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Antibacterial Activity of Cell-Free Culture Supernatant of Bacteriocinogenic *Pediococcus pentosaceus* IO1 against *Staphylococcus aureus* Inoculated in Fruit Juices

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HIGHLIGHTS

- The 10% v/v bacteriocin supernatant exhibited the best antibacterial activity against *Staphylococcus aureus* in fruit juices.
- At lower concentrations, the bacteriocin supernatant had little to no negative effect on sensory properties of fruit juices.
- Bacteriocin from *Pediococcus pentosaceus* IO1 could be used as a biopreservative in fruit juices.

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Acronyms and abbreviations
CFCS=Cell-Free Culture Supernatant
CFU=Colony Forming Units
LAB=Lactic Acid Bacteria

ABSTRACT

Background: *Staphylococcus aureus* is a major food-borne pathogen worldwide and a frequent contaminant of fruit juices. This study aimed to evaluate the antibacterial activity of bacteriocin-containing Cell-Free Culture Supernatants (CFCS) of *Pediococcus pentosaceus* IO_1 against *S. aureus* inoculated in fruit juices, as well as their impact on the juice sensory attributes.

Methods: The orange and watermelon juice samples were treated with bacteriocin supernatant at different concentrations (1, 5, and 10% v/v) and the sensory attributes were evaluated using a 5-point hedonic scale and thereafter inoculated with a culture of *S. aureus*. Then, the inhibitory effect of bacteriocin-containing CFCS of *P. pentosaceus* IO_1 against *S. aureus* in the fruit juices was evaluated by *in situ* and plate assays.

Results: The 10% (v/v) bacteriocin-containing CFCS exhibited the highest antibacterial activity, reducing *S. aureus* counts in pasteurized orange and watermelon juices by 1.42 and 2.12 log Colony Forming Units (CFU)/ml, respectively, and by 1.03 and 0.88 log CFU/ml in unpasteurized orange and watermelon juices, respectively, compared to the control. The taste, colour, and overall acceptability of pasteurized orange and watermelon juices treated with 1% (v/v) bacteriocin supernatant and 0.1% (w/v) sodium benzoate were not significantly different (p=0.228) from those pasteurized orange and watermelon juices without preservative.

Conclusion: The bacteriocin produced by *P. pentosaceus* IO_1 could be used as a natural preservative in fruit juices to control *S. aureus*.

Introduction

Fruit juices are natural liquids extracted from fruit tissue contained antioxidants, vitamins, and minerals which are important for human health and can help preventing heart disease, cancer, and diabetes (Aneja et al., 2014a, b). Fruit juices are becoming more popular as a result of their functional and health benefits. Increased

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consumption of fruit juices have a positive or negative impact on the economy, depending on whether outbreaks of food-borne disease or spoilage problems occur (Aneja et al., 2014a; Tribst et al., 2009).

Food-borne disease caused by bacterial contamination is one of the most serious threats to human health and food safety (Wu et al., 2016). A large number of studies (Aneja et al., 2014a; Tribst et al., 2009) reported outbreaks and the risk of food-borne pathogens and spoilage microorganisms in fruit juices. Consumers and juice stability are both at risk when fruit juices are contaminated by microorganisms.

Staphylococcus aureus is a common contaminant of fruit juices and is a major food-borne pathogen worldwide. More than half of healthy people have *S. aureus* in their nose and throat, and thus on their hands and fingertips, as well as their hair and skin (CDC, 2018; Kadariya et al., 2014). As a result, fruit juices that must be handled during preparation are susceptible to contamination. Staphylococcal enterotoxins are produced by some strains of *S. aureus*, causing staphylococcal food poisoning outbreaks (Wu et al., 2016). The presence of *S. aureus* in fruit juices was reported in previous studies (Sospedra et al., 2012; Tambeker et al., 2009; Titarmare et al., 2009; Wedajo and Kadire, 2019).

In order to reduce the incidence of disease outbreaks, fruit juices are preserved by various techniques, which include the use of chemical preservatives, such as sodium benzoate and potassium sorbate, and also to extend their shelf life (Aneja et al., 2014a; Tribst et al., 2009). However, increasing consumer awareness of the potential health risks associated with chemical preservatives has led researchers to examine the possibility of using bacteriocins produced by Lactic Acid Bacteria (LAB) as an alternative antimicrobial treatment for fruit juices.

Bacteriocins are ribosomally synthesized antimicrobial peptides produced by bacteria that kill some bacteria. The ability to produce bacteriocin is a desired attribute among LAB from the perspective of controlling microbial populations in fermented foods to extend product shelf-life and safety (Field et al., 2018; O' Shea et al., 2013). LAB bacteriocin exhibits killing activity in the nanomolar range against a wide variety of food spoilage microbes and pathogenic bacteria (Field et al., 2018). LAB bacteriocins are known for their activity across a wide pH range and tolerance to high thermal stress. They are also easily broken down by proteolytic enzymes due to their proteinaceous nature and are colourless, odourless, and tasteless, all of which increase their potential usefulness (Perez et al., 2014). Even at very low concentrations, bacteriocins have a quick-acting mechanism that creates pores in the target bacterial membrane (Perez et al., 2014). Bacteriocins are well-accepted natural means of selective microbial inhibition (Settanni and Corsetti, 2008), and they are Generally Recognized as Safe (GRAS).

So far, few studies have investigated the application of bacteriocins in fruit juices, but just a very small number of such studies examined the impact of bacteriocin on the final product quality, including sensory properties. Adesina et al. (2016) previously reported *Pediococcus pentosaceus* IO_1 to be a potent producer of bacteriocin, and it was used in the present study. The purpose of this study was to evaluate the antibacterial activity of bacteriocin-containing Cell-Free Culture Supernatants (CFCS) of *P. pentosaceus* IO_1 against *S. aureus* inoculated in fruit juices, as well as their impact on the juice sensory attributes.

Materials and methods

Bacterial strains and culture conditions

A bacteriocinogenic strain of *P. pentosaceus* IO_1 isolated from the African locust bean "iru" was used (Adesina et al., 2016). The *P. pentosaceus* strain was grown in de Man Rogosa Sharpe (MRS) broth (Oxoid Ltd., Basingstoke, UK) at 30 ^{o}C for 24 h under anaerobic conditions. A food-borne pathogen, *S. aureus*, was used as an indicator strain and was obtained from the Microbiology laboratory at the Federal University of Technology, Akure, Nigeria. *S. aureus* was grown in tryptone soya broth (Oxoid Ltd., Basingstoke, UK) under aerobic conditions until it reached $1x10^8$ Colony Forming Units (CFU)/ml.

Preparation of bacteriocin-containing CFCS

The bacteriocin-producing strain, *P. pentosaceus* IO_1, previously checked for its inhibitory activity in a plate assay using the agar well method (Adesina et al., 2016), was grown in MRS broth at 30 ^{o}C for 48 h under anaerobic conditions, as described by Ogunbanwo et al. (2003). The CFCS was obtained by centrifuging the culture at 4,000xg for 20 min, followed by adjusting to pH 6.5 with 1 N Sodium Hydroxide (NaOH) and filtering the neutralized supernatant through a 0.45 um pore-size membrane filter (Millipore Corporation, Bedford, MA, USA). The bacteriocin-containing CFCS was stored at 4 ^{o}C until use.

Extraction and treatments of fruit juices

Under aseptic conditions, juices were extracted from matured watermelon (*Citrullus lanatus*) and orange (*Citrus sinensis*) fruits (El-Ishaq and Obirinakem, 2015). Five different sterilized glass bottles, each containing 20 ml of the juice samples (watermelon and orange, separately), were pasteurized in a covered water bath at 72 ^{o}C for 2 min. Another 2 different sterilized glass bottles,

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each containing 20 ml of juice samples, were left unpasteurized. The treatments were made as follows: pasteurized juice (control, T1); pasteurized juice+1% bacteriocin supernatant (T2); pasteurized juice+5% bacteriocin supernatant (T3); pasteurized juice+10% bacteriocin supernatant (T4); pasteurized juice+0.1% sodium benzoate (C_6H_5COONa) (T5); unpasteurized juice (T6); unpasteurized juice+10% bacteriocin supernatant (T7).

Inhibition of S. aureus in fruit juices

The method of Kanagaraj et al. (2012) with some modifications was used to assess the efficacy of the bacteriocin-containing CFCS. To inhibit *S. aureus* in fruit juices, watermelon, and orange juices were used. The juice samples (watermelon and orange) treated with different concentrations of bacteriocin-containing CFCS were inoculated each with 100 µl of *S. aureus* at 10^8 CFU/ml. The samples were homogenized and stored for a period of 72 h at room temperature. Samples (1 ml each) were withdrawn at 0, 24, and 72 h, serially diluted with sterile distilled water and plated on mannitol salt agar (Lab M, UK). The CFU of *S. aureus* were counted after that the plates were incubated at 37 °C for 48 h. Juice samples inoculated with *S. aureus* without added bacteriocin supernatant were used as a control.

Sensory evaluation

Sensory evaluation of the fruit juice samples was carried out by 10 untrained panellists. The parameters used were colour, taste, aroma, and overall acceptability. The rating was presented on a 5-point hedonic scale ranging from 5=like extremely to 1=dislike extremely (Mosqueda-Melgar et al., 2012).

Statistical analysis

Statistical analyses of the data were performed using Statistical Package for the Social Sciences (SPSS) software (version 16). Data recorded as mean±standard deviations were analysed by one-way ANOVA followed by Duncan's New Multiple Range test (p<0.05) to determine the significant differences between the mean values.

Results

The inhibitory effects of the bacteriocin-containing CFCS of *P. pentosaceus* IO_1 against *S. aureus* in pasteurized fruit juices are presented in Figure 1. There was a statistically significant difference (p=0.000) between the antibacterial activity of the different concentrations of bacteriocin supernatant. The 10% v/v bacteriocin concentration showed the best antibacterial activity against *S. aureus* in the juices. The log CFU/ml of the *S. aureus* declined to 4.04, 3.95, 3.65, and 3.54 in the pasteurized orange juice containing different concentrations (1, 5, and 10% v/v) of bacteriocin supernatant and sodium benzoate (0.1% w/v), respectively, and reached 5.07 in the control (without preservative) in 72 h. Also, the log CFU/ml of the *S. aureus* declined to 4.40, 4.13, 3.24, and 3.45 in pasteurized watermelon juice containing different concentrations (1, 5, and 10% v/v) of bacteriocin supernatant and sodium benzoate (0.1% v/v) respectively, and reached 5.36 in the control (without preservative) in 72 h. There was no statistically significant difference (p=0.320) between the antibacterial activity of bacteriocin supernatant in orange and watermelon juices. The log CFU/ml of *S. aureus* declined to 4.34 in the unpasteurized orange juice containing 10% (v/v) of bacteriocin supernatant and reached 5.37 in the control in 72 h. Similarly, the log CFU/ml of *S. aureus* declined to 4.59 in unpasteurized watermelon juice containing 10% (v/v) of bacteriocin supernatant and reached 5.47 in the control in 72 h. There was a statistically significant reduction (p=0.025) in the viable cell counts of *S. aureus* in the unpasteurized fruit juices treated with 10% (v/v) bacteriocin supernatant.

The influence of the fruit juices (orange and watermelon juices) pasteurized and treated with or without bacteriocin supernatant of *P. pentosaceus* in comparison with unpasteurized fruit juice samples on the sensory attributes (taste, colour, aroma, and overall acceptability) are shown in Tables 1 and 2, respectively. The taste, colour, and overall acceptability of pasteurized orange and watermelon juices treated with 1% (v/v) bacteriocin supernatant and 0.1% (w/v) sodium benzoate were not significantly different (p=0.228, 0.296) from those pasteurized oranges and watermelon juices without preservative. A significant difference (p=0.001) in the aroma of both pasteurized and unpasteurized orange juices treated with different concentrations of bacteriocin supernatant (O2, O3, O4, and O7) was detected by the panellists in comparison with those without bacteriocin supernatant (O1, O5, and O6; Table 1). On the other hand, the aroma in bacteriocin-treated watermelon juices was not significantly affected (p=0.25) when compared with the watermelon juice without bacteriocin (Table 2). Unpasteurized samples treated with 10% (v/v) bacteriocin supernatant affected the taste, colour, aroma, and overall acceptability of the assayed fruit juices, being those with the lowest score according to the panellists.

Discussion

The results of the present study revealed that different concentrations of bacteriocin-containing CFCS of *P. pentosaceus* IO_1 reduced the counts of *S. aureus* in the

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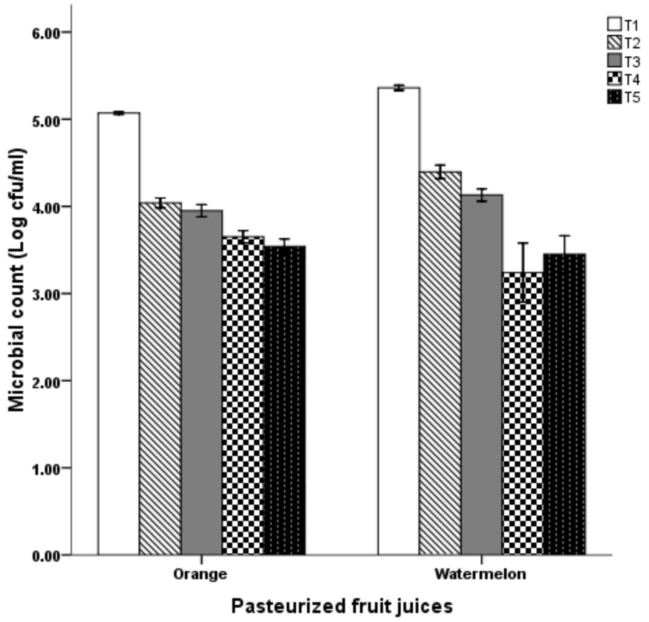

Figure 1: Inhibition of *Staphylococcus aureus* in pasteurized fruit juices containing different concentrations of bacteriocin supernatant of *Pediococcus pentosaceus* IO_1; Samples were incubated at room temperature for 72 h with bacteriocin concentrations of 0% (T1, Control), 1% (T2), 5% (T3), 10% (T4), and sodium benzoate 0.1% (T5). The average data of two determinations±Standard deviation (error bar) are shown.

Table 1: Sensory evaluation of orange juice samples treated with bacteriocin

Treatments	Sensory Properties/Attributes			
	Taste	Colour	Aroma	Overall Acceptability
O1	$5.00^{c}\pm0.00$	$5.00^{b}\pm0.00$	$4.80^{b}\pm0.45$	$4.80^{c}\pm0.45$
O2	$4.40^{c}\pm0.55$	$5.00^{b}\pm0.00$	$3.40^{a}\pm0.55$	$4.40^{c}\pm0.55$
O3	$3.60^{b}\pm0.55$	$4.40^{b}\pm0.89$	$3.20^{a}\pm0.45$	$4.00^{bc}\pm0.71$
O4	$3.40^{ab}\pm0.89$	$3.40^{a}\pm0.89$	$3.40^{a}\pm0.55$	$3.40^{ab}\pm0.55$
O5	$4.80^{c}\pm0.45$	$5.00^{b}\pm0.00$	$4.40^{b}\pm0.55$	$4.80^{c}\pm0.45$
O6	$5.00^{c}\pm0.00$	$5.00^{b}\pm0.00$	$4.60^{b}\pm0.55$	$4.80^{c}\pm0.45$
O7	$2.80^{a}\pm0.84$	$3.40^{a}\pm0.89$	$3.00^{a}\pm0.71$	$3.00^{a}\pm0.71$

Mean values with different superscripts in each column are significantly different ($p<0.05$)
O1:Pasteurized orange juice; O2:Pasteurized orange juice+1% (v/v) bacteriocin-Cell-Free Culture Supernatant (CFCS) of *Pediococcus pentosaceus;* O3:Pasteurized orange juice+5% (v/v) bacteriocin-CFCS of *P. pentosaceus*; O4:Pasteurized orange juice+10% (v/v) bacteriocin-CFCS of *P. pentosaceus*; O5:Pasteurized orange juice+0.1% (v/v) sodium benzoate; O6:Unpasteurized orange juice; O7:Unpasteurized orange juice + 10% (v/v) bacteriocin-CFCS of *P. pentosaceus*

Table 2: Sensory evaluation of watermelon juice samples treated with bacteriocin

Treatments	Sensory Properties/Attributes			
	Taste	Colour	Aroma	Overall Acceptability
W1	$5.00^{c}\pm0.00$	$5.00^{b}\pm0.00$	$4.60^{b}\pm0.55$	$5.00^{c}\pm0.00$
W2	$5.00^{c}\pm0.00$	$5.00^{b}\pm0.00$	$4.60^{b}\pm0.55$	$4.80^{c}\pm0.45$
W3	$4.00^{b}\pm0.71$	$5.00^{b}\pm0.00$	$4.00^{b}\pm1.00$	$4.00^{b}\pm0.71$
W4	$3.40^{a}\pm0.55$	$5.00^{b}\pm0.00$	$3.60^{ab}\pm0.55$	$4.00^{b}\pm0.00$
W5	$4.80^{c}\pm0.45$	$5.00^{b}\pm0.00$	$4.00^{b}\pm1.00$	$4.80^{c}\pm0.45$
W6	$4.40^{bc}\pm0.55$	$5.00^{b}\pm0.00$	$4.00^{b}\pm0.71$	$4.80^{c}\pm0.45$
W7	$3.00^{a}\pm0.00$	$4.60^{a}\pm0.55$	$2.80^{a}\pm0.45$	$2.80^{a}\pm0.45$

Mean values with different superscripts in each column are significantly different ($p<0.05$)
W1:Pasteurized watermelon juice; W2:Pasteurized watermelon juice+1% (v/v) bacteriocin-Cell-Free Culture Supernatant (CFCS) of *Pediococcus pentosaceus*; W3:Pasteurized watermelon juice+5% (v/v) bacteriocin-CFCS of *P. pentosaceus*; W4:Pasteurized watermelon juice+10% (v/v) bacteriocin-CFCS of *P. pentosaceus*; W5:Pasteurized watermelon juice+0.1% (v/v) sodium benzoate; W6:Unpasteurized watermelon juice; W7:Unpasteurized watermelon juice+10% (v/v) bacteriocin-CFCS of *P. pentosaceus*

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juice samples stored at room temperature for 72 h. This study showed that the antibacterial effect of the bacteriocin supernatant in the pasteurized orange and watermelon juices improved with an increase in the concentration of bacteriocin, with the 10% (v/v) bacteriocin exhibiting the best antibacterial activity against the *S. aureus* in both juices. The antibacterial activity of *P. pentosaceus* IO_1 against *S. aureus* determined in this study agree with the findings of other studies with LAB bacteriocins (Jiang et al., 2022; Kaktcham et al., 2019; Kanagaraj et al., 2012; Li et al., 2021; Pei et al., 2022). Kanagaraj et al. (2012) demonstrated the inhibitory effect of bacteriocin produced by *Lactobacillus fermentum* against food spoilage microorganisms such as *S. aureus*. They discovered that a 5% concentration of partially purified bacteriocin from *L. fermentum* KN02 in milk and edible mushrooms reduced the growth of aerobic bacteria. Bacteriocins produced by the LAB strains *Lactococcus lactis* and *Lactobacillus salivarius* inhibit *S. aureus* (Kaktcham et al., 2019; Li et al., 2021). Recently, Jiang et al. (2022) reported the antibacterial activity of a novel bacteriocin LSX01 of *Lactobacillus paracasei* against *S. aureus* and they observed that the *S. aureus* significantly decreases after treating with LSX01 at different concentrations. Bacteriocins' ability to affect *S. aureus* is believed to be largely due to unknown mechanisms. Previous research found that LAB bacteriocins cause metabolic activity disruption, leakage of cellular content, disruption of cell membrane integrity, and DNA damage in *S. aureus* (Jiang et al., 2022; Li et al., 2021; Pei et al., 2022).

The level of food contamination by the target organism has been linked to the inhibitory efficiency of bacteriocins. Bacteriocin activity will be low and unable to prevent the development of contaminating microorganisms if the initial contamination is too high (Balciunas et al., 2013). The concentration of *S. aureus* ($1x10^8$ CFU/ml) used in this investigation was higher compared to those of other studies. The low effectiveness as compared to other studies may be due to the high level of *S. aureus* used in this study. It has also been noted that the application of CFCS (crude bacteriocin) rather than a purified bacteriocin preparation could have the advantage of incorporating different biologically active substances with possible synergistic effects (Hartmann et al., 2011).

In this study, the sensory properties of the fruit juices were also evaluated after the treatment with bacteriocin-containing CFCS of *P. pentosaceus* IO_1. Consumer perception of juice quality is primarily influenced by the organoleptic or sensory properties of the juice. Colour, aroma, and taste are major factors affecting quality perception and consumers' acceptance of fruit juices (Heredia et al., 2013). Our findings revealed that the taste, colour, and overall acceptability of the pasteurized orange and watermelon juices treated with 1% (v/v) bacteriocin-containing CFCS and 0.1% (v/v) sodium benzoate (a chemical preservative) were not significantly different (p=0.228) from the control (juice without preservative). In addition, no statistically significant differences in the aroma of watermelon juice samples treated with 1% and 5% (v/v) bacteriocin-containing CFCS and watermelon juice samples treated with 0.1% (v/v) sodium benzoate were observed compared with the control (watermelon juice with preservative). It is apparent from this study that lower concentrations (1% v/v) of the bacteriocin supernatant had no negative impact on the sensory properties of the fruit juices except for aroma in orange juice, but higher concentrations (5% and 10% v/v) impacted the juices' sensory properties negatively. Juices treated with 1% (v/v) bacteriocin-containing CFCS compete favourably with those treated with a chemical preservative.

Only a few studies showed the sensory evaluation of final bacteriocin-containing products. Bodley (2015) demonstrated that the addition of bacteriocin from *Lactobacillus plantarum* to peach-apricot and orange juices have no alteration in the juice organoleptic properties, which include taste, mouth feel, colour, and smell. Sumonsiri (2019) also showed that the treatment of coconut water samples with 50 and 75 ppm nisin-a bacteriocin derived from *L. lactic* had no alteration in the samples' colour, turbidity, or sensory acceptability.

The ability of bacteriocin to inhibit the growth of pathogenic or spoilage bacteria in food systems is dependent on several factors, including bacteriocin adsorption to food components, inactivation by chemical or enzymatic modification, and poor solubility in the given food matrix (Hartmann et al., 2011). As a result of these factors, bacteriocins should not be used as the only preservative principle in food, but rather as part of a "hurdle" system for developing minimally processed, safe foods with optimal nutrient levels.

Conclusion

The present study was designed to investigate the antibacterial effect of bacteriocin-containing CFCS of *P. pentosaceus* IO_1 against *S. aureus* inoculated in watermelon and orange juices. The 10% v/v bacteriocin supernatant exhibited the best antibacterial activity against *S. aureus* in the fruit juices while lower concentrations (1 and 5% v/v) had little to no negative effect on the sensory attributes of the fruit juices. Hence, the bacteriocin produced by *P. pentosaceus* IO_1 could be used as a natural preservative in fruit juices which are susceptible to *S. aureus* and could meet consumers' demands for juices which are free of chemical preservatives.

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Author contributions

I.A.A. and Y.D.O. designed the study and carried out the work; I.A.A. analyzed the data; I.A.A. and Y.D.O. wrote the manuscript. Both authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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