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Original Article

Antibacterial Activity of the Peptide Microcin J25 Produced by *Escherichia coli*

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ABSTRACT

Background and objectives: Bacteriocins are generally active antimicrobial peptides effective against bacteria closely related to the producer. *Escherichia coli* produce two bacteriocins: colicins and microcins. Microcin J25 (Mcc J25) is an antibacterial peptide that inhibits bacterial transcription by disrupting the nucleotide-uptake channel of bacterial RNA polymerase**.** The objective of this study was to evaluate antimicrobial activity of MccJ25 produced by the bacteriocinogenic *E. coli*.

Methods: In this experimental study, 120 clinical specimens were selected from private diagnostic laboratories in Isfahan (Iran) in 2020. Antagonistic activity of isolates was tested by adopting agar plug method. Total DNA was extracted from clinical specimens and polymerase chain reaction (PCR) was performed using specific primers for amplification of the complete sequence of MccJ25 gene. Accuracy of the PCR products was confirmed by direct sequencing. Homology analysis was performed by using BLAST. Data were analyzed with Chromasv2.1.1 software.

Results: Overall, 120 *E. coli* strains were isolated from the clinical specimens. The antibiotic activity of Mcc J25 was mainly directed at *Enterobacteriaceae*, including several pathogenic *E. coli* strains of which 25 had positive well test samples, and about 5 (20%) of the collected clinical samples that were infected with *E. coli* had the MccJ25 gene.

Conclusions: Based on the results, Mcc J25 has favorable antibacterial potential, which can be further exploited as an alternative to chemical antibiotics.

Keywords: Bacteriocins , Microcin, *Escherichia coli*.

INTRODUCTION

Bacteriocins are generally active antimicrobial peptides against bacteria, closely related to the producer. *Escherichia coli* produce two bacteriocins: colicins and microcins (1). They destroy other bacteria via various mechanisms, including altering membrane permeability and depolarizing membrane ion gradients, or degrading nucleic acids or cell walls (2). The word bacteriocin is typically limited in literature to peptides generated by Gram-positive bacteria, while in Gram-negative bacteria, primarily enterobacteria, toxins are referred to as either colicins i.e. antibiotic proteins targeting *E. coli* or microcins that are characterized by lower molecular mass (3). With only 15 members known since their discovery in 1976, microcins form a very small community of defense peptides (4). They may be highly modified low-molecular-mass peptides below 5 kDa (microcins B17, C7/C51, D93, and J25) or polypeptides between 7 and 10 kDa that may or may not be modified (microcins E492, L, H47, I47, M, 24, and V) (5,6). Some of these peptides were known only on the basis of genetic analysis. Microcins share a conserved organization of their genetic systems in spite of a high structural heterogeneity. A typical gene cluster, located either on a plasmid or on the bacterial chromosome, includes open reading frames encoding the microcin precursor, secretion factors, immunity proteins, and modifying enzymes (6). MccJ25 was first described as a head-to-tail macrocyclic linear peptide (7). Later, with a unique three-dimensional structure, the peptide was described as a 'laso' peptide, revealing that the ring was simply a small cycle arising from a link between the amino group of the N-terminal and the side-chain carboxylate of Glu8 (7, 8). *E. coli* MccJ25 is a cyclic peptide, plasmid-coded, antibiotic composed of 21 unmodified amino acid residues (9). At the beginning of stationary growth, MccJ25 production is induced and adapted to iron-depleted environment (8, 9). MccJ25 is predominantly active on producer strain-related Gram-negative bacteria, with certain pathogenic bacteria hypersensitive to MccJ25 including *Salmonella* and *Shigella* species (10). Bacteriocins are usually safe and stable with therapeutic potential as broad-spectrum antimicrobial agents. The objective of this study was to evaluate antimicrobial activity of MccJ25 produced by the bacteriocinogenic *E. coli*.

MATERIAL AND METHODS

One hundred and twenty strains of *E. coli* were examined in this study. Bacteria strains were isolated from clinical specimens obtained from private diagnostic laboratories in Isfahan (Iran) from May 2020 to October 2020. The bacteria were cultured on blood agar and eosin methylene blue at 35 °C for 18-24 hours. Pure isolates were identified using Gram staining and biochemical tests including catalase test, Simmons citrate agar, sugar fermentation on triple sugar iron agar, gelatin hydrolysis test, indole production, nitrate reduction, urease production, Voges-proskauer test, methyl red, and presumptive test to confirm *E. coli* species.

Antagonistic activity of isolates was tested by adopting agar plug method. This method is commonly used to study antagonism between microorganisms. First, bacterial strain was inoculated onto agar plates previously inoculated with 10^7 -10^9 cfu/ml overnight culture of *E. coli* ATCC 25922. Cell free culture supernatants were obtained by centrifugation of overnight culture (10^9 cfu/ml) of *E. coli* strains at 12,000 g for 10 minutes at 4 ºC. The *E. coli* isolates from clinical specimens secrete molecules that diffuse in the agar medium; this medium was cut and placed on another agar plate inoculated with another microorganism. All the plates were then incubated at 37 ºC for 24 hours. Next, antimicrobial activity was evaluated by measuring diameter of inhibition zone surrounding the agar plug, which may provide an indication of diffused antimicrobial metabolites produced by the growing bacterial culture. The absence of an inhibition zone indicated a negative result for the production of bacteriocins (11, 12).

A set of primer targeting the MccJ25 gene was designed. The original sequence were retrieved from the NCBI GenBank, and Gene runner (version 6.5.52) was applied for qualification of the designed primers (Table 1).

Table 1- Sequences of the primers used in the study

Gene	Sequence (5'→3')	Amplicon	Accession number
FAmcj25	ATGGAACTTCTTGTACTTGTCTG	470 bp	AF061787.1
RBmcj25	CATCCAGATAGCCGTTACCAGC		

One colony of each bacterium was dissolved in 10 µl of sterile water and incubated at 95 °C for 10 minutes. Then, PCR buffer (1X), $MgCl_2$ (1.5 mM), dNTP (200 µM), forward and reverse primers (0.4 µM each), and Taq polymerase enzyme (1 unit) were added to the bacterial colony for polymerase chain reaction (PCR). All reagents were purchased from Sinaclon Co., Iran. Cycling conditions were optimized as follows: initial denaturation for 5 minutes at 95 °C, 35 cycles of denaturation at 94 °C for 35 seconds, annealing at 58 °C for 35 seconds, extension at 72 °C for 30 seconds, and final extension at 72 °C for 5 minutes. The PCR reaction was carried out using the Boecco TC-SQ thermal cycler device (Germany). Finally, PCR products were electrophoresed on 1% agarose gel stained with green viewer fluorescent dye and visualized using an UV transilluminator.

A commercial PCR purification kit was used to purify PCR products, and sequencing was performed by FAZA Biotech Co. (Iran) using forward and reverse specific primers. The nucleotide sequences were analyzed with Chromasv2.1.1 software (http://technelysium.com.au), and sequence homology analysis was performed by using BLAST (http://www.ncbi.nlm.nih.gov/BLAST).

RESULTS

Of 120 clinical isolates, 25 (20.83 %) had large (5-7 mm) inhibition zones in agar well dilution method.

After the initial detection of *E. coli* strains, genomic bacterial DNA was extracted and applied on 1% agarose gel electrophoresis. The PCR products had a size of 470 bp.

Overall, 120 120 clinical specimens were infected with *E. coli*. The MccJ25 gene was detected in about 20% of *E. coli* isolates. All strains detected by the phenotypic methods were confirmed by PCR technique.

DISCUSSION

Antibiotic resistance among pathogenic bacteria is a serious public health concern. Traditional antibiotics must be used with caution to avoid generation and spread of antibiotic resistance, and other viable medications must be sought. Bacteriocins are ribosomally-synthesized proteinaceous compounds that are generally active against bacteria, often closely related to the producer (13). Microcins are bacteriocins produced by *Enterobacteriaceae* that inhibit *E. coli* and closely related strains (3, 14). In this study, the MccJ25 gene from *E. coli* was screened by PCR in 120 clinical specimens. In previous studies, the prevalence of bacteriocinogenic *E. coli* strains ranged from 25 to 55% (15-17). However, previous studies differ in terms of cultivation conditions, indicator bacteria used for detection of bacteriocin production, or the number of detected bacteriocin genes. In a previous study in Isfahan (Iran), about 40% of the clinical specimens contaminated with *Klebsiella pneumoniae* had the microcin E492 gene (15).

In a study by Sable et al., MccJ25 showed inhibitory activity against 12 of the 15 DEC strains (16).

In one study on 105 *E. coli* strains, 4% of the strains contained four types of colicin, while in our study, 20% of the isolates contained MccJ25 (17). In a study by Jeziorowski et al. on *E. coli* 1308 samples, colicin Ia and microcin V were present in 10% and 5% of strains, respectively (18).

In study of Tahamtan et al., all *E. coli* isolates had at least one colicin gene (19).

In a study by Micenkova et al., of 1,181 *E. coli* isolates, 28 samples (7%) contained Microcin H47 and 18 samples (4.5%) contained Microcin V. Moreover, of 179 diarrhea samples, 14 (7.8%) contained microcin H47 and 18 (10.1%) contained microcin V. Of 603 extra-intestinal pathogenic *E. coli* samples, 165 (27.4%) contained microcin H47 and 152 (25.2%) contained microcin V.

CONCLUSION

MccJ25 is an antibacterial peptide that can be used in the next generation of antimicrobials as a good alternative to chemical antibiotics..

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Ethics approvals and consent to participate

The study protocol was approved by the local ethics committee.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding publication of this article.

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