Original Article

Antibacterial Properties of Bacteriocin Purified from Serratia marcescens and Computerized Assessment of its Interaction with Antigen 43 in Escherichia coli

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How to cite this article: Mousavi SM, Archangi B, Zamani I. Antibacterial Properties of Bacteriocin Purified from *Serratia marcescens* and Computerized Assessment of its Interaction with Antigen 43 in *Escherichia coli*. Archives of Razi Institute. 2023;78(6):1738-45.

DOI: 10.32592/ARI.2023.78.6.2694

Article Info:
Received: 28 March 2023
Accepted: 06 June 2023
Published: 30 December 2023

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ABSTRACT

Bacteriocins are a kind of antimicrobial peptides that kill or inhibit the growth of bacterial strains. The purpose of this study was to investigate the antibacterial effect of *Serratia marcescens* on several pathogenic bacterial strains. Bacteriocin produced by *S. marcescens* was purified by chromatography with Sephadex G-75 column, and its antibacterial effect on gram-negative bacteria, including *Escherichia coli* ATCC 700928, *Pseudomonas aeruginosa* PTCC 1707, *S. marcescens* PTCC 1621, *Vibrio fischeri* PTCC 1693, and *Vibrio harveyi* PTCC 1755, were evaluated by the disk diffusion method. The structure of bacteriocin was determined by nuclear magnetic resonance spectroscopy. The interaction of bacteriocin with the antigen 43 (Ag43) of *E. coli* was evaluated by the molecular docking method. Bacteriocin extracted from bacterial isolates had antibacterial activity on *E. coli* strains but not on other studied strains. Bioinformatics analysis also showed bacteriocin docking with Ag43 with an energy of -159.968 kJ/mol. Natural compounds, such as bacteriocin, can be an alternative to common chemical compounds and antibiotics. To reach a definite conclusion in this regard, there is a need for further research and understanding of their mechanism of action.

Keywords: Antibacterial property, Antigen 43, Bacteriocin, Molecular docking, *Serratia marcescens*

1. Introduction

The discovery of antibiotics in the present century has helped to treat various diseases and reduce human mortality. Over time, nevertheless, numerous bacterial strains became resistant to the antibiotics. High production costs, induction of allergic reactions in some people, and changes in the normal human microbiome are other problems associated with antibiotics (1). Bacterial resistance to antimicrobials was observed immediately after their widespread administration. Resistance levels then continued and increased significantly until the World Health Organization in 2000 warned that infectious diseases caused by high levels of resistance to pathogens are incurable (2). To alleviate this problem, the use of most antibiotics has been limited due to the rapid emergence of multidrug-resistant pathogens, thereby directing researchers' efforts to find alternatives to limited antibiotic resources. Novel antibacterial compounds include bacteriophages, probiotic bacteria, antimicrobial peptides, and bacteriocins (3).

Bacteriocins are high-molecular-weight antimicrobial peptides produced by bacterial ribosomes that kill or inhibit strains close to the same bacterial strain but do not damage their producing bacteria despite specific immune proteins (4). Bacteriocins have various applications, including food preservatives (5-8) and antibiotic substitutes (9-11). Compared to chemical drugs, most bacteriocins have high specific activity against various clinical targets, such as antibiotic-resistant strains. Bacteriocins, due to their protein nature, can be genetically engineered and modified according to the researchers' objectives (12).

Over 99% of bacteria can produce at least one bacteriocin. The bacteriocins are extracellular compounds; however, they sometimes remain attached to the surface of their producing bacteria (8). Many recent studies have identified and purified bacteriocins, often with the aim of increasing food shelf life, treating pathogenic diseases, treating cancer, and maintaining human health. Thus, bacteriocins can be predicted to become alternatives to antibiotics in the near future to treat drug resistance of pathogens (13).

Most well-known bacteriocins are generated by gram-positive bacteria and also act against gram-positive bacteria. The majority of bacteriocins do not work against gram-negative bacteria because the outer membrane of this group of bacteria operates as a barrier for the cell and prevents the penetration of molecules such as antibiotics, detergents, and dyes into the cytoplasm (14). However, studies have reported the potential of bacteriocins against these bacteria; for instance, plantaricin 35d produced by *Lactobacillus plantarum* with activity against *Aeromonas hydrophila*, as well as bacteriocin ST151BR produced by *Lactobacillus pentosus* with activity against *Escherichia coli* (9).

Serratia marcescens is a saprophytic gram-negative bacillus classified as a member of the Enterobacteriaceae family. Many bio-compounds are produced by *S. marcescens* species, including biosurfactants, fatty acids, seasonings, enzymes, pigments, and bacteriocins (15).

1.2. Objectives

In the present study, an attempt was made to extract and purify bacteriocin from the bacterial strain of *S. marcescens* and to investigate its antibacterial effect on gram-negative bacteria. Moreover, computerized assessment methods were applied to investigate the mechanism of action of this peptide.

2. Materials and Methods

2.1. Preparation of bacterial strain

The studied bacteriocin was extracted from *S. marcescens*, which was previously isolated and identified from the ponds of shrimp farming by the authors (16).

2.2. Extraction of bacteriocin

S. marcescens was cultured in De Man, Rogosa, and Sharpe (MRS) agar medium with pH=6.5. A volume of 0.1% mitomycin C was added to the medium; this compound induces the production of bacteriocin as well as its separation from the bacterial cell surface

into the medium. Considering that in the previous report, maximum bacterial growth was observed 24 h after culture (16), bacterial cells were precipitated after 24 h by centrifugation at 11,000 rpm for 15 min, and cell-free supernatant was harvested as crude bacteriocin.

2.3. Purification of bacteriocin

For each liter of crude bacteriocin, 400 g of ammonium sulfate was added and kept at 4°C for 24 h. Protein precipitate was collected by centrifugation at 6,000 rpm for 20 min and dissolved in 50 ml of 20 mmol/l of sodium phosphate buffer at pH=6. Purification was performed by chromatography with Sephadex G-75 column using Tris-HCl buffer. The fractions were taken at a flow rate of 0.5 ml/s and concentrated using a rotary evaporator (17). Finally, carbon nuclear magnetic resonance (C-NMR) and proton nuclear magnetic resonance (H-NMR) were applied to determine the secondary structure of purified bacteriocin.

2.4. Evaluation of antibacterial activity

The antibacterial activity of purified bacteriocin on several gram-negative strains, including *E. coli* ATCC 700928, *Pseudomonas aeruginosa* PTCC 1707, *S. marcescens* PTCC 1621, *Vibrio fischeri* PTCC 1693, and *Vibrio harveyi* PTCC 1755, was measured by the disk diffusion method. The plates were incubated at 37°C for 48 h. Water was used as a negative control (18).

2.5. Protocol of molecular docking

According to the results of the previous step, which showed that purified bacteriocin only had an inhibitory effect on *E. coli*, molecular docking was performed between bacteriocin and the antigen 43 (Ag43) in *E. coli* using Molegro Virtual Docker 6 software. Thus, the structure of bacteriocin was drawn based on the NMR spectrum using Mestrelab Mnova v11.0.4 software. The Ag43 structure was also obtained from Protein Data Bank with PDB ID 4KH3. The three-dimensional (3D) structure of bacteriocin docking with the target protein was also plotted using the Molegro Molecular Viewer 2.5 software.

3. Results

3.1. Determining the bacteriocin structure

The bacteriocin structure was plotted in accordance with the obtained H-NMR (Figure 1-A) and C-NMR (Figure 1-B) spectra. As can be seen, the structures

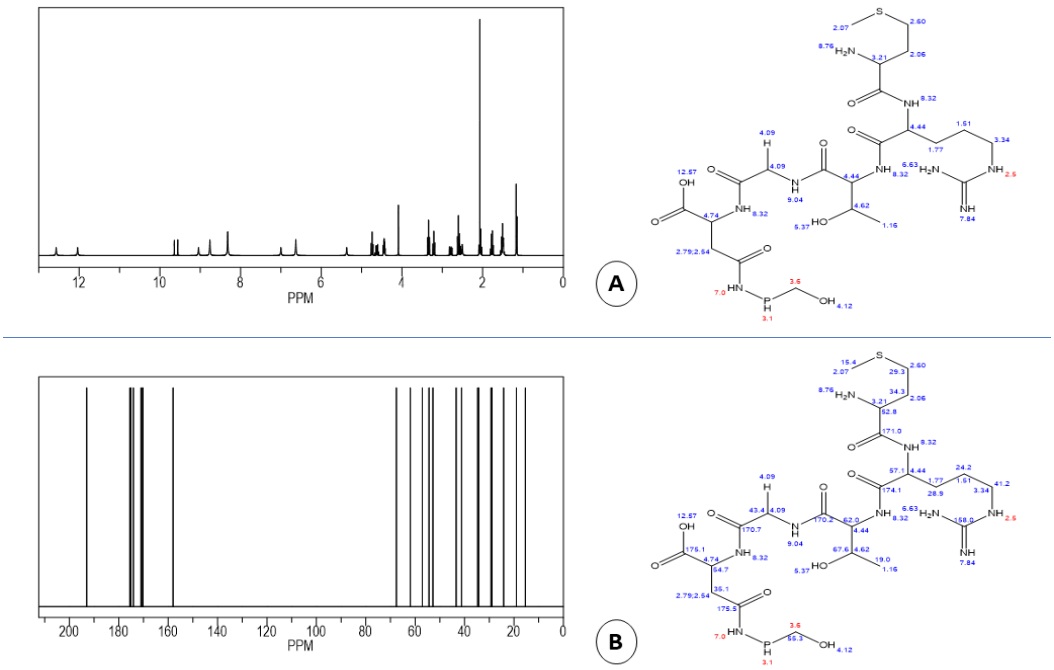

Figure 1. H-NMR (A) and C-NMR (B) spectra and structures obtained for bacteriocin purified from *Serratia marcescens*

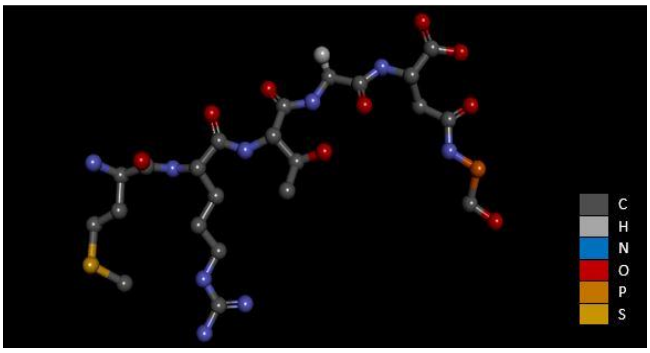

Figure 2. Three-dimensional structure of bacteriocin purified from *Serratia marcescens*

from both H-NMR and C-NMR spectra are identical and confirm the achieved structure. Based on this structure, the 3D morphology of bacteriocin was drawn using Discovery Studio 4.5 software (Figure 2).

3.2. Evaluating the antibacterial activity of bacteriocin

The antibacterial effect of bacteriocin extracted from *S. marcescens* was reported only on the *E. coli* strain (Figure 3). This bacteriocin showed no antibacterial effect on other tested strains.

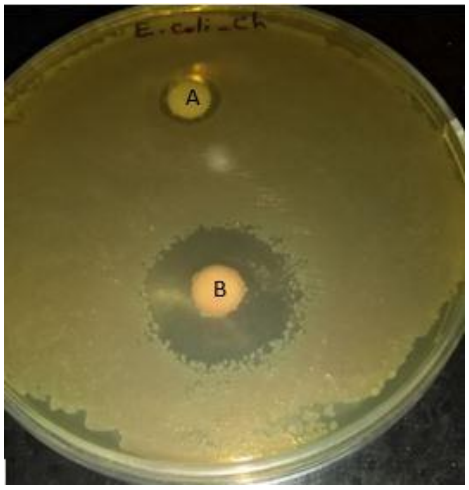

Figure 3. Disk diffusion test to evaluate the antibacterial properties of bacteriocin on *Escherichia coli*; Disk A: Negative Control (Water), Disk B: Bacteriocin

3.3. Assessing the molecular docking

The molecular docking was performed with an energy of -159.968 kJ/mol (Figure 4). The amino acids involved in docking included Asn 205, Asp 189, Lys 243, Trp 224, Tyr 207, Arg 170, and Thr 191. The bonds observed also included hydrogen, electrostatic,

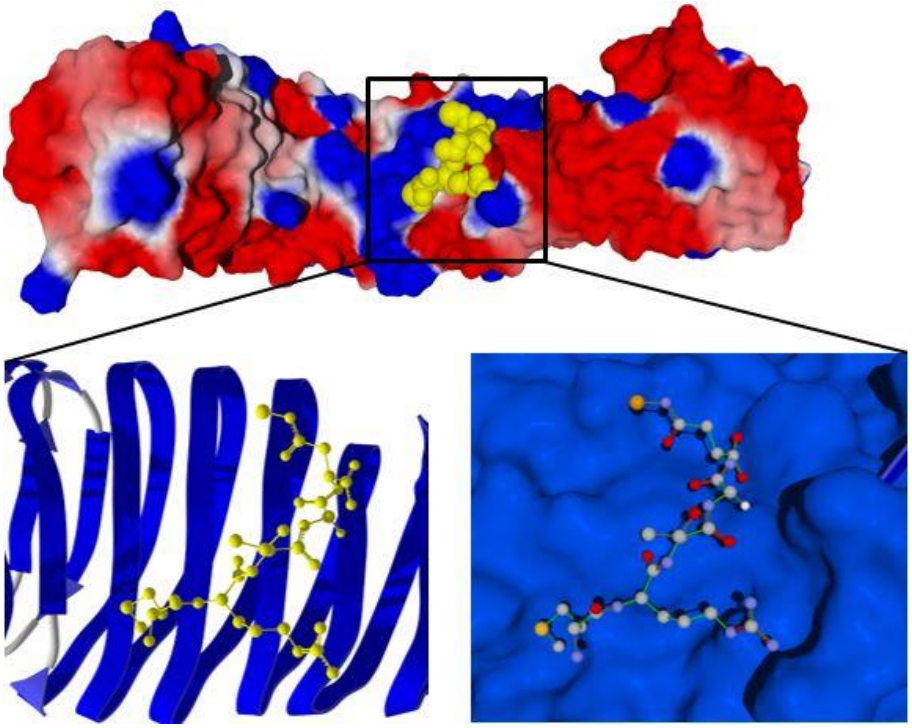

Figure 4. Molecular docking between antigen 43 protein from *Escherichia coli* and bacteriocin purified from *Serratia marcescens*, with energy of -159.968 kJ/mol

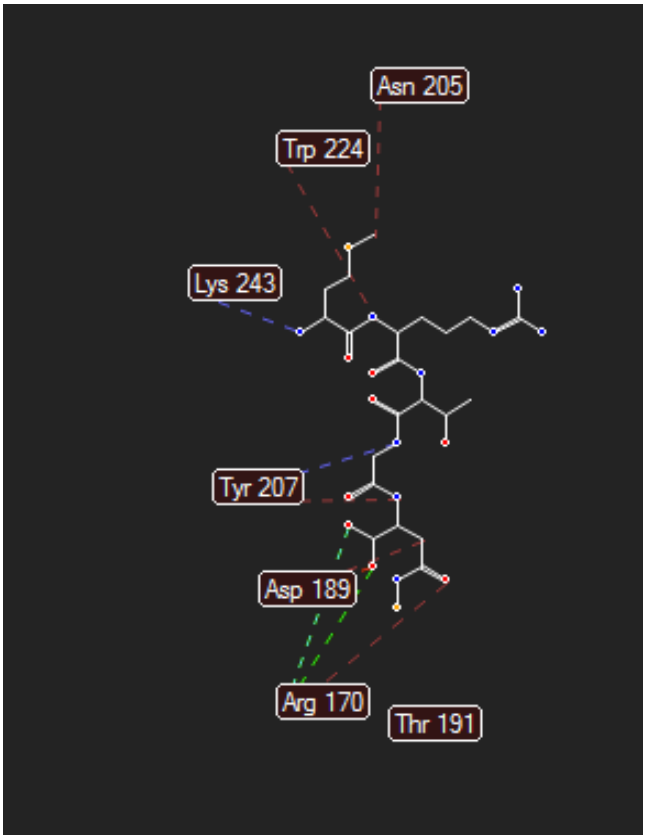

Figure 5. Schematic of some bonds between bacteriocin and amino acids involved in docking (blue dashed line: hydrogen bond, green dashed line: electrostatic bond, red dashed line: ester bond).

and ester bonds (Figure 5).

4. Discussion

Since the identification of bacteriocin, most bacteriocins have been extracted and studied from gram-positive bacteria and less attention has been paid to bacteriocin produced by gram-negative bacteria (19-21). This can be attributed to the abundance and diversity of bacteriocins from gram-positive bacteria (22). However, the value of bacteriocins produced by gram-negative bacteria cannot be ignored (23). Some species of *S. marcescens* are able to synthesize bacteriocin (24). Accordingly, the present study attempted to extract and purify this valuable biological compound from *S. marcescens*.

Similar research suggests that bacteriocin produced by *S. marcescens* often affects other Serratia spp. as well as various strains of *E. coli* (25). Nonetheless, in the current study, in addition to the mentioned strains, its effect was evaluated on several other gram-negative bacteria. Bacteriocin produced by each bacterium affects strains close to the same bacterial strain. *Pseudomonas* and *Vibrio* are not close strains to *S. marcescens* and, as expected, no antibacterial effect was observed on them; nevertheless, *E. coli*, like *S. marcescens*, belongs to the family Enterobacteriaceae, and the bacteriocin produced by *S. marcescens* must stop growing. In the present study, as in other studies in this field, the bacteriocin caused a zone of inhibition in plates in which *E. coli* strains were cultured. Considering the lack of antibacterial effect of bacteriocin on *S. marcescens*, it is assumed that this strain is the same one used to purify bacteriocin, and therefore, has no effects on itself.

In general, limited studies, which are often in the past, have been carried out on the antibacterial potential of bacteriocin produced by *S. marcescent*. Foulds et al. conducted studies on this bio-compound several years ago. They extracted bacteriocin from *S. marcescens* using a salt extraction process and purified it by chromatography (17). They evaluated the effect of bacteriocin on 7 strains of Serratia and 20 strains of *E. coli* and reported that although bacteriocin had no activity on any of the strains of Serratia, it inhibited the growth of 11 strains of *E. coli* (18). The mechanism of action of bacteriocin was also determined by these researchers, who revealed that this bacteriocin strongly inhibits the incorporation of leucine into protein and thymidine into the deoxyribonucleic acid. In addition, adenosine triphosphate levels in bacteria exposed to bacteriocin rapidly reached 10%-15% of control bacteria. Together, these mechanisms inhibited bacterial growth (26).

One of the bioinformatics methods for studying the interaction between molecules is the molecular docking method, which is a key method for predicting the structure of the complex and the interaction of macromolecules with each other

(receptor-ligand) at the atomic level. Basically, the purpose of molecular docking is to achieve a prediction of the complex ligand-receptor structure using computational methods (27). This method was first used in 1982 (28) and today it is widely utilized as a virtual search tool in the early stages of the drug development process (29).

In the present study, molecular docking was applied to evaluate the molecular mechanism of bacteriocin on *E. coli*. Thus, the influence of bacteriocin produced by *S. marcescens* was investigated on the Ag43 in *E. coli*. The Ag43 is a known autotransporter protein. Autotransporters are actually proteins of the type V secretion system. The proteins of the type V secretion system, unlike other proteins in this system, are not in the inner membrane but are located in the outer membrane of bacteria and secrete themselves, that is why they are called autotransporters. Their presence in the outer membrane of bacteria makes them available and prevents the penetration of antibacterial agents into the inner membrane (30).

The Ag43 has been observed in many *E. coli* pathotypes, such as uropathogenic *E. coli* (31). The Ag43 is involved in cell aggregation and biofilm formation (32-34). Therefore, it can be stated that the bacteriocin secreted by *S. marcescens* binds to Ag43 as one of the important virulence factors in *E. coli*, causing its dysfunction and thus inhibiting the growth of *E. coli*. This bacteriocin probably interacts with other virulence agents. This assumption requires further investigation in both in silico and in vitro conditions, as understanding the exact mechanisms of action of bacteriocin can be helpful in its application.

Despite the valuable nature of bacteriocin produced by *S. marcescens* in terms of antibacterial properties, apart from studies by Foulds et al., few extensive studies have been conducted in this field. To achieve the applied purposes of bacteriocin, further and more accurate tests are needed to use this valuable peptide as an alternative antibacterial agent to chemical antibiotics.

Acknowledgment

None.

Authors' Contribution

This article is a part of the Master's thesis of Seyedeh Maryam Mousavi. Bita Archangi was the supervisor and Isaac Zamani was the advisor of this thesis.

Ethics

The authors declare all ethical considerations were respected in the preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

There was no Fund to mention.

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