EFFECT OF pH AND HEAT TREATMENT ON BACTERIOCIN ACTIVITY OF *Pediococcus pentosaceus* IO1, *Tetragenococcus halophilus* PO9 AND *Lactobacillus cellobiosus* BE1

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ABSTRACT

Three lactic acid bacterial (LAB) strains; *Pediococcus pentosaceus* IO1, *Tetragenococcus halophilus* PO9, and *Lactobacillus cellobiosus* BE1 isolated from different traditionally fermented products were evaluated for their bacteriocin activity against *Staphylococcus aureus* and the influence of pH and heat treatment on their bacteriocin activity was assessed. The crude bacteriocins produced by the LAB strains were highly thermostable, retaining their activity even after heat treatment at 115 ^{o}C for 15 min, and stable at pH range of 2 – 9 with higher activity at acidic pH (pH 2 and 5). These results indicate that the bacteriocins produced by bacteriocinogenic LAB isolates could be used as biopreservatives in acidic and heat-processed foods.

Keywords: Bacteriocin, *Lactobacillus cellobiosus, Pediococcus pentosaceus, Tetragenococcus halophilus.*

INTRODUCTION

Bacteriocins are ribosomally synthesized peptides produced by bacteria that exert antimicrobial activities against related and unrelated microorganisms (Cleveland *et al*., 2001; Galvez *et al*., 2007; Gulluce *et al*., 2013). The major producer group for bacteriocins is lactic acid bacteria (LAB) that contain a great variety of microorganisms described as "generally recognized as safe (GRAS)" by the United States Food and Drug Administration. Due to this safety potency of their origin and the wide-range effectiveness on pathogenic or spoilage bacteria, bacteriocins have attracted great research interest as natural antimicrobial agents (Gulluce *et al*., 2013).

Bacteriocins differ from most therapeutic antibiotics in being proteinaceous agents that are rapidly digested by proteases in the human digestive tract. The fact that they are ribosomally synthesized peptides creates the possibility of improving their characteristics to enhance their activity and spectra of action (Saavedra *et al*., 2004). It is desirable to understand the influences that environmental factors may have on the activity of bacteriocins in order to quantitatively estimate their efficacy for future applications in food model systems (Ananou *et al*., 2007).

The LAB strains, *Pediococcus pentosaceus* IO1, *Tetragenococcus halophilus* PO9 and *Lactobacillus cellobiosus* BE1 isolated from "iru", "pito" and "burukutu" respectively, produced bacteriocins with antimicrobial activity against a number of food spoilage and pathogenic bacteria (Adesina *et al*., 2016). The objective of this study was to determine the effect of pH and heat treatment on the bacteriocin activity of *Pediococcus pentosaceus* IO1, *Tetragenococcus halophilus* PO9 and *Lactobacillus cellobiosus* BE1 against *Staphylococcus aureus.*

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

Bacteriocin-producing lactic acid bacterial strains; *Pediococcus pentosaceus* IO1, *Tetragenococcus halophilus* PO9 and *Lactobacillus cellobiosus* BE1, isolated from traditionally fermented products (Adesina *et al*., 2016), were cultured in de Man Rogosa Sharpe (MRS) broth (Oxoid, Hampshire, England) at 30 ^{o}C for 48 h. *Staphylococcus aureus* was used as the indicator organism for the estimation of bacteriocin activity in this study; it was cultured in Tryptone Soya Broth (TSB, Oxoid). Cultures were stored at 4 ^{o}C in the refrigerator.

Preparation of crude bacteriocin

Pediococcus pentosaceus IO1, *Tetragenococcus halophilus* PO9 and *Lactobacillus cellobiosus* BE1 were grown in MRS broth separately for 48 h at 30 ^{o}C. The broth culture was centrifuged at 4000 x g for 20 min. The pH of cell-free culture supernatant was adjusted to 6.5 with 1 M NaOH. Then, catalase (1mg/ml) was added to remove hydrogen peroxide from the supernatant, and thereafter the supernatant was filtered through a 0.45 µm pore-size membrane. The bacteriocin-containing cell-free culture supernatant (crude bacteriocin) was stored at 4 ^{o}C in the refrigerator until required for use (Ogunbanwo *et al*., 2003).

Bacteriocin activity

Agar well diffusion assay was used to study the bacteriocin activity of the LAB strains (Khay *et al*., 2011). An overnight culture of indicator strain (*Staphylococcus aureus*) grown in TSB broth at 37 ^{o}C was diluted to a turbidity equivalent to that of a 0.5 McFarland standard. A lawn of an indicator strain was made by spreading the cell suspension over the surface of Mueller Hinton agar (MHA, LabM, Lancashire, UK) plates with a cotton swab. The plates were allowed to dry and a sterile cork borer of diameter 6.0 mm was used to cut uniform wells in the agar pates. Each well in the MHA plates was filled with 80 µl of untreated (control) or treated crude bacteriocin of the LAB strains. The plates were kept at 4 ^{o}C for 2 h, to ensure diffusion of the supernatant fluid into the agar, and then incubated at 37 ^{o}C for 24 h. The antimicrobial activity was determined by measuring the diameter of zone of inhibition around the wells.

Effect of pH on bacteriocin activity

A 5 ml aliquot of crude bacteriocin from the LAB strains was distributed into different test tubes and the pH values of the contents were adjusted to 2, 5, 7 and 9 individually, using diluted NaOH (1 M NaOH solution) (Joshi *et al*., 2006). The samples were allowed to stand at room temperature for 2 h and the antimicrobial activity was assayed by agar well diffusion method.

Effect of heat treatment on bacteriocin activity

A volume of 5 ml of crude bacteriocin from the LAB strains in different test tubes was taken and heated at 68 ^{o}C and 100 ^{o}C for 10 and 20 min, respectively, and at 115 ^{o}C for 15 min under pressure (Joshi *et al*., 2006). The heat-treated bacteriocin samples were then assayed for antimicrobial activity by agar well diffusion method.

Statistical Analysis

The experiments were carried out in duplicate and the data shown are the means of the duplicates.

RESULTS

Staphylococcus aureus was chosen as the indicator strain to test the effect of heat and pH on bacteriocin activity. Table 1 shows the effect of heat treatment on the bacteriocin activity of LAB strains; *Pediococcus pentosaceus* IO1, *Tetragenococcus halophilus* PO9 and *Lactobacillus cellobiosus* BE1. The crude bacteriocins of the LAB strains were highly thermostable, retaining 100 % of their activity at 68 ^{o}C for 10 min, and even after treatment with 115 ^{o}C for 15 min, the bacteriocins could still retain more than 80 % of their activity against the indicator strain.

The crude bacteriocins of the LAB isolates were active in a pH range of 2 - 9, but the maximum activity was observed at acidic pH (pH 2.0 and 5.0) (Figure 1).

Table 1. Effect of heat treatment on the bacteriocin activity of LAB strains against *S. aureus*

Temperature (^{o}C)	Time (min)	Zone of Inhibition (mm)		
		***P. pentosaceus* IO1**	***T. halophilus* PO9**	***L. cellobiosus* BE1**
68	10	18.5 (100)	19.0 (100)	15.0 (100)
	20	17.0 (92)	18.5 (97)	14.5 (97)
100	10	17.0 (92)	18.0 (95)	14.5 (97)
	20	16.5 (89)	17.5 (92)	13.5 (90)
115	15	16.5 (89)	17.5 (92)	12.5 (83)
Control (without heat treatment)	-	18.5	19.0	15.0

Values are means of duplicate determinations [values in parentheses represent % antimicrobial activity]

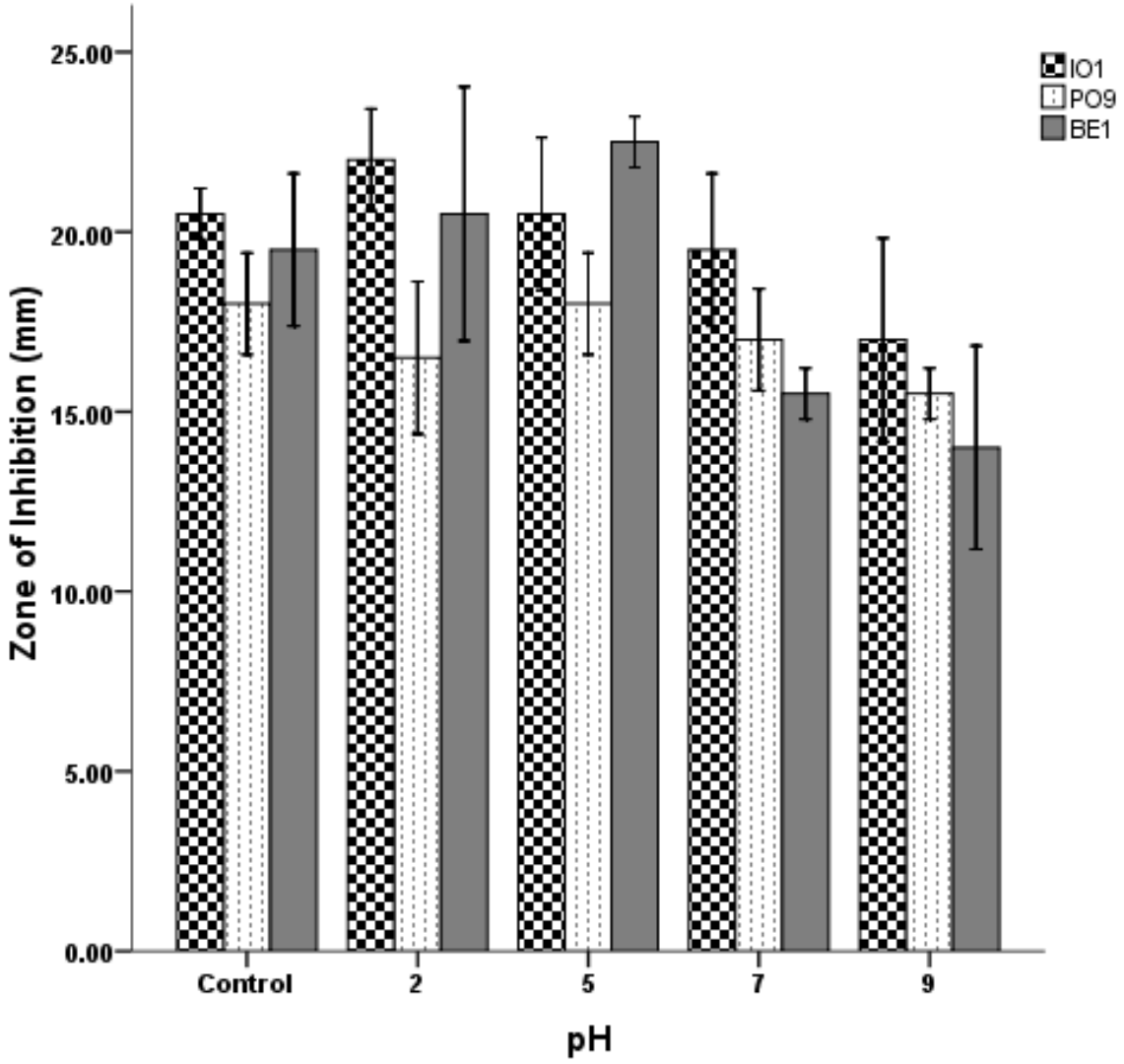

Figure 1. Effect of pH on the bacteriocin activity of LAB strains against *S. aureus*.

Error bars represent standard deviations of mean of duplicate determinations. IO1 – *P. pentosaceus*; PO9 – *T. halophilus*; BE1 – *L. cellobiosus.*

DISCUSSION

The thermal stability at 68 and 100 ^{o}C (up to 20 min) and at 115 ^{o}C for 15 min of bacteriocins produced by bacteriocinogenic LAB isolates may constitute an advantage for potential use as biopreservatives in combination with thermal processing in order to preserve food products. These findings are consistent with the stability of bacteriocins reported by other researchers. Yang *et al*. (2012) reported that bacteriocin-like substances produced by eight LAB isolates retained their activity after heat-treatment at 80 and 100 ^{o}C for 60 and 90 min. However, the bacteriocin-like substances were sensitive to autoclaving at 121 ^{o}C for 15 min displaying either smaller or no inhibition zones compared to the control. Joshi *et al*. (2006) also reported that a bacteriocin produced by *Lactobacillus* CA44 was found to be stable at 68 ^{o}C for up to 20 min, and at 100 ^{o}C for 10 min it could retain 55 % of antimicrobial activity, while at the same temperature for 20 min, only 28 % of activity could be retained. Similar results were reported for bacteriocin-like substances from *Lactobacillus plantarum*, *L. fermentum*, and *L. acidophilus* (Aslim *et al*., 2005). Todorov and Dicks (2009) reported that bacteriocin ST44AM remained stable at 25, 30, 45, 60, and 100 ^{o}C for 120 min. The thermotolerance feature might be related to the molecular structure of the bacteriocin, usually composed by small peptides without tertiary structure (Parada *et al*., 2007).

Bacteriocins produced by the LAB isolates studied were noted to have maximum activity at acidic pH (pH 2 and 5), so the result proved that they could be used in acidic food products like fruit juices. This result is supported by findings of Joshi *et al*. (2006). Messens and De Vuyst (2002) reported that many LAB and bacteriocins display greater antibacterial activity at lower pH values (pH 5 and below). The stability of bacteriocin to different heat-treatment and pH conditions reflects that such compounds can withstand the conditions normally encountered in food processing, so would remain effective during processing.

CONCLUSION

This study revealed that bacteriocins produced by *Pediococcus pentosaceus* IO1, *Tetragenococcus halophilus* PO9 and *Lactobacillus cellobiosus* BE1 were highly thermostable up to 115 ^{o}C and active at wider pH range from 2 to 9.

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