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Isolation, characterization, and antimicrobial evaluation of bacteriocin produced by lactic acid bacteria against *Erwinia carotovora*

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Abstract

This study aimed to estimate the antimicrobial efficiency and stability of the crude bacteriocin produced by dadih's LAB against *Erwinia carotovora* under different pH, temperature, and enzymatic treatments. Twelve strains of LAB were isolated from the dadih including four strains were *Leuconostoc sp.* and eight were *Streptococcus sp.* The antimicrobial effects of bacteriocins from the genus Streptococcus sp. against *Erwinia carotovora* were higher than the genus *Leuconostoc* sp. The isolated bacteriocins showed potential stability indifferent pH (3 to 11) and temperature conditions (30 to 121 °C). Six of nine bacteriocins included *Enterococcus faecalis* subsp. *liquefaciens* R-19, R-55, R-32, *Streptococcus lactis* subsp. *diacetylactis* R-41, R-43, and *Leuconostoc paramesenteroides* R-45 induced the highest stability under different temperatures and pH. Analysis of the 16s rDNA of isolates R-43 and R-55 showed that these bacteria belong to *Enterococcus hirae* and *Pediococcus pentosaceous*, respectively. The treatment with amylase, proteinase K, and trypsin revealed that R-43 and R-55 probably belong to the group IIa classification of bacteriocins (*Antilisteria-Pediocin-like bacteriocins)*. It could be concluded that dadih's LAB is a promising source for bacteriocins used safely in the bio-preservatives of the food products under different storage conditions.

Keywords: lactic acid bacteria; *Erwinia carotovora*; bacteriocin; bio-preservative; antimicrobial activity.

Practical Application: Bacteriosin could be used as natural preservative for foods.

1 Introduction

Foremost, the application of technology in the agricultural and the processing of food products is conducted to produce fresh, nutritious, and safe food. Nevertheless, the development of advanced methods and technology to provide fresh products from vegetables or fruits still encounter significant economic losses due to microbial spoilage and pathogen. The Food and Agriculture Organization (FAO) 2016 estimated the total global production of 1.07 billion tons of fresh vegetables and 865 million tons of fresh fruits. Unfortunately, more than 50% of the losses of fruits and vegetables even before they reach the consumers (Elik et al., 2019). Fruits and vegetables are usually infected through surface injuries during or after harvesting, and several fungal and bacterial colonizations occur (Elik et al., 2019).

Erwinia carotovora is a gram-negative pathogenic bacterium with rod-shaped cells that usually attack various types of vegetables and fruits. These bacteria can induce a decay of agricultural products in the field, in storage, or during transportation and causes several common problems in vegetables or fruits. Vegetables and fruits are contaminated by bacteria, mostly through water after irrigation or heavy rains (Muimba-Kankolongo, 2018). For instance, *E. carotovora* is responsible for tomato damage during transportation or storage, and soft rot and blackleg disease in potatoes (Blancard, 2012). These bacteria are degrading the cell wall of the host plant, then colonizing the intercellular spaces and delivering robust molecules. They are known as *Avirulence effectors* (Avr) through a type III secretion system (Aizawa, 2014). The blackleg disease is primarily caused by *E. carotovora* subsp. *atroseptica, E. carotovora* subsp. *carotovora.* However, *E. carotovora* subsp. *carotovora* is considerably the most ubiquitous and have a rapid rate of growth in contaminated potato (De Boer et al., 1996).

The application of bio-preservation and antimicrobial compounds has been part of human life since the rise of human civilization. There is sufficient evidence to believe that human has profited from the bio-preservation of fermented food products such as bacteriocin from dadih's LAB (Pato et al., 2020). Bacteriocins are common antimicrobial peptides produced by different microorganisms. Almost all bacterial species have the ability for the production of bacteriocins as a part of the defending molecules. LAB can produce several organic substances accountable to the sensory and preservation attributes. The preservative characteristics of LAB are mainly based on antimicrobial metabolites, including organic antifungal peptides, hydrogen peroxide, acids, reuterin, diacetyl, and bacteriocins (Heredia-Castro et al., 2015). Bacteriocins are peptides synthesized by ribosomes with antimicrobial properties produced by different

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LAB (*Lactobacillus, Lactococcus, Streptococcus, Pediococcus, Leuconostoc,* and *Enterococcus*).

Bacteriocins synthesized by LAB showed great interest because of their practical use as bio preservatives in the food industry. The application of bacteriocins as a food preservative has increased, due to their safety and stability in the variants of pH and temperature. The bacteriocins are efficacious against Gram (+) and Gram (-) bacteria. For instance, *Clostridium tyrobutyricum, Staphylococcus aureus, Listeria monocytogenes, Listeria innocua, Escherichia coli, Bacillus cereus, Salmonella typhimurium, Campylobacter jejuni* and *Helicobacter pylori* NCIPD 230 (Heredia-Castro et al., 2015). However, only *Pediococcus acidilactici* (podocin PA-1) and *Lactococcus lactis* (nisin) are permitted and approved as food preservatives. The composition of proteins, lipids, manufacturing process, enzymatic degradation, additives present, and pH are the significant factors affecting the effectiveness of bacteriocin in different food systems. This work aimed to estimate the antibacterial properties of bacteriocin synthesized by LAB isolated from dadih's against *E. carotovora* under different conditions.

2 Materials and methods

2.1 Activation of the LAB and E.carotovora

The active culture was conducted by mixing 0.1 mL of the dadih's LAB culture in a test tube containing 5 mL of MRS (De Man, Rogosa, and Sharpe) broth, and the mixture was shaken and incubated for 18 h at 37 °C. All isolates of LAB were identified by API50 CH for their morphological and biochemical properties including the Gram-reaction, motility, spore formation, oxidase, and catalase production (Hosono et al., 1989). The isolates which showed Gram-positive, oxidase, and catalase-negative were considered LAB and were used for further studies. The pathogenic bacteria were activated by the inculcation of 0.1 mL of the bacterial sample into 5 mL of the nutrient broth, shaken, and incubated at 37 °C for 18 h.

2.2 Determination of the antimicrobial activity of LAB in vitro

The antimicrobial activity of LAB isolated from dadih's was determined using the paper disk diffusion method reported by Syukur et al. (2014). The cultures of dadih's LAB have been incubated aerobically for 24 h at 37 °C. However, *E. carotovora* was grown in nutrient broth and incubated for 24 h at 37 °C then 100 µL were placed and spread on the surface of MRS agar. A sterile paper disc (6 mm) was immersed into the supernatants of LAB, and a sterile MRS broth was used as a negative control. The discs were placed on the surface of MRS agar plates that were seeded previously with *E. carotovora*. Then, all plates were incubated for 24 h at 37 °C, and the diameter of the zone of growth inhibition was measured.

2.3 Preparation and purification of crude bacteriocin

The preparation and purification of the crude bacteriocin were carried out as described in detail in our previous work (Pato et al., 2022).

2.4 Bacteriocin characterization

Effect of pH

The effect of pH on bacteriocin was carried out by the addition of 0.5 mL of purified bacteriocin to 4.5 mL of nutrient broth with different pH values (3, 5, 7, 9, and 11) then incubated for 30 min at 30 °C. Each sample with a different pH value was examined against the indicator of bacteria by the agar diffusion method suggested by Syukur et al. (2014).

2.5 Effect of temperature

One-half ml of purified bacteriocin was added to nutrient broth (4.5 mL) in test tubes, and the tubes and overlaid with paraffin oil to prevent evaporation before heating for 10 min at 30, 50, 70, 90, 100, and 121 °C. The preparations containing 0.5 mL bacteriocin and 4.5 mL nutrient broth were plugged carefully using non-absorbent cotton, coated with aluminum foil, and kept in the autoclave for 10 min at 121 °C to evaluate its activity at very high autoclaving (Syukur et al., 2014).

2.6 Effect of enzymes treatments

The effect of enzymes treatment on crude bacteriocin activity was carried out according to Zhou et al. (2014). In brief, the bacteriocin was treated with 5 mg/mL amylase, 5 mg/mL trypsin, and 5 mg/mL proteinase while 0.5M, pH 7.0 phosphate buffer was used as a control. Additionally, the agar diffusion method was carried out to study the effect of enzymes treatment on the activity of bacteriocin according to Syukur et al. (2014) using the above preparations against *E. carotovora* as an indicator bacteria.

2.7 Production of bacteriocin

Crude bacteriocin synthesized by different strains of LAB was propagation in 1000 mL MRS broth seeded with a 10% inoculum of overnight culture before being incubated for 24 h at 37 °C. Then the whole broth for each strain was centrifuged for 15 min at 10,000 × g, and the cell-free supernatants were collected. The supernatants were saturated with ammonium sulfate (70%) and kept at 4 °C for the precipitation of proteins. The ammonium sulfate-saturated supernatant was centrifuged (10,000 × g at 4 °C for 30 min), and bacteriocin was collected (Ogunbanwo et al., 2003; Sankar et al., 2012).

2.8 Identification of bacterial isolate

Isolation of bacterial genome DNA

Isolation of DNA of *Streptococcus faecalis* subsp. *liquefaciens* (R-55) and *Streptococcus lactis* subsp. *diacetylactis* (R-43), was performed using the GES method (Pitcher et al., 1989). The DNA was prepared by the use of three other reagents (0.25 mL cold 7.5 mol/l ammonium acetate, 0.5 ml chloroform and 2-pentanol (24: 1) mixture, and 0.54 volumes of cold 2-propanol) and one high-speed centrifugation step. This method was applied to both Gram-negative and Gram-positive bacteria. It eliminated endogenous nuclease activity and avoided the need for phenol, RNase, and protease treatments. The DNA was of high purity

high molecular mass and double-stranded. The isolated DNA was stored in 1.5 mL microtubes at -20 °C.

2.9 Amplification and sequence analysis using 16S rDNA gene

PCR amplification on 16S rDNA was carried out using Primer 27 F: 5'-AGA GTT TGA TCCTGG CTC AG - 3' and Primer 1492 R: 5 - GGT TAC CTT GTT ACG ACT T - 3' (O'Donnell, 1993; White et al., 1990). PCR product purification was carried out using the polyethylene glycol (PEG) precipitation method (Hiraishi et al., 1995) and continued with a sequencing cycle. For performed sequencing of 16S rDNA, the primer used was Primer 27 F: 5 - AGA GTT TGA TCC TGG CTC AG - 3 and 1492 R: 5 - GGT TAC CTTGTT ACG ACT T-3'. The results of these sequencing cycles were re-purified by the ethanol purification method.

The process of DNA sequencing was done by ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). The results of subsequent determination of DNA base sequence were reducing DNA baser program. Furthermore, the matching process was done by selecting the menu *Alignment* with *Bio Edit* (National Library of Medicine, 2021). Matching results were used to search for the DNA base sequence of genes that are similar or similar to DNA base sequence data for genes from an international gene bank database through the National Center for Biotechnology Information (NCBI) with the BLAST method. Based on phylogenetic trees, the DNA sequence was used as a data reference. DNA sequence data can be retrieved from the international database software NCBI. The selected bacterial DNA sequence was stored as Phylogenetic and molecular evolutionary analyses were conducted using MEGA version X (Stecher et al., 2020) and followed by the neighbor-joining method suggested by Saitou & Nei (1987).

2.10 Statistical analysis

The data obtained from this study were tabulated, calculated the mean and standard error values of three experiments were calculated using Microsoft Excel®. One way analysis variance (ANOVA) was performed using PASW-Statistics18-SPSS software (Hong Kong) to determine statistically significant difference (95% confidence interval) among experimental variables.

3 Results and discussion

Twelve strains of LAB were isolated from the dadih and were identified by the API 50 CH methods as *Leu. paramesenteroides* (R-8), *St. cremoris* (R-14), *St. faecalis* subsp. *Liquefaciens* (R-19), *St. lactis* subsp. *diacetylactis* (R- 22), *Leu. paramesenteroides* (R-31), *St. faecalis* subsp. *liquefaciens* (R-32), *St. lactis* subsp. *diacetylactis* (R-41), *St. lactis* subsp. *diacetylactis* (R-43), *Leu. paramesenteroides* (R-45), *Leu. paramesenteroides* (R-49), *St. faecalis* subsp. *liquefaciens* (R-55) and *St. faecalis* subsp. *liquefaciens* (R-56).

The antimicrobial effects of the isolated strains of LABs were evaluated against the pathogenic *E. carotovora*, gram-negative (-) bacteria. It is well documented that LABs can produce inhibitory substances such as bacteriocins, organic acids, and H_2O_2 in the growth media. The antibacterial property of the bacteriocins synthesized by these twelve isolated strains against *E. carotovora* is presented in Figure 1. These results indicated that all the isolated strains inhibited *E. carotovora* growth by various degrees. Moreover, the strains from the genus *Streptococcus sp*. displayed a noticeable inhibitory effect against *E. carotovora* growth compared with the genus *Leuconostoc sp*. Additionally, the bacteriocin from the genus *St. faecalis* subs. *liquefaciens* (R-55) exhibited the maximum inhibition zone; whereas, the genus *Leu. paramesenteroides* (R-8) showed the minimum activities against *E. carotovora*. In our previous work, we showed that bacteriocin from *Leu. paramesenteroides* exhibited minimal antimicrobial effects against *Listeria monocytogenes* (Pato et al., 2020). Moreover, Nes et al. (2007) stated that the *Streptococcal* isolates have high-frequency peptide bacteriocin as manifested by the genome sequencing of *Streptococcus* from the 45 fully sequenced gram-positive (+) genomes studied. These authors found that 80% of the double-glycine GG motif candidate peptides were referred to as *Streptococcal* genomes.

A previous study reported that the GG motif in Gram-positive bacteria plays a crucial role in the synthesis of many peptides implicated in quorum sensing and bacteriocin production (Dirix et al. 2004). Hence, the antimicrobial stability of the nine bacteriocins from *St. faecalis* subsp. *liquefaciens* (R-19), *St. lactis* subsp. *diacetylactis* (R-22), *St. faecalis* subsp. *liquefaciens* (R-32), *St. lactis* subsp. *diacetylactis* (R-41), *St. lactis subsp. diacetylactis* (R-43), *Leu. paramesenteroides* (R-45), *Leu. paramesenteroides* (R-49), *St. faecalis* subsp. *liquefaciens* (R-55) and *St. faecalis* subsp. *liquefaciens* (R-56) was further evaluated against *E. carotovora* at different levels of pH (Figure 2). The results also showed that the antimicrobial property of bacteriocin against *E. carotovora* at different pH levels mostly remained stable at low pH (acidic) conditions. But, some bacteriocins lost the antimicrobial effect under neutral and alkalic conditions (≥ pH 7). Moreover, the strains R-22, R-49, and R-56 completely lost their antimicrobial property when the pH of the supernatants was set at pH 7 to 11; however, the other strains showed potential activity and increased the inhibition zone compared to the control condition by increasing pH (Figure 2).

Additionally, 6 of 9 bacteriocins induced the highest antimicrobial effect against *E. carotovora* in the wide pH level

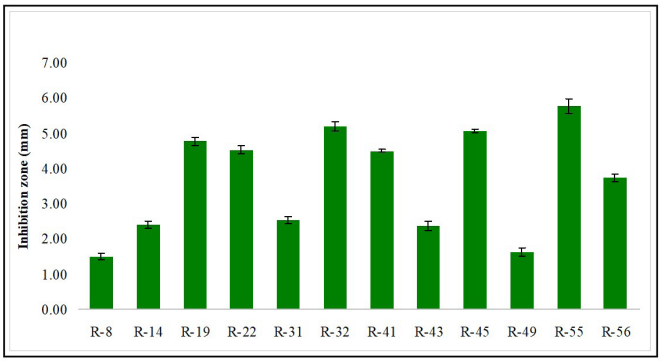

Figure 1. Antimicrobial activity of cell-free supernatant from dadih's lactic acid bacteria against *Erwinia carotovora*.

condition (pH 3 – 11) including R-55, R-32, R-19, R-41, R-43, and R-45. These bacteriocins were also stable in various pH conditions against *Listeria monocytogenes,* as reported in our previous study (Pato et al., 2020). In this concern, Abanoz & Kunduhoglu (2018) suggested that bacteriocins that are stable over wide ranges of pH have a crucial advantage as bio-preservatives in food products and fermented foods. Consequently, the bacteriocins synthesized by isolates R-55, R-32, R-19, R-41, R-43, and R-45 are considered promising bio preservatives in foods.

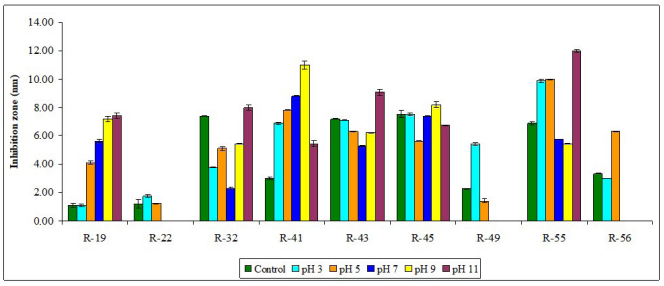

Figure 2. The sensitivity of antimicrobial activity of supernatant of dadih's LAB against *E. carotovora* at a different pH level.

It was reported that the production of bacteriocins depends on the growth conditions including medium composition, pH, temperature, water activity, and others (Nes et al., 2007). In this study, the stability of the bacteriocin and supernatant of the six promising isolates against *E. carotovora* was evaluated under various temperatures (30, 50, 70, 90, 100, and 121 °C) for 10 min. The results presented in Figure 3 suggested that crude bacteriocins and supernatants isolated from *St. faecalis* subsp. *liquefaciens* R-19 (Figure 3A), R-32 (Figure 3B), *St.*

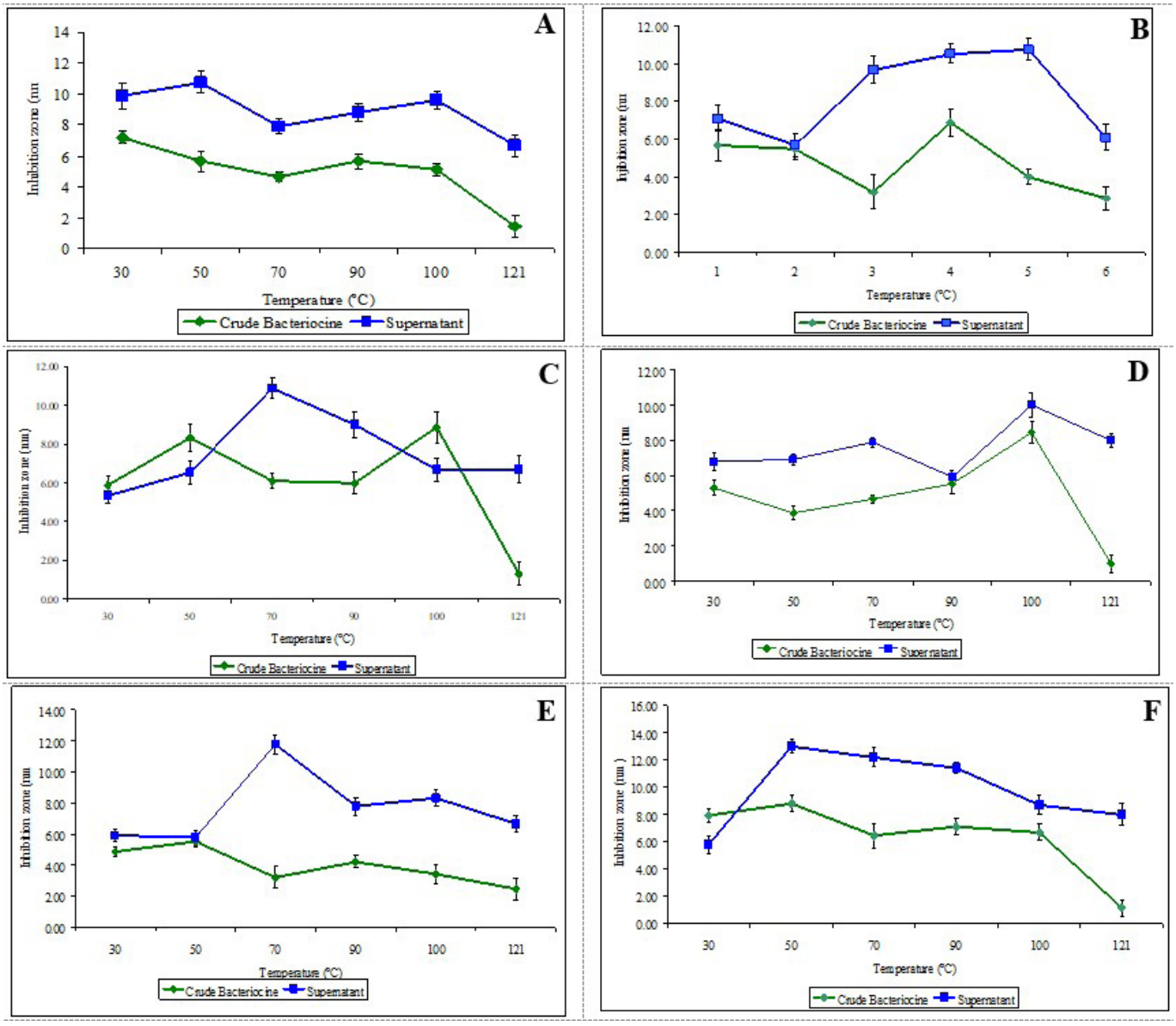

Figure 3. Effect of temperatures on the sensitivity of antimicrobial activity of crude bacteriocin and supernatant from dadih's LAB against *E. carotovora*; (A) *St. faecalis* subsp. *liquefaciens* R-19; (B) *St. faecalis* subsp. *liquefaciens* R-32; (C) *St. lactis* subsp. *diacetylactis* R-41; (D) *St. lactis* subsp. *diacetylactis* R-43; (E) *Leu. paramesenteroides* R-45 and (F) *St. faecalis* subsp. *liquefaciens* R-55.

lactis subsp. *diacetylactis* R-41 (Figure 3C), R-43 (Figure 3D), *Leu. paramesenteroides* R-45 (Figure 3E) and *Liquefaciens* R-55 (Figure 3F), were stable and not influenced by heating at 30 – 100 °C since the inhibitory effect against *E. carotovora* was not significantly changed. However, the exposure to heat at 121 °C diminished the inhibitory effect of all tested samples by about 64% for crude bacteriocin and around 20% for the supernatant. According to these results, the inhibitory activity of the supernatant of 6 isolates was more stable and more effective and the inhibitory activity of the supernatant was 65% higher than the crude bacteriocin. Interestingly, the data presented in Figures 3A and 3E indicated that the supernatant of *St. faecalis* subsp. *liquefaciens* R-19 and *Leu. paramesenteroides* R-45 showed the best antimicrobial stability against *E. carotovora* under 90, 100, and 121 °C, as well as at room temperature (30 °C). The results also suggested that these strains are suitable bio-preservatives for fresh fruits and vegetables, ready-to-eat products, or food process products under different storage conditions.

A previous report revealed that proteolytic enzymes and high lipids composition reduces the antimicrobial effectiveness of bacteriocin (Bogsan et al., 2015). The effects of enzymes (amylase, proteinase K, and trypsin) on the antimicrobial activities of bacteriocin compared to the control condition (samples without enzymes treatment) at different temperatures are shown in Figure 4. These results revealed that treatment with these proteolytic enzymes (proteinase K and trypsin) resulted in a complete loss of the antimicrobial effect of the bacteriocin synthesized by *St. faecalis subsp. liquefaciens* R-55, R-32, R-19, *St. lactis subsp.diacetylactis* R-41, R-43, and *Leu. paramesenteroides* R-45. The loss of the antimicrobial property of the bacteriocin is related to the proteinaceous part of the bacteriocin molecule and confirmed the proteinaceous nature of the inhibitory substances (Heredia-Castro et al., 2015). The determination of the proteinaceous nature of the inhibitory substances confirmed that these six isolates produce bacteriocin-like substances (BLS) as suggested by Heredia-Castro et al. (2015).

Furthermore, treatment with amylase enhanced the antimicrobial activity for crude bacteriocin isolated from all strains except that isolated from *St. lactis* subsp. *diacetylactis* R-43 and *St. faecalis* subsp. *liquefaciens* R-55 induced less antimicrobial effect than the control (Figure 4). The decrease of antimicrobial activity of bacteriocin from R-43 and R-55 suggested that this bacteriocin is glycoproteins (carbohydrate moiety), which require both the glyco and the protein portion of the molecule to induce its activity. These results also indicated that these bacteriocins may be classified as group IV which contains lipids and carbohydrates in their molecular structure (Heredia-Castro et al., 2015). Several glycoprotein bacteriocins sensitive to amylase were isolated such as *Enterococcus faecium* DB1, *Lactobacillus Brevis* DF01, leuconocins produced by *Leuconostoc paramesenteroides*, and carnosum 54 produced by *Leuconostoc carnosum* (Heredia-Castro et al., 2015).

The 16S rDNA gene is a gene that is generally the target of several amplifications for DNA sequence analysis. This technique also has high discriminatory power with 100% type ability and good reproducibility. Similarity scores <97% represent that

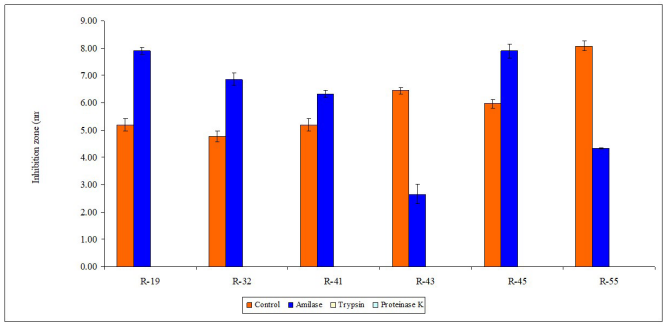

Figure 4. Effect of enzyme treatments on the antimicrobial activity of crude bacteriocin from Dadih's LAB against *E. Carotovora*.

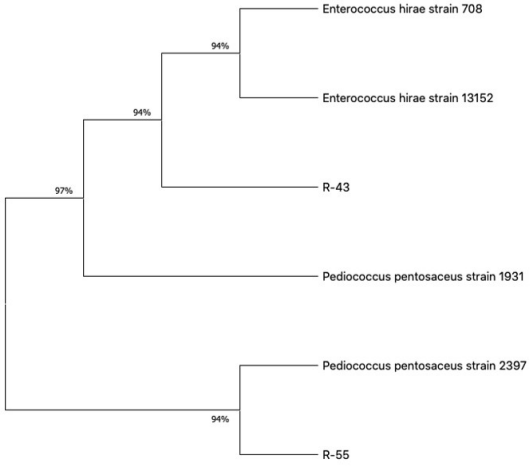

Figure 5. Evolutionary relationships of isolate R-43 and R-55 regarding bacterial strains in Genebank NCBI by using the neighbor-joining method.

Table 1. Data collection results of bacterial isolate R-43 and R-55 based on 16S rDNA sequence analysis.

Sample code	Sequence code	Nearest bacterial taxon results from BLAST homology in NCBI
R-55	Contig-R55	*Pediococcus pentosaceus* strain 239716S ribosomal RNA Gene (Accessionno: MT604839.1) [Homologi: 100.00%; Max score: 2582; Total score: 2582; Querycoverage: 100%; E value: 0.0; Max identities: 1398/1398 (100%) ; Gaps 0/1398(0%)]
		Pediococcus pentosaceus strain 193116S ribosomal RNA Gene (Accessionno: MT597748.1) [Homologi: 100.00%; Max score: 2582; Total score: 2582; Querycoverage: 100%; E value: 0.0; Max identities: 1398/1398(100%); Gaps0/1398(0%)]
R-43	Contig-R43	*Enterococcus hirae* strain 708 chromosome, complete genome (Accessionno: CP055232.E) [Homologi: 100.00%; Max score: 2604; Total score: 15618; Querycoverage: 100%; E-value: 0.0; Max identities: 1410/1410(100%); Gaps 0/1410(0%)]
		Enterococcus hirae strain 13152 chromosome (Accessionno: CP055230.1) [Homologi: 100.00%; Max score: 2604; Total score: 15618; Querycoverage: 100%; E value: 0.0; Max identities: 1410/1410 (100%); Gaps 0/1410(0%)]

these isolates represent a new species, whereas >97% can also specify a new species or as an alternative to a new cluster in a previous taxonomic (Janda & Abbott, 2007). The identification of a new bacterial strain is still unknown regarding the genetic information and its position in the taxonomy based on sequence analysis of 16S rRNA. However, Janda & Abbott, (2007) stated that the isolates with at least 99% or >99.5% similarities should be identified as strains or species similar to that referenced by the Gene bank.

The results of the analysis of the 16S rDNA gene of the two isolates (R-55 and R-43) were uploaded into the database Genebank (www.ncbi.nlm.nih.gov/BLAST) and analyzed for sequence homology with species of reference. Based on the identification of these isolates with 16S rDNA gene analysis, 1 of 2 isolates belong to species *Pediococcus pentosaceus* (similarity level 100%) homology with *P. pentosaceus* strain 23971 and strain 1931, while another isolate was identified as *Enterococcus hirae* (similarity level 100%) homology with *E. hirae* strain 708 and strain 13152 (Table 1).

In this research, the bacteriocin synthesized by isolate R-43 was identified as *Enterococcus hirae* (Figure 5). The enterocins are the bacteriocins synthesized by the genus *Enterococcus spp* with potent applications in food, human, and veterinary medicine (Nes et al., 2014; Rehaiem et al., 2016). Studies of these bacteriocins synthesized by *E. hirae* are still limited due to most of the enterocins commonly produced by *E. faecium* and *E. faecalis* (Cavicchioli et al., 2019). Enterocins produced by *E. hirae* be belongs to class IIa: Anti-listeria-Pediocin-like bacteriocins (Nes et al., 2014). Sánchez et al. (2007) indicated that the bacteriocin synthesized by *E. hirae* DCH5 isolated from the mallard ducks inhibits the diversified number of food spoilage and food-borne pathogenic bacteria, for instance, *C. botulinum, L. monocytogenes*, *S. aureus*, and *E. carotovora* reported in this research.

As a result of the 16S rDNA analysis sequence of isolate R-55, the isolate is identified as *Pediococcus pentosaceus* (Figure 5). Among these known *Pediococcus* strains, *P. pentosaceus* can synthesize bacteriocin (pediocin) used as culture or their products as bio-preservatives in several foods. Additionally, *P. pentosaceus* are used in the fermentation of silage, dough, and fruit juices (Papagianni & Anastasiadou, 2009). Rodríguez et al. (2002) reported that pediocin PA-1, an antimicrobial peptide synthesized by *Pediococcus acidilactici* PAC 1.0, showed particularly strong activity against *L. monocytogenes*, a food-borne pathogen of particular concern among the food industries. Pediocin is an extensively studied class IIa bacteriocin (*Antilisteria-Pediocin-like bacteriocins*), and is well-characterized as a food bio-preservative. Pediocin GS4 also is a novel bacteriocin synthesized by *P. pentosaceus* GS4, MTCC 12683 (Ghosh et al., 2019). The optimum antibacterial properties of pediocin GS4 were achieved at 50 °C and pH 5.0 and 7.0, did not denature by the treatment of lysozyme or amylase treatment and were inactive in the organic solvents.

4 Conclusion

In the current study, 12 strains of dadih's LAB can synthesize bacteriocin with potential antimicrobial properties against *E. caratovora.* The inhibition zone of bacteriocin from the genus *Streptococcus* ssp. gave an excellent ability to suppress the growth of *E. carotovora.* The antimicrobial effects of *St. faecalis* subsp. *liquefaciens* R-55, R-32, R-19, *St. lactis* subsp. *diacetylactis* R-41, R-43, and *Leu. paramesenteroides* R-45 bacteriocins showed high stability against *E. carotovora* under different pH (3-11). The supernatant of the isolated LAB was more stable under various temperature conditions (30-121 °C) compared to the crude bacteriocin. Analysis of the 16S rDNA of isolates R-43 and R-55 showed that these bacteria belong to *Enterococcus hirae* (R-43) and *Pediococcus pentosaceous* (R-55), while amylase, proteinase K and trypsin analysis revealed that bacteriocin produced by R-43 and R-55 probably belongs to group IIa. Additionally, dadih showed a promising source for the isolation of LAB has stable antimicrobial effects against *Erwinia caratovora* under different conditions, and can be used effectively as bio preservative for food products.

Conflict of interest

The authors declare no conflicts of interest.

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