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Original article

ANTIMICROBIAL EFFECT OF THE POSTBIOTIC PRODUCED BY *PEDIOCOCCUS ACIDILACTICI* ON *ESCHERICHIA COLI* ATCC 25922

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Summary

Ghasemi, A., S. M. H. Karouei, D. Bastami, A. R. Rad, H. Foadaddini, H. Azad, A. Aghajani Amiri & I. Gholamour Azizi, 2026. Antimicrobial effect of the postbiotic produced by *Pediococcus acidilactici* on *Escherichia coli* ATCC 25922. *Bulg. J. Vet. Med.,* **29**, No 1, 171–179.

The increasing resistance of pathogenic bacteria to conventional antibiotics has necessitated the search for alternative antimicrobial agents. This study investigated the antimicrobial activity of postbiotic metabolites produced by *Pediococcus acidilactici* against *Escherichia coli* (ATCC 25922). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the postbiotics were determined using the broth microdilution method. Additionally, the disk diffusion and well diffusion methods were employed to evaluate the antimicrobial activity. The results demonstrated that the postbiotic metabolites exhibited significant antimicrobial effects, with a MIC of 62.5 µg/mL and a MBC of 125 µg/mL. Inhibition zones of 9.33 mm, 12.33 mm, and 12.33 mm were observed in the disk diffusion method at concentrations of 230, 250, and 270 µg/mL, respectively. In the well diffusion method, inhibition zones of 18 mm, 18.33 mm, 21.33 mm, and 23 mm were observed at concentrations of 250, 260, 270, and 280 µg/mL, respectively. These findings suggest that postbiotic metabolites from *Pediococcus acidilactici* have potential as alternative antimicrobial agents against *Escherichia coli*.

Key words: antimicrobial activity, *Escherichia coli*, *Pediococcus acidilactici*, probiotic, postbiotic

INTRODUCTION

Pediococcus acidilactici is a Gram-positive bacterium belonging to the group of homofermentative lactic acid bacteria, considered to be one of the most important known members of probiotic bacteria that can prevent the growth of dangerous pathogenic microbes (Hill *et al.*, 2014). These bacteria have antimicrobial proper-

ties and can also reduce the growth and proliferation of pathogenic and spoilage microorganisms and neutralise the metabolites and toxins (Gaggìa *et al.*, 2010). The antimicrobial activity of these bacteria is caused by the production of inhibitory substances such as organic acids (lactic and acetic acids), oxygen compounds like hydrogen peroxide, protein compounds such as bacteriocins, low molecular weight peptides, and antifungal proteins (Żółkiewicz *et al.*, 2020).

Postbiotics produced by *Pediococcus acidilactici* include a variety of bioactive compounds, such as bacteriocins (e.g. pediocin), organic acids (e.g. lactic and acetic acids), and other metabolites with antimicrobial, immunomodulatory, and anti-inflammatory properties. These postbiotics have shown promising results in inhibiting the growth of foodborne pathogens and spoilage microorganisms, making them potential candidates for use in food preservation and therapeutic applications (Aguilar-Toalá *et al.*, 2018).

Pediococcus species produces small, heat-resistant, non-lanthionine bacteriocins that contain class 2 peptides. These bacteriocins have demonstrated antimicrobial activity against foodborne pathogens such as *Listeria monocytogenes, Clostridium perfringens*, *Bacillus cereus*, and *Staphylococcus aureus* (Klaenhammer, 1988). *Escherichia coli* is a common cause of various bacterial infections in both humans and animals. It is the primary agent behind enteritis and is also responsible for septic urinary tract infections, as well as other clinical infections, including neonatal meningitis. Additionally, it can lead to diarrhoea in animals (Rahmani *et al.*, 2023).

The rise of antimicrobial resistance has significantly complicated the treatment of bacterial infections. Globally, there is an increasing prevalence of multidrug-resistant strains of *Escherichia coli*. This rise is primarily attributed to the dissemination of mobile genetic elements, such as plasmids (Caldwell *et al.*, 2019). The efficacy of antibacterial drugs has significantly decreased, largely due to the rise in bacterial resistance, which is currently one of the major public health concerns (Alpert, 2017). Most infections caused by antibiotic-resistant microorganisms do not respond to conventional treatments, and even last-resort antibiotics have lost their effectiveness. The increase in multidrug-resistant bacteria over recent decades has led to serious problems (Khameneh *et al.*, 2016).

Postbiotics refer to the non-living functional components of probiotic cells, typically produced by living bacteria during the fermentation process or through various physical and chemical methods in a laboratory setting. Postbiotics can include both bacterial and fungal species (Kazem Alilou *et al.*, 2021).

MATERIALS AND METHODS

Bacterial strains and culture conditions

The study utilised *Escherichia coli* (ATCC 25922) as the target pathogen. The strain was obtained from the Iranian Biological Resource Center (IBRC) in Tehran, Iran. The strain was cultured on Mueller-Hinton agar (MHA) and maintained at 37 °C for 24 hours. For experimental purposes, a single colony was inoculated into Mueller-Hinton broth (MHB) and incubated at 37 °C for 18–24 hours to achieve a turbidity equivalent to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL).

Preparation of postbiotic metabolites

The postbiotic metabolites were obtained from *Pediococcus acidilactici*, a probiotic strain cultured in De Man, Rogosa, and Sharpe (MRS) broth at 37 °C for 48 hours under anaerobic conditions. After incubation, the culture was centrifuged at 10,000 × g for 15 minutes to separate the bacterial cells from the supernatant. The supernatant, containing the postbiotic metabolites, was filtered through a 0.22 µm membrane filter to ensure sterility. The concentration of the postbiotic metabolites was determined using spectrophotometry at a wavelength of 600 nm, and the final concentration was adjusted to 250 mg/mL for further experiments.

Extraction of metabolites

The contents of the Erlenmeyer flasks, containing approximately 100 mL of MHB culture medium were divided equally into 10 centrifuge tubes. The tubes were centrifuged three times at 3000 rpm for 10 minutes each. After centrifugation, the supernatant was carefully extracted using a Landa 1000 sampler, excluding the bottom 1 mL containing bacterial cells. The extracted supernatant, containing the bacterial metabolites, was transferred to separate Erlenmeyer flasks for further use (Darshit & Pandya, 2018).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC of the postbiotic metabolites against *E. coli* were determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Briefly, two-fold serial dilutions in MHB of the postbiotic metabolites were prepared, ranging from 500 µg/mL to 7.8 µg/mL. Each well was inoculated with 100 µL of bacterial suspension (1.5×10^6 CFU/mL) and incubated at 37 °C for 24 hours. The MIC was defined as the lowest concentration of the postbiotic that completely inhibited visible bacterial growth. For MBC determination, 10 µL from each well showing no visible growth was subcultured on MHA plates and incubated at 37 °C for 24 hours. The MBC was defined as the lowest concentration that resulted in no bacterial growth on the agar plates.

Disk diffusion and well diffusion assays

The antimicrobial activity of the postbiotic metabolites was also evaluated using the disk diffusion and well diffusion methods. For the disk diffusion assay, sterile paper discs (6 mm diameter) were impregnated with 10 µL of the postbiotic metabolites at concentrations of 230, 250, and 270 µg/mL. The discs were placed on MHA plates inoculated with *E. coli* (0.5 McFarland standard) and incubated at 37 °C for 24 hours. The zones of inhibition were measured in millimeters. For the well diffusion assay, wells (6 mm diameter) were punched into MHA plates inoculated with *E. coli*. Each well was filled with 100 µL of the postbiotic metabolites at concentrations of 250, 260, 270, and 280 µg/mL. The plates were incubated at 37 °C for 24 hours, and the zones of inhibition were measured.

Statistical analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism, version 9.0. The significance of differences between groups was determined using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. A P-value <0.05 was considered statistically significant.

RESULTS

In this study, the minimum inhibitory concentration (MIC) of *Escherichia coli* in the presence of the metabolite obtained from *Pediococcus acidilactici* was determined to be 62.5 µg/mL. The minimum bactericidal concentration (MBC) of the *Pediococcus acidilactici* metabolite against *Escherichia coli* was 125 µg/mL.

The effect of extract concentrations of 230, 250, and 270 µg/mL on the growth inhibition of *E. coli* bacteria was evaluated using the disk diffusion method. The non-growth halo test results indicated that *Pediococcus acidilactici* metabolites at concentrations of 230, 250, and 270 µg/mL produced halos with diameters of 9.33, 12.33, and 12.33 mm, respectively (Table 1). An ANOVA test was conducted to assess the differences in average growth inhibition zones among the various extract volumes. The statistical analysis revealed a significant difference (P=0.006) between the concentrations tested in the antibacterial assessment. Volumes of 250 and 270 µg/mL did not exhibit a significant difference in their inhibition zones when compared to each other; however, both of these volumes demonstrated a significant increase in the inhibition zone compared to the 230 µg/mL volume.

The effect of extract concentrations of 250, 260, 270, and 280 µg/mL on the inhibition of *E. coli* growth was evaluated using the well method. The results of the bacteria growth halo test indicated that the wells with 250, 260, 270, and 280 µg/mL metabolite concentrations produced growth halos measuring 18, 18.33, 21.33, and 23 mm in diameter, respectively. (Table 2). An ANOVA statistical test was employed to assess the differences in the mean sizes of the non-growth halos. The statistical analysis revealed a significant difference among the concentrations used in the antibacterial tests (P=0.001). *Post hoc* analysis indicated that the non-growth halos created by the 250 µg/mL and 260 µg/mL concentrations were the smallest and did not show a significant difference from each other. In contrast, the non-growth halos at concentrations of 270 µg/mL and 280 µg/mL were significantly

Table 1. Zones of inhibition (mm) of *Pediococcus acidilactici* metabolites at different concentrations against *Escherichia coli* ATCC 25922 using the disk-diffusion method. Data are presented as mean ± SD (n=3)

Concentrations (µg/mL)	Zones of inhibition (mm)	P value
230	9.33±0.58	0.006
250	12.33±1.15	
270	12.33±0.58	

Table 2. Zones of inhibition (mm) of *Pediococcus acidilactici* metabolites at different concentrations against *Escherichia coli* ATCC 25922 using the well-diffusion method. Data are presented as mean ± SD (n=3)

Concentrations (µg/mL)	Zones of inhibition (mm)	P value
250	18.00±1.00	0.001
260	18.33±0.58	
270	21.33±1.15	
280	23.00±1.00	

larger compared to those at 250 µg/mL and 260 µg/mL, but there was no significant difference between the halos produced at 270 µg/mL and 280 µg/mL.

DISCUSSION

The antimicrobial activity of *Pediococcus acidilactici* metabolites is attributed to a variety of bioactive compounds produced during fermentation. These metabolites include organic acids, bacteriocins, hydrogen peroxide, and other antimicrobial peptides, which collectively contribute to their inhibitory effects against pathogenic bacteria such as *Escherichia coli* (Arena *et al.*, 2018).

Pediococcus acidilactici produces significant amounts of lactic acid and acetic acid during fermentation. These organic acids lower the pH of the environment, creating unfavourable conditions for bacterial growth. The undissociated forms of these acids can penetrate bacterial cell membranes, disrupting intracellular pH homeostasis and leading to cell death (Garsa *et al.*, 2014). One of the most well-known bacteriocins produced by *Pediococcus acidilactici* is pediocin, a class IIa bacteriocin. Pediocin exhibits strong antimicrobial activity against Gram-positive bacteria, but it can also affect Gram-negative bacteria when combined with other compounds. Pediocin disrupts bacterial cell membranes by forming pores, leading to leakage of intracellular contents and cell death (Arena *et al.*, 2018).

Pediococcus acidilactici also produces hydrogen peroxide as a byproduct of aerobic metabolism. Hydrogen peroxide induces oxidative stress in bacterial cells, damaging DNA, proteins, and lipids, which ultimately results in cell death (Sharma *et al.*, 2014).

In addition to the above, *Pediococcus acidilactici* produces other bioactive compounds, such as diacetyl, reuterin and exopolysaccharides, which contribute to its antimicrobial and immunomodulatory properties. These compounds can inhibit bacterial adhesion, biofilm formation, and quorum sensing, further enhancing their antimicrobial efficacy (Garsa *et al.*, 2014).

The results of our study demonstrated that the secondary metabolites of *Pediococcus acidilactici* exhibited significant antimicrobial activity against *Escherichia coli*. The minimum inhibitory concentration (MIC) was determined to be 62.5 µg/mL, while the minimum bactericidal concentration (MBC) was 125 µg/mL. In the disk diffusion assay, the metabolite produced inhibition zones of 9.33 mm, 12.33 mm, and 12.33 mm at concentrations of 230, 250, and 270 µg/mL, respectively. Similarly, in the well diffusion method, concentrations of 250, 260, 270, and 280 µg/mL resulted in inhibition zones measuring 18 mm, 18.33 mm, 21.33 mm, and 23 mm, respectively.

The antimicrobial action of *Pediococcus acidilactici* metabolites against *E. coli* is likely mediated through multiple synergistic mechanisms. Organic acids lower the intracellular pH and disrupt membrane integrity, bacteriocins form pores in the bacterial cell membrane, and hydrogen peroxide induces oxidative stress. These combined effects lead to substantial growth inhibition, as evidenced by the MIC and MBC values (Sharma *et al.*, 2014; Arena *et al.*, 2018).

The increasing prevalence of antibiotic-resistant *E. coli*, particularly in hospital-acquired infections and urinary tract infections (UTIs), underscores the urgent need for alternative antimicrobial agents. Probiotic-derived compounds, such as those from *Pediococcus acidilactici*, rep-

resent promising candidates due to their efficacy and safety profile (Irfani *et al.*, 2008). The findings of this study highlight the potential of these metabolites as a viable strategy to combat resistant bacterial strains.

In a study by Hossein Afzali and colleagues, an analysis of 391 positive urine culture samples (72.1% from women, 27.9% from men) identified *E. coli* as the most common uropathogenic bacterium. The study also reported significant resistance rates to ciprofloxacin among various bacterial strains: *E. coli* at 37.8%, *Klebsiella* at 22.5%, *Enterococcus* at 36.8%, and S. *baumannii* at 62.5%. These findings underscore the growing challenge of antibiotic resistance and highlight the need for innovative antimicrobial compounds (Afzali *et al.*, 2013).

Similarly, the study by Adetunji & Isola (2011) demonstrated the ineffectiveness of many commonly used antibiotics against *E. coli* strains isolated from a meat cutting board. More than 60% of the tested antibiotics, including ciprofloxacin, tetracycline, amoxicillin, chloramphenicol, ampicillin, and gentamicin failed to inhibit bacterial growth. The only antibiotic to which the bacteria showed high sensitivity was cefuroxime, which produced a growth inhibition zone 10 mm in diameter. In contrast, our study achieved a growth inhibition zone of 12.33 mm using a metabolite of *Pediococcus acidilactici* at a concentration of 250 µg/mL. This finding highlights the potential of *Pediococcus acidilactici* metabolites as potent antimicrobial agents.

Several studies have investigated the antimicrobial potential of probiotic metabolites, and our findings are consistent with and expand beyond these previous works. For instance, a study by Arena *et al.* (2018) demonstrated that *Pediococcus acidilactici* strains produced bacteriocins with strong inhibitory activity against *Listeria monocytogenes* and *Staphylococcus aureus*. While their study focused on Gram-positive bacteria, our results extended these findings to Gram-negative bacteria, specifically *E. coli,* highlighting the broad-spectrum potential of *Pediococcus acidilactici* metabolites.

In another study, Garsa *et al.* (2014) reported that *Pediococcus acidilactici* metabolites, particularly pediocin, exhibited significant antimicrobial activity against the foodborne pathogens *Salmonella* and *Clostridium*. Their findings align with our results, as we observed similar inhibitory effects against *E. coli*. However, our study provided additional insights by quantifying the MIC and MBC values, which are crucial for understanding the therapeutic potential of these metabolites.

Sharma *et al.* (2014) explored the antimicrobial effects of *Lactobacillus plantarum* metabolites against multidrug-resistant *E. coli* strains. They reported MIC values ranging from 50 to 100 µg/mL, which are comparable to our findings (MIC of 62.5 µg/mL). However, our study achieved a larger zone of inhibition (12.33 mm) compared to their results (8–10 mm), suggesting that *Pediococcus acidilactici* metabolites may be more potent in certain contexts. Interestingly, a recent study by Kim *et al.* (2022) investigated the synergistic effects of combining *Pediococcus acidilactici* metabolites with conventional antibiotics. They found that the combination of pediocin and ciprofloxacin significantly enhanced the antibacterial activity against *E. coli*, reducing the required antibiotic concentration by 50%. This finding supports the potential of *Pediococcus acidilactici* metabolites as adjuvants in combination therapy, which

could be a promising avenue for future research.

The antimicrobial activity of *Pediococcus acidilactici* metabolites is likely mediated by multiple mechanisms. These metabolites may include organic acids (e.g., lactic acid), bacteriocins, hydrogen peroxide, and other bioactive compounds. Further research is needed to isolate and characterise the specific compounds responsible for these effects and to elucidate their precise mechanisms of action. The antimicrobial properties of *Pediococcus acidilactici* metabolites make them promising candidates for the treatment of infections by multidrug-resistant *E. coli*, particularly in urinary tract infections. They could also be used in the food industry as natural preservatives to inhibit the growth of pathogenic bacteria in food products. Given their potential safety and efficacy, these metabolites could be developed into novel therapeutic agents or incorporated into probiotic formulations to enhance gut health and prevent infections.

While our study provides valuable insights into the antimicrobial potential of *Pediococcus acidilactici* metabolites, it has certain limitations. For instance, the study was conducted *in vitro*, and further research is needed to evaluate the efficacy and safety of these metabolites *in vivo*. Additionally, the specific bioactive compounds responsible for the observed effects need to be isolated and characterised. Future studies should also explore the synergistic effects of these metabolites with existing antibiotics, as well as their potential applications in clinical and industrial settings.

CONCLUSION

In conclusion, the secondary metabolites of *Pediococcus acidilactici* exhibited significant antimicrobial activity against *Escherichia coli*, making them a promising candidate for the development of novel therapeutic agents. Their natural origin, combined with potent antimicrobial effects, place them as a viable alternative to traditional antibiotics in the fight against antibiotic-resistant infections. Further research is warranted to fully explore their potential and translate these findings into practical applications.

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