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Screening and Molecular Dynamic Simulations of Colicin Produced by Commensals *Escherichia coli* Isolated from Animals

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Abstract

E. coli strains isolated from animal feces were subjected to detection of colicin production. The produced colicin was prepared as a disc to serve as an inhibitor for other types of bacteria on Mueller-Hinton agar. The result indicates that the produced colicin has an inhibition effect against *Staphylococcus sp.* with inhibition zone vary from 1.3 to 2.5 cm. Moreover, the result of electrophoresis of total crude protein after cell lysis with sonication verify the presence of 130kD bands in the supernatant. The results of docking were analyzed and compared to predict the potential binding affinities of the colicin types against the FH VH at lipid Π protein.

Keywords: Colicin, *Escherichia coli,* docking**.**

Introduction

Bacteria produce proteins called bacteriocins that can inhibit or even kill closely related species. Colidins are the most well-characterized group among the bacteriocins. They are generated by *E. coli* and other Enterobacteriaceae family members, and they are effective against them (1, 2). Researchers have identified at least 34 colicins, thoroughly studying 21 of them, and they share an uncommon set of properties. A group of carcinogenic bacteria generates colicin proteins under stressful conditions. Thus, the kind of stress that is causing the inductive activity must be specified. Colicins released by colicinogenic bacteria are protected and have plasmid-

encoded gene clusters, preventing cell death due to their special protection and plasmid-encoded nature (2, 3). Colicins have various applications, including food preservatives and managing diarrheal illnesses caused by enteropathogenic bacteria due to their broad range of action (2, 4). This type of bacteria and bacteriocine production bacteria were extensively studied in our Lab, such as *Bacillus cereus* (5, 6,10)

Materials and Methods

Stimulation the bacteria for secreted Colicin protein

All the *E. coli* strains, previously isolated (11), were grown in LB broth at 37ºC with mitomycin-C (0.25µg/ml) which was added to induce colicin production. Incubation was continued overnight at 37^{O}C to reach the final concentration of 2x105CFU/ml. Then, the cells were transferred into LB agar plates and grown overnight at 37^{O}C.

The putative colicin-producing E. coli strains were killed using Sonicator, filtered using a 0.45 filter, and dispensed into a paper disc. The crude colicin was then placed on Muller Hinton agar plates infected with E. coli O157:H7 and Staphylococcus spp. The strain isolated from a clinical sample was incubated at 37°C for 24 hours, revealing the activity of colicin produced by E. coli strains. A potential colicinogenic strain was selected for further studies and stored in glycerol broth at -20ºC.

SDS-polyacrylamide Gel Electrophoresis

The specific protein band was observed using vertical gel electrophoresis using A10% SDSPAGE according to the methods described in (14).

Docking: Docking simulations were performed using the Hdock web server (http://hdock.phys.hust.edu.cn) to predict the interactions between colicin types A (PDB ID: 1col), E1 (PDB ID: 2i88), M (PDB ID: 2xmx), S4 (PDB ID: 3few), and N (PDB ID: 1a87) against the FHUA protein from *E. coli* (PDB ID: 1by3). The FHUA protein was prepared by removing all water molecules and heteroatoms from the crystal structure and adding hydrogen atoms using the protein preparation wizard in Maestro (Schrödinger, LLC). The protein was then minimized using the OPLS3e force field.

The same docking protocol was performed for the same set of proteins (colicin types A, E1, M, S4, and N) against the protein Libid II (PDB ID: 46173749) using the Hdock web server. The protein Libid II was prepared in the same way as the FHUA protein. The docking results were analyzed and compared to predict the potential binding affinities of the colicin types against the FHUA and Libid II proteins.

Material and methods of docking

The Computed Atlas of Surface Topography of proteins (CASTp)server was used to identify the binding site of the selected proteins. The protein structures were obtained from the Protein Data Bank (PDB) with the following PDB IDs : 1BY3 and 2xmx. The CASTp server was accessed online at http://sts.bioe.uic.edu/castp/calculation.php.

↗ The protein structures were uploaded in PDB format, and the server was set to detect

binding sites with a probe radius of 1.4 Å. The server generated output files that included the binding site's location and size, as well as the amino acid residues involved in the binding site.

Results

The *E.coli* strains isolated previously from animal feces were subjected for detection of colicin production. The produced colicin was prepared as a disc to serve as an inhibitant for other types of bacteria on Mueller-Hinton agar. The results indicate that the colicin produced has an inhibitory effect against Staphylococcus sp. The inhibition zone varies from 1.3 to 2.5 cm, as presented in Table (1) and Figure (1). **SDS Page Analysis of Colicin Protein**

The presence of 130 kD bands in the supernatant after cell lysis with sonication confirmed the electrophoresis of total crude protein as shown in Figure (2).

Result of Docking of colicin protein

The docking results were analyzed and compared to predict the potential binding affinities of the colicin types against the FH VH at lipid Π protein.

Binding site detection results

The 3D spherical representation for pocket amino acids (red) is placed inside the proteins of interest, with the right-sided key amino acid positions highlighted in blue. Prediction of binding sites shows amino acids, as shown in Figures (3 and 4). In addition, there is a preference for collecting amino acid residues within a secondary structure. The residues of amino acids are shown in Figure (5).

Table 1: The result of the colicin inhibition against pathogenic bacteria.

No.	Strain number	*Staphylococcus sp.*
1	A5	1.3 cm
2	A6	1.9 cm
3	A8	1.9 cm
4	A9	1.8 cm
5	A10	2.5 cm
6	A11	1.8 cm
7	A13	1.5 cm
8	A17	1.9 cm
9	A18	1.9 cm
10	A23	1.5 cm

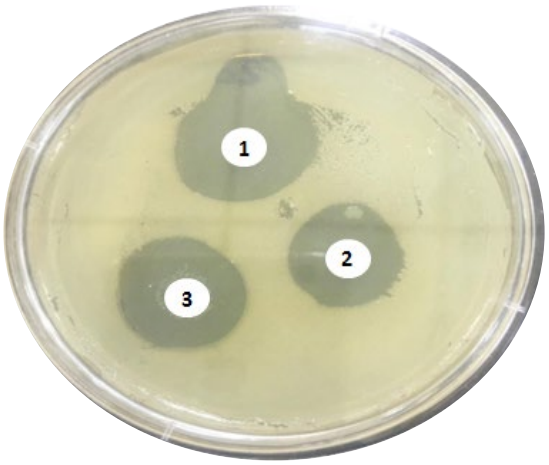

Fig 1: Inhibition zone of the colicin product against pathogenic bacteria (*Staphylococcus sp.)*.

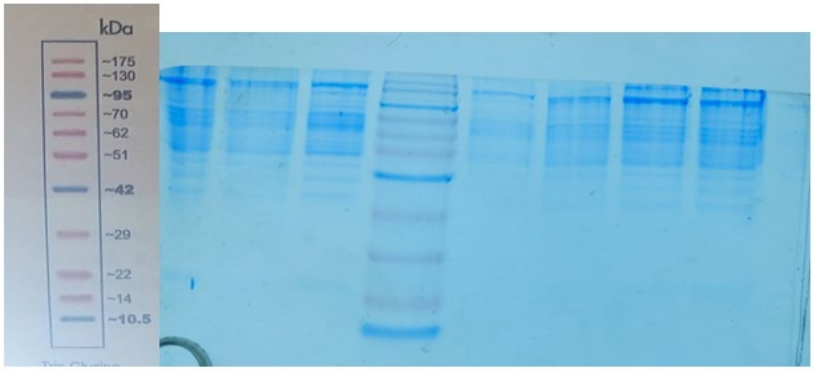

Figure 2. SDS Page Analysis of the Colicin Protein.

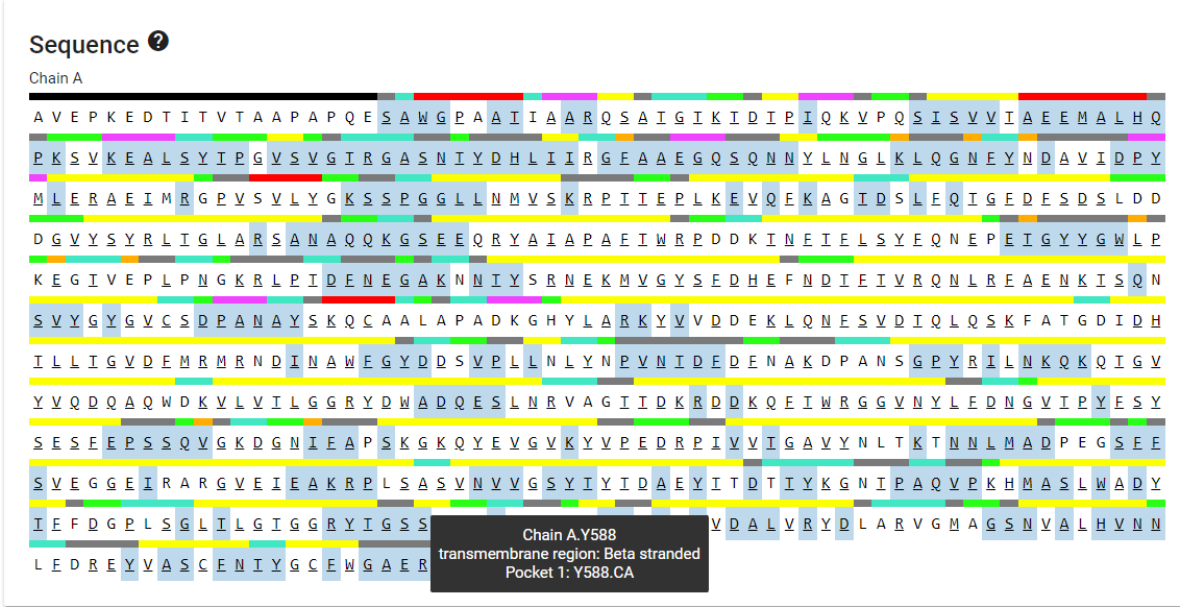

Fig: 3: Prediction of binding sites (red color) of interested proteins and amino acids involved in active site were predicted (blue color) .

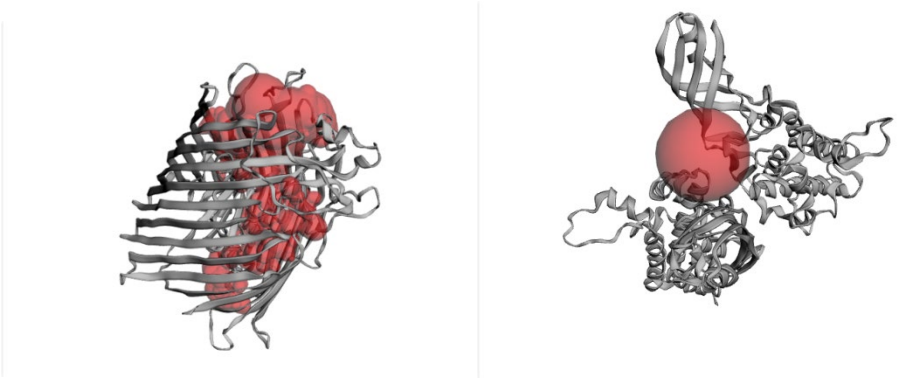

Fig : 4. Determination of active site amino acids of an interested protein using the CASTp server. The 3D spherical display highlights the large active sites in red and the amino acids in dark gray.

Fig 5. The legend of secondary structure (chain A and chain B).

Discussion

Bacteria produce bacteriocins, which can inhibit or kill closely related species, with colidins being the most well-characterized group among these bacteriocins. . E. coli and other members of the Enterobacteriaceae family generate these colidins, which are effective against them (1 , 2). At least 34 colicins have been identified, with 21 more studied. These unique properties are due to a group of carcinogenic bacteria producing colicin proteins under stressful conditions. The release of these colicins doesn't always lead to cell death, and the resulting colicins are protected.

Colicins are a class of antimicrobial proteins produced by *Escherichia coli (E. coli)* to suppress the growth of other *E.coli* strains and closely related bacterial species (3). The proteins have undergone evolutionary changes in order to provide a competitive advantage to the host bacterium in its interactions with closely related bacterial species (15). Many bacteria in the Enterobacteriacea family can make colicins. In fact, tests have shown that about 30% of *E.coli* isolates can make at least one type of colicin (16).

By looking at evolutionary origins through phylogenetic analysis, it is possible to tell whether E. coli isolates are pathogenic (17). *E. coli* isolates can be assigned to one of the four major phylogenetic groups A, B1, B2, and D, which contain seven subgroups based on phylogenetic studies (18). Previous research on the correlation between colicin production and virulence factors was restricted since it mostly examined UPEC isolates and found varying numbers of colicin and virulence genes (19)

The least commonly observed phenomenon involves the degradation process, which catalyzes the hydrolysis of the b-1,4 bond between N-acetyl glucosamine and N-acetylmuramic acid in the glycan backbone of the bacterial cell wall. Another method is to suppress the production of wall peptidoglycan or murein, which results in

the creation of spheroplasts and, ultimately, cell death. (2, 20).

Conflicts of interest

The authors declare that there is no conflict . of interest

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الفحص والمحاكاة الديناميكية الجزيئية لإنتاج الكوليسين عن طريق الإشريكية القولونية التعايشية المعزولة من الحيوانات

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الخلاصة

تم إخضاع سلالات الإشريكية القولونية التي تم عزلها سابقا من براز الحيوانات للكشف عن إنتاج الكوليسين. تم تحضير الكوليسين المنتج على شكل قرص لاستخدامه كمثبط لأنواع أخرى من البكتيريا على أكار مولر-هينتون. أشارت النتائج إلى أن مادة الكوليسين المنتج لها تأثير تثبيطي ضد المكورات العنقودية *Staphylococcus* sp. مع منطقة تثبيط تتراوح من 1.3 إلى 2.5 سم. نتيجة الترحيل الكهربي للبروتين الخام الكلي بعد تحلل الخلية باستخدام الموجات الصوتنة، أثبتت وجود حزم 130 كيلو دالتون. كما تم تحليل نتائج الالتحام الجزيئي (Docking) ومقارنتها للتنبؤ بالارتباطات الملزمة المحتملة لأنواع الكوليسين ضد FH VH في البروتين الدهني Π.

الكلمات المفتاحية: الكوليسين ، الاشيريشيا القولونية، تحليل الالتحام.