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Identification of Genes Encoding the Production of Bacteriocin from *Lactiplantibacillus plantarum* and its Activity against some Pathogenic Bacteria in Kirkuk City.

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Abstract

Our previous research results showed that through microscopic and biochemical detection of single pure colonies,we obtained 45 positive samples of lactic acid bacteria isolated from cow milk in Kirkuk City In addition 40 isolates of pathogenic bacteria (10 isolates each of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Escherichia coli*) were obtained from Kirkuk City Hospitals during the period from December to February 2021.The results were displayed subsequent to their comparison with the non-inoculated culture medium. The same extraction procedures were conducted on this medium to assess the inhibitory effects of both the initial(supernatant)and final(deposits) extraction steps against two isolates of each gram-positive and gram-negative pathogenic bacteria. Consequently, we conducted molecular studies to evaluate the effectiveness of *Lactobacillus* isolates against these pathogenic strains. Geneious Prime 2021version 2.2 software was utilized to identify genes associated with bacteriocin production, specifically PlnK, PlnN, PlnEF, and PlW.

1. Introduction:

Lactic acid bacteria is one of the most important groups of bacteria in the food industry that has been used for a long time in dairy products by humans all over the world. It was classified as the first class as " Generally recognized as safe" (GRAS) [1] because it is a non-pathogenic bacterium suitable for technological and industrial processes. In addition, it has characterized by its ability to tolerate bile acids and plays a role in the production of antimicrobial substance [2].

In the medical field, Lactobacillus Plantarum can be used to control the risk of cardiovascular and blood vessel diseases, cytokine production, and the production of many exopolysaccharides that have anticancer activity in addition to a number of juvenile diseases as well as to lower the level of cholesterol in fat tissue [3], [4], [5].

Despite producing substances likebacitracin, hydrogen peroxide, diacetyl fatty acids reutterin (3-hydroxypropionaldehyde) ethanol, [6] such bacteria exhibit antibacterial properties against food-borne and clinically important human pathogens [7]. Natural antimicrobial elements synthesized by lactic

acid bacteria (LAB) are generally inhibitory to pathogens and significantly impede the action of food spoilage organisms.Bacteriocins and other LAB metabolites have been commercially exploited for their antimicrobial properties and used in many applications in the dairy industry to prevent the growth of undesirable microorganisms [8]. Many strains of lactic acid bacteria related to food groups could produce bacteriocins or antibacterial proteins highly effective against foodborne pathogens such as *Staphylococcus aureus*, *fluorescens Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexneri*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, and Clostridium botulinum [9].

Therefore, this study was designed in order to use molecular means to identify bacteriocin producing genes in bacteriocin producing *Lactiplantibacillus plantarum* strains studded by (Fahem et al.,2021) [10].

2. Materials and Method:

2.1 Sample Collection and Isolation of Lactic Acid Bacteria:

All microorganisms(*lactobacillus* strains and pathogenic bacteria) obtained from [10].

2.2 Extraction and Partial Purification of Bacteriocin:

Bacteriocin was extracted using Chloroform and cooling centrifuge [11] and as follows:MRS Liquid medium Prepared (250 ml) in a 500ml flask and inoculated with bacteria isolated from milk and incubated for 24-48 hours at 37°C. Then, culture media was centrifuged using special tubes and by a cooling centrifuge at a speed of 7100 rpm at 12°C for 15 minutes. Supernatant was collected using a sterile pipette, the separation process was repeated a number of times and collected in a sterile flask. For this part, the activity tests conducted for both all the sediment parts after it was dried and ground into a powder form and dissolved in a certain volume of physiological saline to obtain a concentration of 100%. Chloroform (125 ml) was added to the supernatant and mix with the electric mixer for 20 minutes. The operation was repeated once others using a centrifuge at a speed of 10400 rpm for 20 minutes also tested the activity for this part.

2.3 Estimation of the Extracted Bacteriocin Activity:

The activity of bacteriocin was estimated by the well diffusion method using a Muller-Hinton agar according to the methodology cited by (Sgouras et al., 2004) [12].

2.4 Detection of Bacteriocin-Producing Genes in Lactic Acid Isolates:

The location of several bacteriocin-producing genes was investigated after the determination of DNA sequencing using software (Geneious prime 2021 2.2 software) by alignment DNA sequencing with gene sequencing for special bacteriocin (Bacterial Database BAGEL4). The detection of the location of bacteriocin-producing genes in the DNA sequence of isolates was followed by (Jafari et al., 2021) [13]. As well as drawing the neighborhood tree for genes.

3. Results and Discussion:

Bacteriocin generated from Lb Plantarum 17 and Lb. Plantarum 8 was effective against the Gram-positive bacteria (Staph.aureus and Staph.epidermidis) and gram-negative bacteria (Pseudomonas aeruginosa and E.coli) as it clear in [10], For the filtrate portion, the largest inhibition diameter was 20 mm and the lowest was 8 mm, while the residue portion displayed the highest inhibition of 20 mm and the lowest of 10 mm against gram positive bacteria and for gram- negative bacteria (9-21)mm. This was consistent with (Han et al.,203) [14].

Despite the fact that certain studies have shown that bacteriocin is ineffective against Gram- negative bacteria; the existence of this effect may set the stage for the use of this bacillus' extract in the pharmaceutical industry or as natural food additives.The reason could be that E. coli has a particular protein that protects it against bacteriocins produced by other types of bacteria [15]. Since bacteria that create bacteriocins have a specialized immunity against them because they contain genes that encode immune proteins and so assist them to resist bacteriocins

Moreover, the locations of a number of bacteriocin-producing genes in the isolates' DNA were examined, and the DNA sequence was established using a program (Geneious Prime 2021 2.2 software) by aligning the DNA sequence with the gene sequence of the Bacterial Database BAGEL4 (Figures 1, Figure 2, and Figure 3).

Within the genes of the Pln in Lactobacillus plantarum, DNA sequences are organized into 5–6 triggers to regulate several promoters in about 22–25 genes. The presence of dipeptide bacteriocins generated from Plantaricin A, which is encoded by two peptides (Plan A, Plan B), and Plantaricin W, which is encoded by Plan A and Plan B, suggests that they are in charge of numerous bacteriocins' gene expression [16] Additionally, numerous strains of Lb. plantarum include genes that produce the bacteriocin dipeptides Plantaricin JK, Plantaricin EF, and Plantaricin S. While Plantaricin EF and Plantaricin JK both contain peptides (pin E and pin F), and (pin K and pin J) respectively [17]. As a result, it has an alignment of the neighbor trees of the four genes, Lactobacillus plantarum WSAK1. As indicated in Table 1, the gene sequences in the isolate under research exhibit 99% similarity with genes isolated from other worldwide species with the use of a number of known gene sequences in the Genes Bank for Special Bacteriocin (Bacterial Database BAGEL4) database.

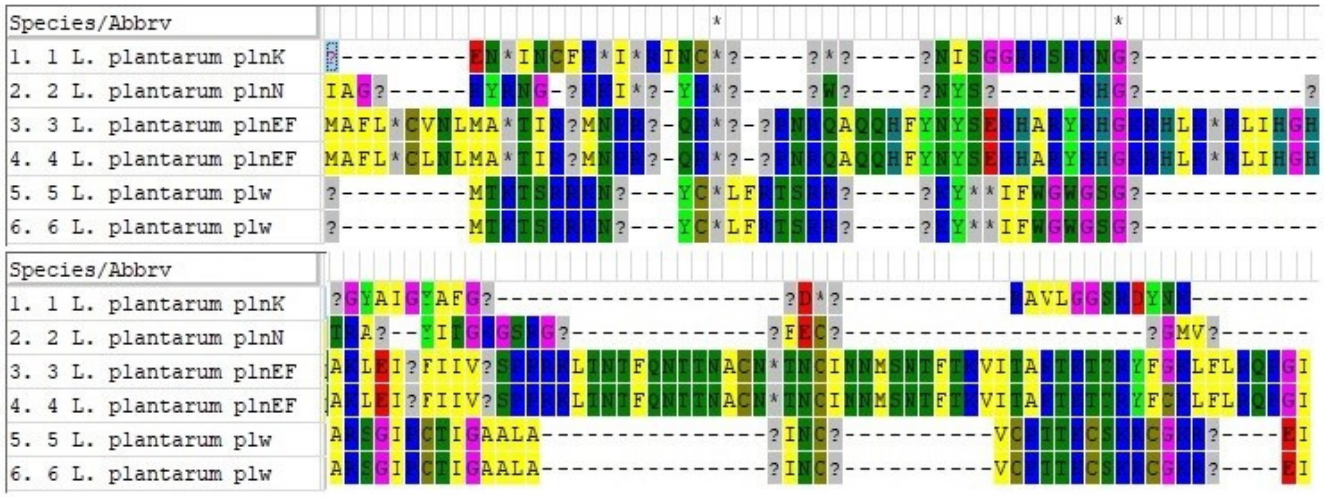

Figure 1. 1Alignment of the DNA sequences of the amino acids of the bacteriocin genesplnK, plnN, plnEF and plnW in bacteria *Lactobacillus plantarum* WSAK1

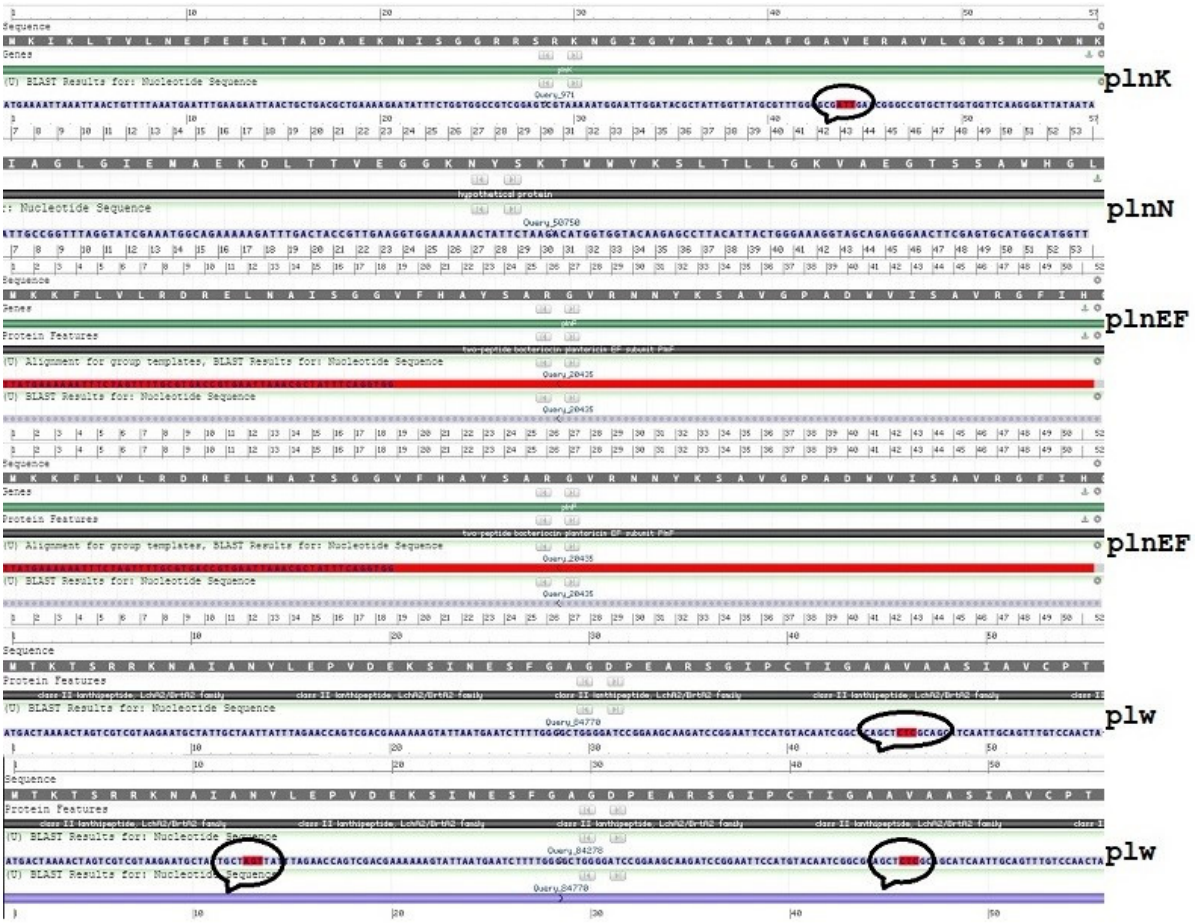

Figure 2. Alignment of DNA sequences of bacteriocin genesplnK, plnN, plnEF and plnW in bacteria *Lactobacillus plantarum* WSAK1.

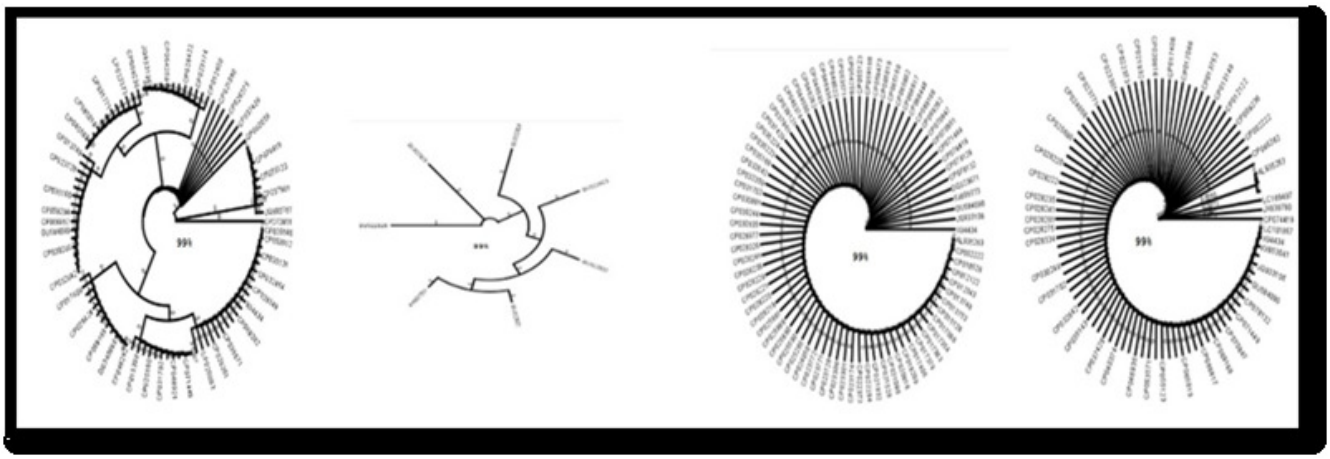

Figure 3. Neighbor tree of bacteriocin genes in isolation *Lactobacillus plantarum* WSAK1.

Table 1. Bacterial genes matching ratio Under study with bacteria genes in the database of the National Center for Biotechnology.

Identities	Sequence ID with compare	Gene	Amino acid change	Nucleotide	Location	Type of substitution	No.of sample
99%	ID:KF802197.1	plnK	Valine Isoleucine	G	166	Transition	1
99%	ID:GU584090.1	Pln	Glycine	G	3237	Translation	2
99%	ID:LC169496.1	PlnEF	Misc_feature	T	19	Translation	3
99%	ID:GU322926.1	PLW	Asparagine	A	72	Transition	4

4. Conclusions:

In conclusion, it was revealed that the most isolated strains of lactic acid bacteria by (Fahem et al.,2021) [10]. followed Lb plantarum with the plantaricin genes where they were responsible for producing plantaricin C bacteriocin. In addition, plantaricin C had inhibitory effect against anumber of bacteria such as S. aureus and other bacteria. So, the production of bacteriocin is a useful source of natural food preservatives to reduce the effect of chemical preservation. Since the increasing demand for products associated with health benefits has increased the research on underexplored foods and their potential as probiotic sources.

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Data Availability Statement: All of the data supporting the findings of the presented study are available from corresponding author on request.

Declarations:

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: The manuscript has not been published or submitted to another journal, nor is it under review.

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تشخيص الجينات المشفرة لانتاج البكتيريوسين من *Lactiplantibacillus plantarum* وتقييم فعاليتها ضد بعض البكتيريا المرضية في مدينة كركوك

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الخلاصة

أظهرت نتائج دراسة سابقة لنا الحصول على ٤٥ عينة موجبة من بكتريا حامض اللاكتيك التي عزلت من حليب الابقار في مدينة كركوك، وذلك بعد الاختبارات المجهرية والكيموحيوية للمستعمرات النقية المفردة اضافة الى الحصول على 40 عزلة من البكتيريا المرضية في مستشفيات مدينة كركوك للمدة من كانون الأول 2020 الى شهر شباط 2021 . وشملت 10 عزلات من كل *Staphylococcus aureus* ، *Staphylococcus epidermidis* ، *Pseudomonas aeruginosa* ، *E.coli* .تم تشخيص البكتريا المرضية بالاعتماد على الفحوصات المجهرية والكيموحيوية، كما أظهرت النتائج بعد مقارنتها مع الوسط الزرعي غير الملقح والذي اجري عليه نفس الإجراءات الخاصة بطرائق الاستخلاص حيث ان تاثيرات مثبطة لنمو بكتيريا الاختبار لكل من الراشح الاولي والراسب النهائي ضمن خطوات التنقية الجزئية للبكتيريوسين من عزلات بكتيريا حامض اللاكتيك على كل من نوعين لكل من البكتيريا المرضية الموجبة والسالبة لصبغة كرام لذلك تم اختيار عزلات *Lactobacillus* ذات الفعالية الاكثر ضد العزلات المرضية من خلال استخدام (*Software* 2.2 2021 *prime geneous*) للكشف عن الجينات المسؤولة عن انتاج البكتريوسين اذ امكن الحصول على الجينات المشفرة لانتاج البكتيريوسين *PlnK* و *PlnN* و *PlnEF* و *PlW* .

الكلمات الدالة: بكتيريا حامض اللاكتيك ؛البكتيريوسين ؛ *Lactiplantibacillus plantarum* ؛ كركوك.

التمويل: لايوجد.

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول.

اقرارات:

تضارب المصالح: يقر المؤلفون أنه ليس لديهم تضارب في المصالح.

الموافقة الأخلاقية: لم يتم نشر المخطوطة أو تقديمها لمجلة أخرى، كما أنها ليست قيد المراجعة.