nutrient medium where an antibacterial effect is achieved at 1.0 mg/mL PBA (Table 1). The discrepancy can be explained by the data of Virto *et al*. (*35*), which showed that inactivation of *E. coli* by chlorine dissolved in distilled water is significantly more pronounced than the inactivation of bacteria that were exposed to chlorine in an organic medium. Their results suggest that bacterial cell membrane damage is greater in water compared to the organic medium that prevented cell membrane permeability and chlorine penetration into the *E. coli* cell. Hence, our results are consistent with that study (*36*).

CONCLUSIONS

In this study we have revealed PBA as a promising antibacterial agent of medical importance due to two of its properties. Firstly, PBA has antibacterial effects on *E. coli* and its enterobacterial relatives. We determined the PBA concentrations with bacteriostatic/bactericidal effects against *E. coli*. Secondly, we have shown that PBA is effective against multidrug resistant *E. coli*, including resistance to modern antibiotics. Moreover, we have used PBA for an efficient decontamination of *E. coli* from fresh tomato fruits, thus disclosing the potential of PBA usage in raw food decontamination.

This is the first study of the antibacterial effect of PBA on the *E. coli* and its pathogenic relatives, which is supplemented with the determination of practical usage of PBA for decontamination of (even multiple-antibiotic resistant) bacteria on fresh tomato fruits and therefore opens up a perspective of PBA application in food processing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

D. Đermić and E. Đermić designed the research. D. Đermić, K. Martinko and B. Bedenić performed experiments. S. Ivanković and M. Miloš participated in data analysis. D. Đermić, K. Martinko, Isidoro Feliciello, and L. Vujić performed the statistical analysis. D. Đermić and K. Martinko wrote the manuscript. All authors revised and approved the final submitted version of the manuscript.

ORCID ID

B. Bedenić https://orcid.org/0000-0002-9975-6830

K. Martinko https://orcid.org/0000-0002-8526-8457

E. Đermić https://orcid.org/0000-0002-0786-9062

L. Vujić https://orcid.org/0000-0002-8755-9960

S. Ivanković https://orcid.org/0000-0001-7551-5111

M. Miloš https://orcid.org/0000-0001-9439-9400

I. Feliciello https://orcid.org/0000-0002-2588-9366

D. Đermić https://orcid.org/0000-0002-5263-2750

REFERENCES

1. Tack ILMM, Nimmegeers P, Akkermans S, Hashem I, van Impe JFM. Simulation of *Escherichia coli* dynamics in biofilms and submerged colonies with an individual-based model including metabolic network information. Front Microbiol. 2017;8:2509.

https://doi.org/10.3389/fmicb.2017.02509

2. Zhang H, Dolan HL, Ding Q, Wang S, Tikekar RV. Antimicrobial action of octanoic acid against *Escherichia coli* O157:H7 during washing of baby spinach and grape tomatoes. Food Res Int. 2019. 108523.

https://doi.org/10.1016/j.foodres.2019.108523

3. Nguyen C, Carlin F. The microbiology of minimally processed fresh fruits and vegetables. Crit Rev Food Sci Nutr. 1994;34(4):371–401.

https://doi.org/10.1080/10408399409527668

4. Rowe P. *Escherichia coli* O157:H7, other verotoxin-producing *E. coli* and the hemolytic uremic syndrome in childhood. Can J Infect Dis. 2009;6:105–10.

https://doi.org/10.1155/1995/803560

5. Burger R. EHEC O104:H4 in Germany 2011: Large outbreak of bloody diarrhea and haemolytic uraemic syndrome by Shiga toxin–producing *E. coli via* contaminated food. *In:* IOM (Institute of Medicine) Improving food safety through a One Health approach. The National Academies Press, Washington, DC, USA; 2012. pp. 115-30.

6. Shiomi D, Mori H, Niki H. Genetic mechanism regulating bacterial cell shape and metabolism. Commun Integrative Biol. 2009;2(3):219–20.

https://doi.org/10.4161/cib.2.3.7930

7. von Wulffen J, RecogNice-Team, Sawodny O, Feuer R. Transition of an anaerobic *Escherichia coli* culture to aerobiosis: Balancing mRNA and protein levels in a demand-directed dynamic flux balance analysis. PloS One. 2016;11(7):e0158711.

https://doi.org/10.1371/journal.pone.0158711

8. Clesceri LS, Greenberg AE, Eaton AD. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, USA, 1998.

9. Gupta D, Madramootoo CA. Fate and transport of *Escherichia coli* in tomato production. Expo Health*.* 2017;9:1-13.

https://doi.org/10.1007/s12403-016-0217-7

10. Ibekwe AM, Watt PM, Shouse PJ, Grieve CM. Fate of *Escherichia coli* O157:H7 in irrigation water on soils and plants as validated by culture method and real-time PCR. Can J Microbiol. 2004;50(12):1007-14.

https://doi.org/10.1139/w04-097

11. Gu G, Hu J, Cevallos-Cevallos JM, Richardson SM, Bartz JA, van Bruggen AHC Internal colonization of *Salmonella enterica* serovar Typhimurium in tomato plants. PLoS One. 2011;6(11):e27340.

https://doi.org/10.1371/journal.pone.0027340

12. Pianietti A, Sabatini L, Bruscolini F, Chiaverini F, Cecchetti G. Faecal contamination indicators, *Salmonella, Vibrio* and *Aeromonas* in water used for the irrigation of agricultural products. Epidemiol Infect. 2004;132(2):231–38.

https://doi.org/10.1017/s095026880300181x

13. Solomon EB, Pang HJ, Matthews KR Persistence of *Escherichia coli* O157:H7 on lettuce plants following spray irrigation with contaminated water. J Food Prot. 2003;66(12):2198–202.

https://doi.org/10.4315/0362-028x-66.12.2198

14. Guo X, Chen JR, Brackett RE, Beuchat LR. Survival of *Salmonellae* on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. Appl Environ Microbiol. 2001;67:4760–4.

https://doi.org/10.1128/AEM.67.10.4760-4764.2001

15. Shu P, Li YJ, Wang XY, Yao L, Sheng JP, Shen L. Exogenous ferulic acid treatment increases resistance against *Botrytis cinerea* in tomato fruit by regulating nitric oxide signaling pathway. Postharv Biol Technol. 2021;182:111678.

https://doi.org/10.1016/j.postharvbio.2021.111678

16. Praeger U, Herppich WB, Hassenberg K. Aqueous chlorine dioxide treatment of horticultural produce: Effects on microbial safety and produce quality–a review. Crit Rev Food Sci Nutr. 2018;58(2):318–33.

https://doi.org/10.1080/10408398.2016.1169157

17. Ölmez H, Kretzschmar U. Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. LWT-Food Sci Technol. 2009;42(3):686–93.

https://doi.org/10.4236/fns.2017.810068

18. Martinko K, Ivanković S, Đermić E, Đermić D. Phenylboronic acid as a novel agent for controlling plant pathogenic bacteria. Pest Manag Sci. 2022;78:2417-22. https://doi.org/10.1002/ps.6872

19. Martinko K, Ivanković S, Đermić E, Đermić D. *In vitro* antifungal effect of phenylboronic and boric acid on *Alternaria alternata*. Arch Industr Hygiene Toxicol. 2022;73:83-7. https://doi.org/10.2478/aiht-2022-73-3620

20. Martinko K, Ivanković S, Đermić E, Đermić D. Control of early blight fungus (*Alternaria alternata*) in tomato by boric and phenylboronic acid. Antibiotics. 2022;11**:**320.

https://doi.org/10.3390/antibiotics11030320

21. Soriano-Ursúa MA, Farfán-García ED, López-Cabrera Y, Querejeta E, Trujillo-Ferrara JG. Boron-containing acids: preliminary evaluation of acute toxicity and access to the brain determined by Raman scattering spectroscopy. Neurotoxicology. 2014;40:8–15.

https://doi.org/10.1016/j.neuro.2013.10.005

22. Marasović M, Ivanković S, Stojković R, Đermić D, Galić B, Miloš M. *In vitro* and *in vivo* antitumour effects of phenylboronic acid against mouse mammary adenocarcinoma 4T1 and squamous carcinoma SCCVII cells. J Enzyme Inhib Med Chem. 2017;32:1299-304.

https://doi.org/10.1080/14756366.2017.1384823

23. Michaelis A, Becker B. Ueber monophenylborchlorid und einige derivate desselben. Ber Dtsch Chem Ges. 1882;15:180–5.

24. US Environmental Protection Agency. Boronic acid, phenyl 98-80-6| DTXSID9059179. CompTox Chemicals Dashboard: Release Notes. Ver.2.2.1. 2023; Available at https://comptox.epa.gov/dashboard/chemical/details/ DTXSID9059179 [displayed 13 March 2022].

25. Liu X, Laks PE, Pruner MS. A preliminary report on the wood preservative properties of phenylboronic acid. Forest Prod J. 1994;44(6):46-8.

26. Freeman A, Village Y, Segal R, Dror Y Methods and compositions for treating fungal infections, Pub. No.: US 2004/022014.6 A1. 2010

27. Blattner FR, Plunkett G, Bloch CA, Perna NT, Burland V, Riley M, *et al.* The complete genome sequence of *Escherichia coli* K-12. Science. 1997;277: 1453-1462. https://doi.org/ 10.1126/science.277.5331.1453

28. Miller JH A Short Course in Bacterial Genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA. 1992.

29. Krilanović M, Tomić-Paradžik M, Meštrović T, Beader N, Herljević Z, Conzemius R, Barišić I, Vraneš J, Elveđi-Gašparović V, Bedenić B. Extended-spectrum beta-lactamases and plasmid diversity in urinary isolates of *Escherichia coli* in Croatia: a nation-wide, multicentric, retrospective study. Folia Microbiol. 2020;65(4):649-67.

https://doi.org/10.1007/s12223-019-00769-1.

30. Clinical Laboratory Standard Institution. Performance Standards for Antimicrobial Susceptibility Testing, 28th ed.; Approved Standard M100-S22; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2018.

31. Guzmán C, Bagga M, Kaur A, Westermarck J, Abankwa D. ColonyArea: An ImageJ plugin to automatically quantify colony formation in clonogenic assays. PLoS One. 2014;9(3):e92444.

https://doi.org/10.1371/journal.pone.0092444

32. Tukey JW. Comparing individual means in the analysis of variance. Biometrics. 1949;5(2):99–114.

33. IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp. 2021.

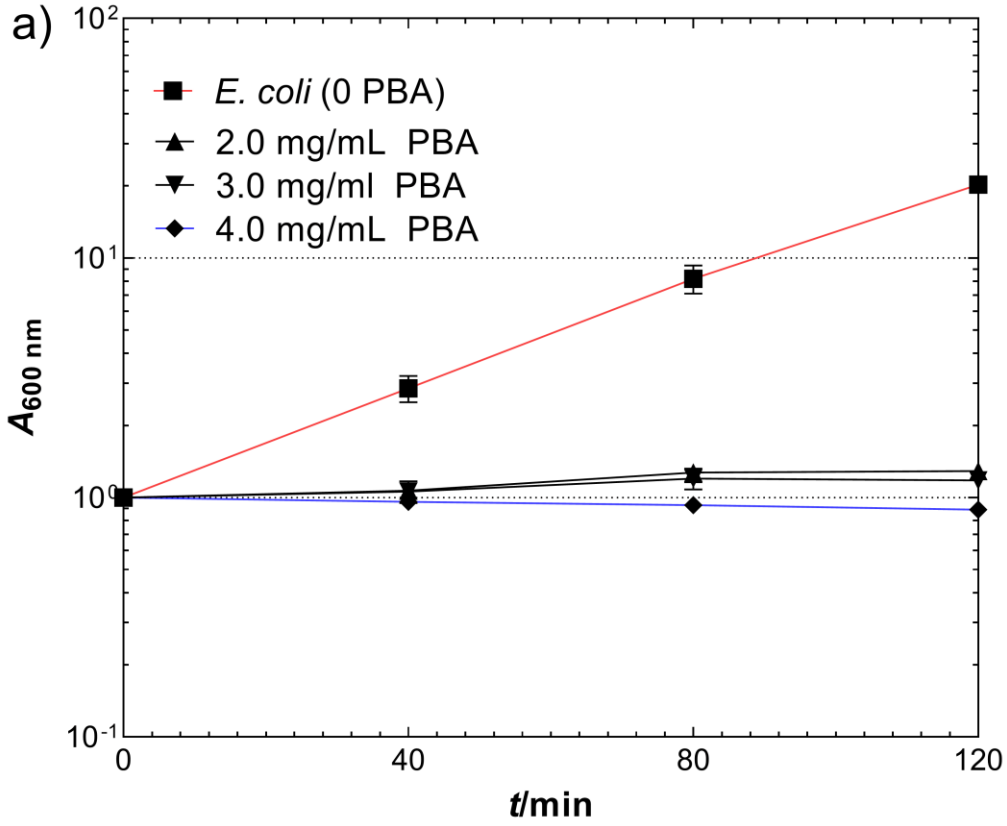
34. Shen C, Luo Y, Nou X, Wang Q, Millner P. Dynamic effects of free chlorine concentration, organic load, and exposure time on the inactivation of *Salmonella*, *Escherichia coli* O157:H7, and non-O157 Shiga toxin–producing *E. coli*. J Food Prot. 2013;76(3):386–93.

https://doi.org/10.4315/0362-028X.JFP-12-320

35. ECHA (European Chemicals Agency) Sodium chloride. EC number: 231-598-3| CAS number: 7647-14-5 (toxicological information). 2023. Available at https://echa. europa.eu/registration-dossier/-/registered-dossier/15467/1/2# [displayed 1 November

36. Virto R, Mañas P, Alvarez I, Condon S, Raso J. Membrane damage and microbial inactivation by chlorine in the absence and presence of a chlorine-demanding substrate. Appl Environ Microbiol. 2005;71(9): 5022–8.

https://doi.org/10.1128/AEM.71.9.5022-5028.2005



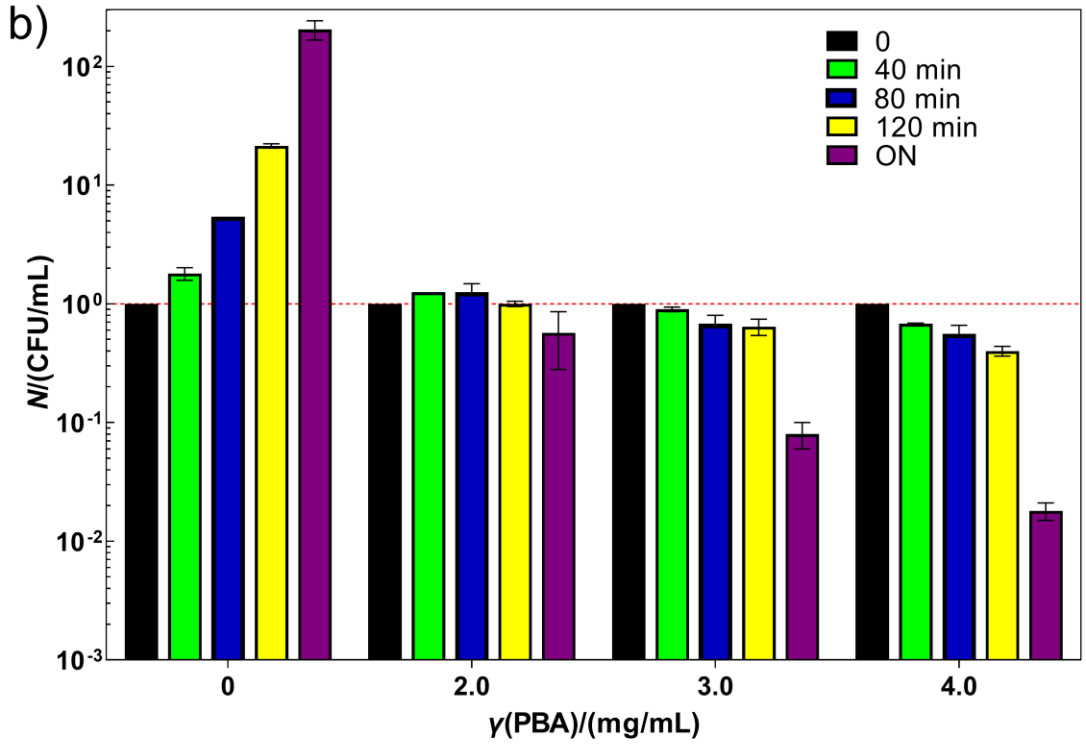


Fig. 1 Kinetics of wild-type *E. coli* MG1655 growth (a) and viable cell count (CFU, Colony Forming Units) (b) in a liquid LB medium supplemented with phenylboronic acid, at 37 °C (duration of exposure to PBA is expressed in minutes). Serial dilution of bacterial cultures was applied on LB plates (with no PBA added) and incubated for 48 h at 37 °C. Optical density and viable cells titer at the start of incubation were used as a reference for expressing their changes during the incubation. Each value is a mean of three independent experiments, with error bars representing standard deviation

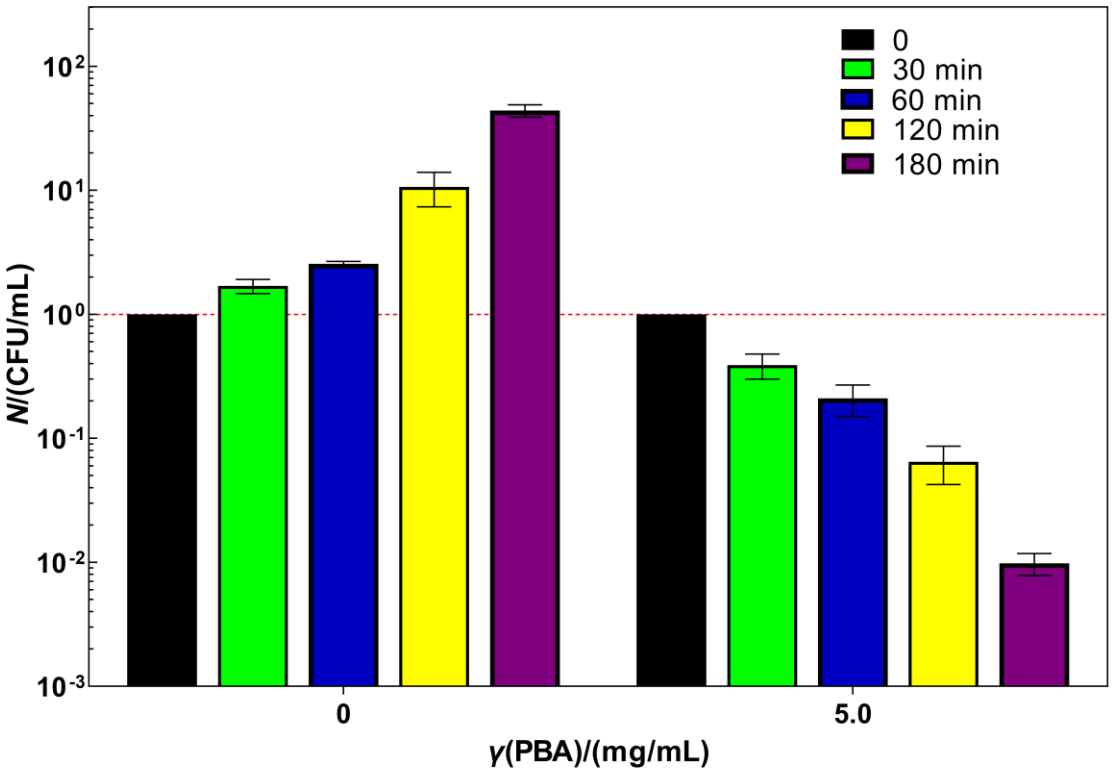


Fig. 2. Kinetics of multiple-antibiotic resistant *E. coli* DE728 viable cell count (CFU) in a liquid LB medium supplemented with phenylboronic acid, at 37 °C (duration of exposure to PBA is expressed in minutes). Bacteria were resistant to tetracycline, ampicillin, chloramphenicol, kanamycin, rifampicin, streptomycin and nalidixic acid. Serial dilution of bacterial cultures was applied on LB plates (with no PBA added) and incubated for 48 h at 37 °C. Viable-cells titer at the start of incubation was used as a reference for expressing its changes during the incubation. Each value is a mean of three independent experiments, with error bars representing standard deviation

Table 1. Inhibitory effect of PBA on human pathogenic bacteria growth on agar plates after 24 h of incubation at 37 °C

γ(PBA)/ (mg/mL) / Organism	0.7	0.8	0.9	1.0	1.1	1.2	1.5	2.0	2.5
Yersinia enterocolitica	+	-	-	-	-	-	-	-	-
Escherichia coli	+	+	+	-	-	-	-	-	-
Salmonella enteritidis	+	+	+	-	-	-	-	-	-
Shigella sonnei	+	+	+	+	+	-	-	-	-

Table 2. Inhibitory effect of PBA on growth of *Escherichia coli,* producing extended-spectrum beta-lactamases (ESBL), on agar plates after 24 h of incubation at 37 °C

Strain	Additional resistance[a]	*γ*(PBA)/(mg/mL)								
		0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5
5	CAZ, CTX, CRO, FEP, GM, CIP	+	-	-	-	-	-	-	-	-
6	CAZ, CTX, CRO, FEP, GM, CIP	+	-	-	-	-	-	-	-	-
11	CAZ, CTX, CRO, FEP, GM, CIP	+	-	-	-	-	-	-	-	-
2	CAZ, CTX, CRO, FEP, GM, CIP	+	+	+	+	-	-	-	-	-
3	CTX, CRO, FEP, GM, CIP	+	+	+	+	-	-	-	-	-
4	CAZ, CTX, CRO, FEP, GM, CIP	+	+	+	+	-	-	-	-	-
1	CAZ, CTX, CRO, FEP, CIP	+	+	+	+	+	-	-	-	-
12	CAZ, CTX, CRO, FEP, CIP	+	+	+	+	+	-	-	-	-
ATCC25922		+	+	+	+	+	-	-	-	-

8	CAZ, CTX, CRO, FEP, CIP	+	+	+	+	+	+	-	-	-

[a]CAZ-ceftazidime, CTX-cefotaxime, CRO-ceftriaxone, FEP-cefepime, IMI-imipenem, MEM-meropenem, GM-gentamicin, CIP-ciprofloxacin, TZP-piperacillin-tazobactam

Table 3. Effect of PBA on growth of *Escherichia coli* washed from tomato fruits and incubated either 0 min (top row) or 120 min (bottom row) in the PBA-containing solutions, compared to control washings with dH_2O and EtOH (1.0 %) after 72 h incubation

γ(PBA)/(mg/mL)	*E. coli* + dH_2O	*E. coli* + EtOH	*E. coli* + 0.5	*E. coli* + 1.0	*E. coli* + 2.0	*E. coli* + 3.0
Mean value of colony area (cm^2) ± SD	$(3.4\pm0.2)^{e}$	$(2.5\pm0.1)^{d}$	$(2.0\pm0.2)^{c}$	$(1.4\pm0.2)^{b}$	$(1.6\pm0.4)^{b}$	$(0.5\pm0.2)^{a}$
	$(3.4\pm0.2)^{d}$	$(2.5\pm0.1)^{c}$	$(0.1\pm0.0)^{a}$	$(1.1\pm0.1)^{b}$	$(0.2\pm0.0)^{a}$	$(0.1\pm0.0)^{a}$

*Different letters indicate a significant difference according to the Tukey test at the level of $p<0.05$

Supplementary material

Table S1. Additional antibiotic and genomic traits of the clinical isolates of ESBL-producing *Escherichia coli* strains

Strain		Minimal inhibitory concentration (mg/mL)[a, b]										DNA profile	
		CAZ	CTX	CRO	FEP	IMI	MEM	GM	CIP	TZP	Beta-lactamase content	Plasmid type	PFGE cluster
1	3459	32 (R)	16 (R)	128 (R)	2 (I)	0.06 (S)	0.06 (S)	4 (S)	128 (R)	4 (S)	CTX-M-15, TEM-1	X, FIA	E XXIV
2	7377	8 (I)	8 (R)	>128 (R)	64 (R)	0.06 (S)	0.06 (S)	64 (R)	>128 (R)	4 (S)	CTX-M-15, TEM-1	FIA	E XVIIIb
3	16416	4 (S)	16 (R)	128 (R)	4 (R)	0.06 (S)	0.06 (S)	64 (R)	128 (R)	2 (S)	CTX-M-15	X, FIA	E XXXII
4	1358	64 (R)	>128 (R)	>128 (R)	64 (R)	0.06 (S)	0.06 (S)	128 (R)	>128 (R)	32 (S)	CTX-M-15, TEM-1	X	E IX
5	23407	64 (R)	>128 (R)	>128 (R)	64 (R)	0.06 (S)	0.06 (S)	128 (R)	128 (R)	2 (S)	CTX-M-15, TEM-1	L/M	E XXVII
6	3263	16 (R)	128 (R)	64 (R)	64 (R)	0.06 (S)	0.06 (S)	64 (R)	>128 (R)	1 (S)	CTX-M-15	FIA	E II
8	21646	16 (R)	64 (R)	16 (R)	4 (R)	0.06 (S)	0.06 (S)	1 (S)	>128 (R)	0.5 (S)	CTX-M-15	X	E XXVII
11	8537	8 (I)	128 (R)	>128 (R)	64 (R)	0.06 (S)	0.06 (S)	128 (R)	>128 (R)	4 (S)	CTX-M-15	NEG	E XIX
12	4145	128 (R)	>128 (R)	>128 (R)	16 (R)	0.25 (S)	0.25 (S)	2 (S)	32 (R)	2 (S)	CTX-M-15, TEM-1	N	E XIV

[a] CAZ-ceftazidime, CTX-cefotaxime, CRO-ceftriaxone, FEP-cefepime, IMI-imipenem, MEM-meropenem, GM-gentamicin, CIP-ciprofloxacin, TZP-piperacillin-tazobactam

[b] R-resistant, I-intermediately sensitive, S-susceptible

The isolation and characterization of the *E. coli* strains was reported earlier (*29*)