Original Article

Purification of the biologically active peptide from a new strain of *Lactobacillus delbrueckii JKD5* isolated from local yogurt and study of its antimicrobial properties against some microorganisms

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Abstract

Organisms produce a large variety of biologically active peptides and have high inhibitory activity against other organisms that cause spoilage and spoilage of many foods. *Lactic acid* bacteria are considered one of the most important single-celled organisms that are characterized by their ability to produce biologically active peptides. They are of great importance because they are useful and widespread. Most types of lactic acid bacteria can produce bioactive peptides that inhibit peptides bactericidal bioactive bacteria (bacteriocins), which can control the growth of pathogenic bacteria and the formation of (biofilm), this mechanism by these bacteria not only generates the free amino acids needed by bacteria but also It produces a variety of peptides, some of which have biological activities. These are called "bioactive peptides (BAPs) and are interesting from a nutrition and health care perspective, as they have many functions such as antimicrobial antioxidant, anticholesterol and anti-hypertension. Their activities vary according to the strain. Bacteria in terms of containing proteinase enzymes, which leads to a large variety of proteolytic activities. Through this study, *Lactobacillus delbrueckii* JKD5 bacteria were isolated from local yogurt in the city of Basrah in southern Iraq. It was isolated and purified under anaerobic conditions, its morphology was studied and biochemical tests were conducted for it. It was genetically diagnosed and registered in the NCBI Gene Bank with the number OM278543.1 or original Purely under the name *Lactobacillus delbrueckii* JKD5 .Partial purification was carried out by precipitation with ammonium sulphate at 75% saturation, centrifugation at 10000 rpm for 10 minutes, then the bacterial filtrate was taken, then membrane osmosis was carried out using dialysis bags, and the peptides were extracted and purified by HPLC and ÄKTA pure devices to reach the total purification. A characterization study was conducted for the produced peptide and it showed that it has a molecular weight of 6.5 kDa and was stable at 60 and 80 °C for 60 minutes, which indicates that the peptides can withstand high temperatures in the sterilization process, which is one of the very important processes in food production. It retains its effectiveness at pH 3 and 5. The activity of the peptide produced from the antibacterial isolate JKD5 was characterized and examined against two types of bacterial strains, one positive and the other negative for Gram stain. They are *Bacillus cereus* and *Escherichia coli*.

Keywords: biologically active peptide, *Lactobacillus delbrueckii*, yogurt, purification.

Introduction

The addition of starters is a safe way to protect food from pathogenic bacteria, increase storage time by inhibiting spoilage microorganisms or undesirable biological reactions, and in addition to extending shelf life,

lactic acid enhances nutritional value and adds flavour and texture to food (Gillliland, 1990)). The late last century and the beginning of this century witnessed a clear development in the methods of treatment and food preservation, especially the use of microorganisms and their important metabolic products. Where the attention of many people, especially lactic acid bacteria was drawn to its beneficial effect and to meet the market need for healthy products in addition to its high inhibitory effectiveness against types of pathogenic bacteria and bacteria that cause food spoilage, and this important effect is due to the ability of bacteria to produce metabolites through the fermentation process and its production of lactic acid, hydrogen peroxide and acetic acid, as well as bacteriocins with beneficial inhibitory effects in the host (Dinev *et al*., 2018)). Lactic acid bacteria produce through their pathways a wide range of metabolites, some of these metabolites play an important role in inhibiting the growth of many bacteria that cause many diseases as well as many microorganisms that cause food spoilage during storage. It exceeds the use of conventional chemical preservatives (Abo-Amer *et al*., 2007; *Cintas et al*., 1998; Zottola *et al.*, 1994). Lactic acid peptides are low-molecular-weight protein compounds that have an anti-bacterial activity such as food spoilage bacteria and foodborne pathogenic bacteria, gram-positive including sporophyte-forming, and also extends the range of some of them to include gram-negative bacteria (Hanlin *et al.*, 1993; Riley, 1998). Inhibitory means peptides, i.e. a group of microorganisms that peptides can influence and inhibit. Most of the peptides have limited inhibitory action, as their effect is limited to bacteria closely related to the bacteria that produce them. Based on this, this statement applies to peptides of gram-negative bacteria more than peptides of gram-positive bacteria (Hardy, 1986), especially lactic acid peptides, where most of them are characterized by a broad inhibitory action that extends to include unrelated bacteria from gram-positive bacteria and pathogenic or pathogenic bacteria Food spoilage and sometimes also Gram-negative bacteria (Da Silva *et al*., 2022). Access (2000) Serkedjieva *et al*. Isolation and characterization of a new antiviral substance from *Lactobacillus delbeueckii*, the inhibitor of which complies with the criteria of bacteriocins, and was effective against species of lactic acid bacteria and several food-borne pathogens.

Hassan *et al*. (2020) From isolate of *Lactobacillus spp*. From Pakistani yogurt, about 50 samples were identified including 12 strains of lactic acid bacteria that were isolated and tested for their antimicrobial activity.

The use of peptides remains a matter of great importance in food preservation in terms of providing food safety, ensuring the quality of the food supplied and extending the shelf life (Perpetuini *et al*., 2020). It can also be used in different applied fields such as controlling feed contamination and environmental pollution with waste (Laukova *et al*, 2000).

The main objective of this study was to examine the antimicrobial components produced by *Lactobacillus delbeueckii* and isolate the peptide produced by it with antibacterial activity, in addition to studying the optimum pH of peptide stability and thermal stability, which are important aspects in food production through thermal and sterilization processes to which food is exposed to.

Materials and working methods

Bacteria isolation

Dairy products were used to isolate bacteria, represented by braids cheese, soft cheese and yogurt. The isolates were grown on a liquid MRS medium, then they were grown in a solid MRS medium, by planning method, for 48 hours, at 37 °C, and under anaerobic conditions (Harrigan *et al*., 1976).

1- Bacterial diagnosis

Phenotypic, microscopic and biochemical examinations were carried out according to what was mentioned in Bergey's Manual for the year (Garrity *et al*., 2005).

2- Preliminary screening of the isolates based on the inhibitory action of the metabolites of *Lactobacillus spp*.

The good diffusion method mentioned by Gasu et al. (2018) and followed by (Al-Seraih *et al*., 2017) to detect the inhibitory activity of lactic acid bacteria, as isolated bacteria were activated on MRS liquid medium at a temperature of 37 °C for 24 hours under anaerobic conditions, and food spoilage bacteria were also activated. *E. coli* and *B. cereus* 24 hours before the start of the inhibitory action, after that all isolated bacteria cells were separated from the growth medium using a centrifuge at 4 ° C at a speed of 10000 rpm, then the biomass was neglected and the filtrate containing the products was used metabolically.

The sifting process was carried out on a Mueller Hinton Agar medium, where 0.1 ml of food spoilage bacteria was transferred to the medium and spread by a glass diffuser (L-shaped). Then holes were made using a 6 mm diameter cork auger and the holes were filled with the prepared bacterial filtrate of 0.05 ml, then the dishes were placed in the refrigerator for 2 hours and then placed in the incubator for 24 hours at a temperature of 37° C. After incubation, the diameter of the formed and growth-free areola was measured and compared with the inoculum-free control treatment.

3- Diagnosis of the genetically selected isolate by 16SrRNA test

The bacterial isolate was selected based on its high yield of peptides and its ability to inhibit some pathogenic microorganisms to perform a 16SrRNA test to confirm its resistance to *Lactobacillus delbrueckii* using the primer AGAGTTTGATCCTGGCTCAG-3'-5'Fand GGTTACCTTGTTACGACTT. 94°C for 3 minutes thereafter followed by 35 cycles of denting at 94°C for 45 seconds, annealing at 56°C for 45 seconds, extension at 72°C for 1 minute, then one cycle of final extension at 72°C for 7 minutes. After performing the electrophoresis of the PCR samples, the genetic sequencing process was carried out in Korea in the technology laboratory at the National Instrumentation Center for Environmental Management (NICEM) and the type of bacterial isolate was determined after conducting the research using the (Basic Local Alignment Search Tool BLAST) program. Available at the National Center for Biotechnology Information (NCBI) at www.ncbi.nlm.gov/nucleotide and the Bio ID program (Tamura *et al*., 2011).

5 - Extraction and purification of peptides for selected bacterial isolates

5-1 precipitation with ammonium sulfate

After growing the selected bacterial isolates in a liquid MRS medium for 24 hours, at a temperature of 37 °C and under anaerobic conditions, the purification process was carried out according to the mentioned method by Levieux *et al*. (2006) to precipitate proteins from the bacterial filtrate by adding 27 gm of ammonium sulphate (NH4)2SO4 per 50 ml of the filtrate for 24 hours with stirring with the help of magnetic stirrer to obtain a saturation percentage of 75%. Then centrifugation was carried out at 10,000 rpm for 10 minutes, then the bacterial filtrate was taken.

5-2 Determination of total protein concentration (Buriate reagent)

The total protein in the bacterial filtrate was measured using the Biuret kit supplied by the French company BIOLABO and by following the steps indicated by the company, where the absorbance was measured at a wavelength of 550 nm and the following equation was applied in (Wotton, 1964):

$$\text{Total protein g/100 ml} = \frac{\text{Sample absorbance}}{\text{absorbance of standard solution}} \times \text{Standard solution concentration}$$

5-3 Membranous exudation

Membrane exudation was performed according to the method described by Brewer *et al*. (1974) and the method presented by Al-Hatim *et al*. (2020) for 18 hours and replaced the solution every 6 hours to increase separation efficiency and to get rid of ammonium sulphate.

6- Secondary screening based on HPLC technology

HPLC Diagnostics

According to the method mentioned by Pritchard (2012), the isolation was prepared according to the methods mentioned in the previous paragraphs from the sedimentation process, centrifugation, dialysis to lyophilization. The filtrate was prepared by performing a filtration process using Millipore with a volume of 0.45 µl and then injected with an HPLC device. Vydac C18®, Protein and Peptide. The separation conditions of the device are as follows: a flow rate of 1 ml/min, a wavelength of 220 nm, a flow rate of 0.5 ml/min, a column temperature of 40 ° C, and an operating time of 20 minutes. The solvents used are as follows:

The first solvent: acetonitrile-water TFA with proportions of 5%, 95% and 0.1%, respectively

The second solvent: acetonitrile, water as well as TFA in the proportions 95%, 5% and 0.1%, respectively.

The mixing ratios were as follows: the first solvent was 80%, and the second solvent was 20%, according to the method used in Anonymous, 2019)).

7- Gel filtrations with ÄKTA pure 25. technology

The total purification process was carried out by separating and purifying the peptides obtained from the lyophilized bacterial filtrate, as the sample prepared the most inhibiting action and the highest peak after being injected with HPLC and injected with gel filtration technique using ÄKTA Pure 25 device, based on the method mentioned by Ling et al. (2018) and featured by Yang et al. (2020) with some modifications. A Superdex TM peptide 10/300 GL column with a diameter of 10 mm and a volume of 23.562 ml filled with agarose gel and Dextran and with dimensions (10 × 30) mm was used. 0.5 of the prepared sample was injected into the column after it was filtered using a filter with a diameter of 0.22 mm micron and the column was equilibrated with a buffer sodium phosphate solution prepared at a ratio of 0.15 M and pH 7. The conditions of the purification and separation process were fixed under a pressure of 1.3 MPa and a flow rate of 0.5 ml/min the separated peaks at a wavelength of 220 nm were followed up through the chromatograph

that appears on the computer screen. Then, the resulting peaks were collected after each separation process at a rate of 2 ml/part through the F9-R fraction collector, then the peptides obtained from the isolation were lyophilized. The most efficient product was produced by Freeze- a drying device and kept in the refrigerator for testing.

8- Characterization of the product peptide

8-1 Determination of the molecular weight of a peptide by electrophoresis

Molecular weight estimation was carried out in the laboratories of the Ministry of Science and Technology, Department of Environment and Water, Food Contamination Research Center, and the electrophoresis method was used in a polyacrylamide gel with a concentration of 15%, following the method mentioned by Blum *et al*., 1987; Laemmli, 1970 (in the presence of SDS) Polyacrylamide Gel Electrophoresis to estimate the molecular weight of the peptide produced by lactic acid bacteria, as 100 µl of the sample solution was added to the gel in the tubes, then the storage solution was placed so that it covered the surface of the tubes, and an electric current was delivered with a power of 2 mA, for each tube. In the stacking phase for 30 minutes and 5 mA/tube (with a potential difference of 240V) in the separation phase for 4-5 hours with cooling at 4°C, then remove the gel from the tubes and immerse in the dye fixing solution for 30 minutes and then wash with distilled water For 10 minutes several alterations were made with distilled water, after which the gel was taken out and washed in distilled water 3-4 times for 1-2 minutes and a Gel documentary system was used to photograph and see the firmness after the migration process was completed. Then the molecular weight of the separated peptides was determined and determined the beams were measured by measuring the distance traveled by the dye before dyeing and the distance traveled by the beams after dyeing. The relative movement was calculated according to the following equation:

Relative movement RF = distance traveled by beams(cm)/distance traveled by dye (cm)

8-2 Study of the inhibitory action of the produced peptide

The Well diffusion method followed by (NCCLS, 2002) and mentioned by (Al-Seraih *et al*., 2017) was used with some changes, as the dishes containing Mueller Hinton Agar were inoculated in the activated liquid bacterial culture at 24 hours and 0.1 diffusion ml of the bacterial suspension mediated by the bacterial vector with a vaccine size of CFU/ml 1 x 108 and holes were made using a 6 mm diameter cork borer and the holes were filled, and the incubation was done at 37°C for 18 hours. The inhibitory activity of the peptide was studied by measuring the diameter of the inhibitory areola around the growth pits and comparing it with the vaccine-free control treatment.

8-3 Determination of the optimum pH for the stability of the produced peptide

1 ml of the purified peptide from the gel filtration step was incubated with 1 ml of buffer solutions of different pH in a range of (3-11) and with a concentration of 0.1 mo. Sodium acetate buffer solution of pH 3 and 5 and phosphate buffer solution was used. Sodium pH 7, while Tris-HCl buffer solution pH 9 and 11 was used in a water bath at 37 °C for 60 minutes, then the tubes were transferred to an ice bath and the inhibitory activity of the produced peptide was estimated against *Bacillus cereus* and *E. coli* based on residual activity (%), then plotted the relationship between different pH values against residual activity (%) to determine the optimal pH for the stability of the peptide. According to the method used by (Osmanağaoğlu *et al*., 1998)**.**

8-4 Set the optimum stability temperature for the product peptide

1 ml of the purified peptide from the gel filtration step was incubated at different temperatures (60, 80, 100, 120) Ċ for 60 minutes in a water bath and then the tubes were directly cooled in an ice bath and the inhibitory activity of the produced peptide was estimated against both *Bacillus cereus* and *E. coli* based on the residual activity (%), then the relationship between the different temperature values and the residual activity (%) was plotted to determine the optimum temperature for the stability of the peptide.

Results and discussion

The results showed that the sources of isolated lactic acid bacteria that were grown using selective medium MRS Broth that were isolated from dairy products belonged to the lactic acid bacteria. The acid-producing colonies were captured and indicated by the formation of a clear halo around the colonies due to the dissolution of calcium carbonate. The colonies of lactic acid bacteria appeared on the MRS solid medium through the initial diagnosis as being circular-convex, while others were flat with equal ends, smooth and shiny. As for their colour, the colonies were cream in colour. It can be said that the isolates belong to the genus *Lactobacillus spp*. It agrees with what was mentioned by researchers Holt and Krieg, (1986), according to what was mentioned in Bergey's Manual of 1986. All isolates appeared their cells varied in shape, as some of them were rods, while others were spherical, and some were single, some were bilateral, and others were in the form of long chains. Or short adhesive, most of which are opaque. In addition, it is gram-positive, non-sporulating and immobile, and this corresponds to what was mentioned (Harley and Prescott 2002; Buchanan and Gibbons 1974). Microscopy results are in agreement with Gutiérrez-Cortés *et al*. (2017) When conducting biochemical tests for bacterial isolates, it was found that all isolates do not produce catalase due to their inability to produce the enzyme catalase, which converts hydrogen peroxide into water and oxygen (Baron and Feingold, 1994). With what was mentioned (Atlas *et al*., 1995) and non-producing ammonia because the orange colour of the medium did not change to violet, which indicates the inability of bacteria to produce ammonia from arginine. On the liberation of ammonia from arginine. This indicates the absence of a bacteria with forced heterogeneous fermentation, *L. reureri*, which is one of the bacteria associated with lactic acid bacteria that inhabit the intestinal lumen. The inability of bacteria to produce the enzyme tryptophanase that breaks down the amino acid tryptophan and to produce indole agreed with what was stated (Murray *et al*., 2007). And it is not reducing nitrate because the tubes are not coloured red, which indicates the inability of bacteria to reduce nitrate to nitrite. The ability of bacteria to change the colour of the index from purple to yellow is caused by a decrease in the pH from 6.5 to 4.8, and this is consistent with what was stated in (Holt et al., 1994). The results agreed with (Gammoh *et al*., 2020) in the physiological and biological characterization of *Lactobacillus delbrueckii subsp*. All isolates obtained were lactic acid bacteria according to Bergey's guide, 2009 (Garrity, 2005)). Also, some isolates showed the ability to ferment different types of sugars.

Confirmation of the isolation diagnosis by 16S rRNA

DNA extraction of lactic acid bacteria After conducting the previous tests and choosing the most productive isolate of lactic acid JKD5 according to the sifting in the HPLC device, the diagnosis of the isolation based on the type was confirmed by molecular diagnosis, as the technique of relaying DNA bundles to isolate bacteria on agarose gel 1.5% showed that the process of The extraction was good and the beams appeared with a high degree of clarity as shown in Figure (1).

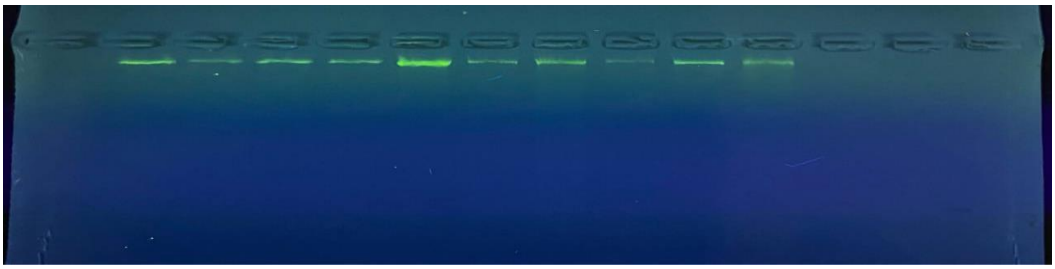

Figure (1) Electrophoresis using 1% agarose gel at a rate of 5 volume/cm for 1:15 hours to extract DNA from bacteria

Amplifying of the 16S rDNA gene by (PCR) Technique. by using a universal primer, results have obtained the required band of 16SrDNA for each isolate with along electrophoresed ladder in the region of 1500bp (fig. 2).

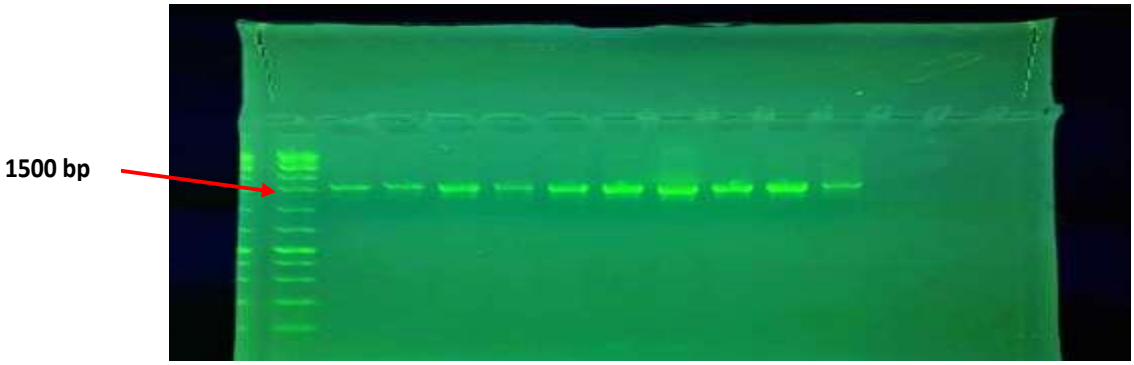

Fig. (2): Agarose gel electrophoresis of 16S rRNA gene with 100bp DNA ladder, showing a single amplification band of 1500 bp for isolates.

16S rRNA sequences were used to determine the diversity of the *Lactobacillus* strain as well as detection about 16S rRNA and the information related to *Lactobacillus* spp. for precisely identification, this strain was registered new strains due to genetic identification dependent on 16SrRNA. According to information available, after alignment with other 16SrRNA sequences in Gene Bank, they showed a high degree of similarity to references strains. The similarity of bacteria isolated from local yogurt was 94% due to bacteria.

The figures (3,4) shows the sequence of nitrogen bases sequence in a gene of 16S rRNA of JKD_5. It turned out to be a *Lactobacillus delbrueckii* and registered in the NCBI Gene Bank with the accession number OM278543.1 and became under the name *Lactobacillus delbrueckii* JKD5 on the website https://www.ncbi.nlm.nih.gov/nuccore/OM278543.

In addition to the isolate JKD_5 which had identity of 94% with the reference strain *Lactobacillus delbrueckii*, which led us to suggest the probability of obtained new strain.

ATCGTTTTAAGGGCAATTGAGAACATGCAGTCGTACGCTTCTTTCATCCCGAGTGCTTGCTTAC
AAACTCTCGAGGAGTGGCGGACGGGGGGGTCACAGCCGGGGAACGTATCCATCGGAGGGGG
ATGATCTTGGAAGCAAGCTATAATACGGCATATGAGTTTATGCCGCAGCCTCAATACTGACAG
GGGCTAGCTTGAAGAGAATTGCATGACCTCCCGGGCTAGTTAGCTAGTGTGCTTCCCATTGTA
GCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGACTTGCCGTCGTCCCCACCTTCCTCCGG
GTTGTCACCGGCAGTCTCATTAGACTGCCCGACTTCATGCTGGCTACTAATAATAAGGGTTGC
GCTCGGTGCGGGACTTAACGCAGCATCTCACGACACGAGCTGACGACAGCCATGCACCACCT
GTCACTTTGTCACTGAAGGTACCCCGATGTCTTCTAAGCAGAAAGACACTGTTAAGTCCGTGC
CAGCTTCTGCGCTAATACGCGGATTGCACGACTTGCCCGACTTTATTGGGCGCACAGCGTGAA
CACCTTTGAGCTTAAGTCTGATGTGAAAGCTCCCGCTCAACCGGGTTGGGTCATTGGAAACTG
GGATACTGGAGTGAACCCACGAGAGCTGAACTCTATGTGTTGCGGTGAAGTACGAAGATAGA
TGGAGAACCAGTATTCGCGAAGCCTTTCTATCGTCTGTAACTGACGCTGACGAGAGAAAGCCT
TCGCGCAACAGTACTAGATAATCTGGTAGTCTACGCCGTAAACGATGAGTGCTCAGTGTCCTC
TGTGCACTCCTTCAGCGCTGTTGCAAACGCATTACGCAGTCGAGCTGGGGGTACCACAGCAGC
TGAAAAACAAGTATTGACGTTACCCGCAAAACGCGGACATGTGGTAACTCGAGTACCGAATA
CTGACATGCTGCATCCTTTACACTCAAAACAGGAGCTACCTTAGTACAACGTCATGTCTTCTA
GACTACGAGTCCGATCGGAGACTTCGTTAAGTCGGGATCGTGGACGATATCTCCATTGCCAAA
TACTTAGCCTCTCTCGAAGAGCGTGACAACTGA

Fig. 3 The **Sequencing of *Lactobacillus delbrueckii* JKD5**

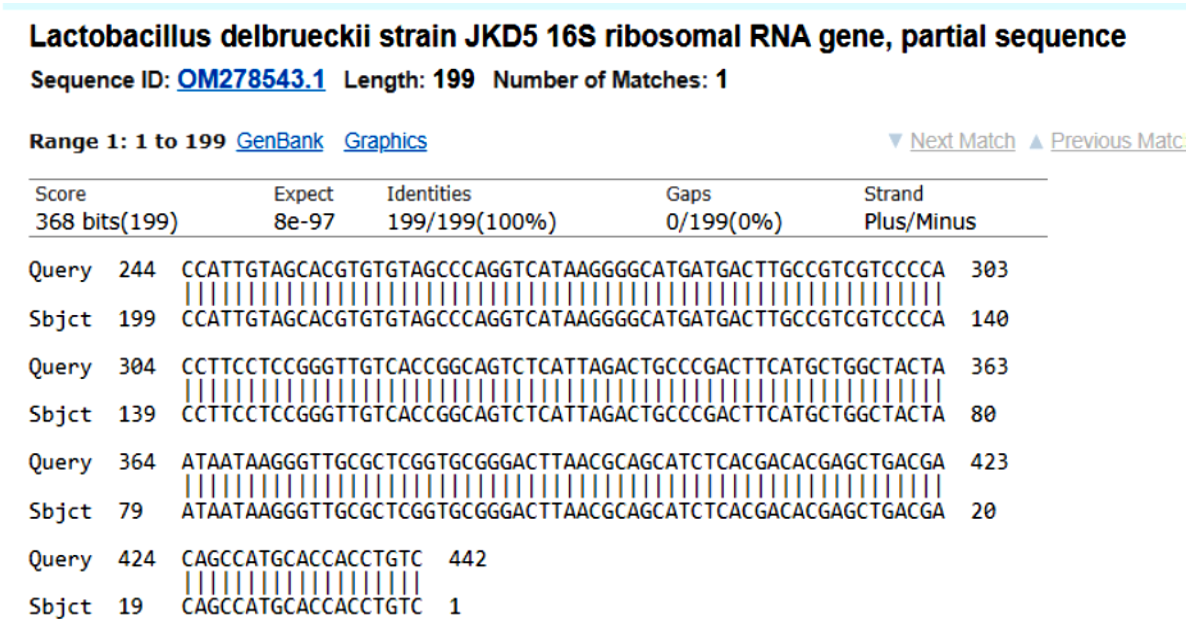

Figure 4: Blasting identifying result from NCBI of *Lactobacillus delbrueckii* JKD5

HPLC Diagnostics

Diagnostic results using HPLC technology. The concentration of the most effective sample is inhibited by the appearance of peaks. The isolate JKD5 had the highest concentration of 53988200 at a holding time of

4.50 minutes, knowing that it is isolated from local yogurt sources. As shown in Figure No. (5) the results of the diagnosis by HPLC device for the most productive isolate of JKD_5 peptide.

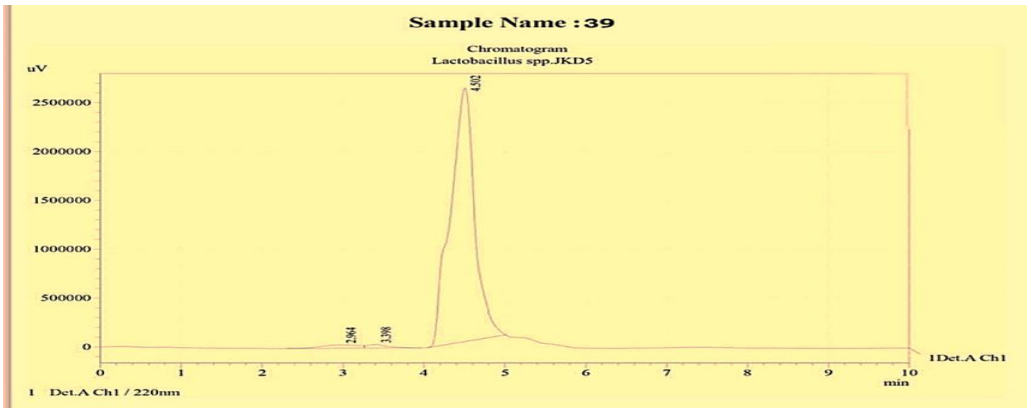

Figure No. (5) Diagnostic results by HPLC device for the most productive isolate of JKD_5

Wu *et al*. (2019) of the isolation of ACE-inhibiting peptides from milk fermented with *Lactobacillus delbrueckii* QS306. The peptide was purified using an HPLC column. Serkedjieva *et al*. (2000) using HPLC C18 on a single vertex of *delbrueckii* bacteria. Pingitore *et al*. (2007), used the same conditions to purify antimicrobial peptides from lactic acid bacteria extracted and purified from saliva.

Gel filtration with ÄKTA pure 25

Figure (6) indicates the separation and purification of peptides using gel filtration technology in ÄKTA pure 25 devices for partially purified bacterial filtrate of JKD5 isolate and isolated from local yogurt after this isolate appeared the most efficient, most inhibitory and highest peak when injected with HPLC, and the results indicated the emergence of Five clear peaks represented the peptides produced from the above isolation, as the second peak was chosen, it gave the highest enzymatic activity at a retention time of 18 minutes.

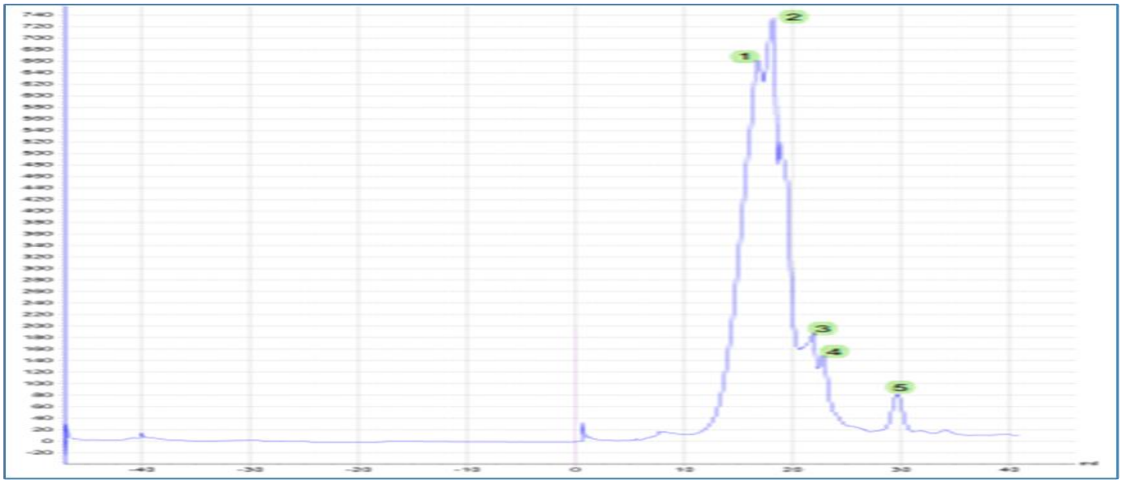

Figure 6. Gel filtration profile of a peptide prepared from the bacterial filtrate of JKD5 lactic acid bacteria using the ÄKTA Pure 25 apparatus.

The result was similar to the study of Al-Sahlany *et al*. (2020) Where study showed that the purification of the biologically active peptide produced from the yeast Saccharomyces cerevisiae has a molecular weight of 9.77 kDa and exerts an inhibitory activity against Gram-negative and Gram-positive bacteria,

The results were close to what was indicated by Alkotaini *et al*. (2013) purified using Sephadex G-25 gel filtration chromatography. Peaks were collected and concentrated using lyophilized, then tested for antimicrobial activity.

Zhang *et al*. (2013) by using gel filtration technology and a Sephadex-G15 column to isolate 6 peaks of pure peptides from cow's milk whey proteolytic at a wavelength of 220 nm with high antioxidant activity.

Characterization of the product peptide

Molecular Weight Diagnostics

Figure No. (7) shows the standard curve for the determination of the molecular weight of peptides separated from isolated JKD5 by gel filtration technique upon electrophoresis on acrylamide gel in the presence of SDS. It was noted that the molecular weight of the peptide obtained from isolate JKD5 was 6.5 kDa.

The results were similar to Niamah (2018), as it obtained a molecular weight of the produced pediocin, which was 6094.8 Daltons. The results are close to that of Qian *et al*. (2011) who were able to obtain 6 peptide peaks with high inhibitory activity and different molecular weights from skim milk fermented with *Lactobacillus delbrueckii ssp*. LB340. Obtained through the study of Al-Sahlany *et al*. (2020) on peptides from yeast *Saccharomyces cerevisiae*, where the study showed that the purification of the biologically active peptide produced from yeast has a molecular weight of 9.77 kDa and exerts an inhibitory activity against Gram-negative and Gram-positive bacteria. Find *Serkedjieva et al*. (2000) indicated that the purified molecular weight of *Lactobacillus delbrueckii* was 6 kDa.

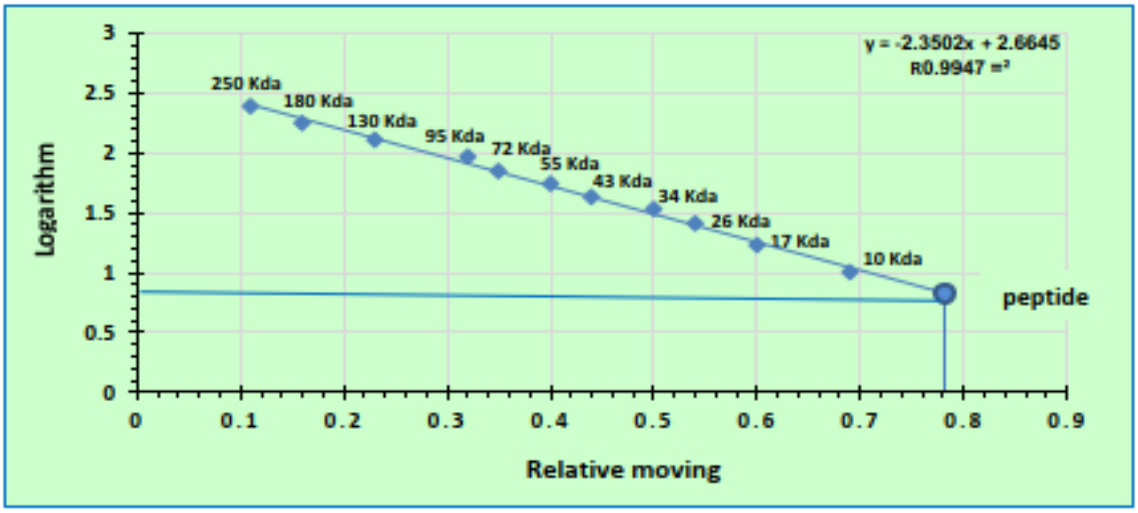

Figure (7) Standard curve for molecular weight determination of peptides separated from isolated JKD5 upon electrophoresis on acrylamide gel in presence of SDS.

Through Figure No. (8) it can be seen that one bundle of peptides was obtained from local yogurt, because the results are close, and the molecular weight was 6.5 k Da.

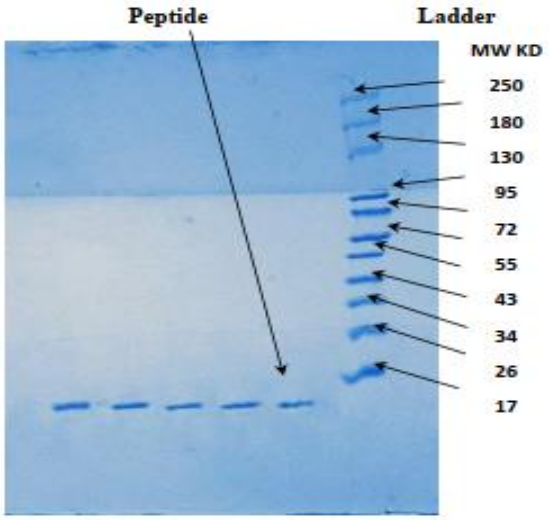

Figure (8) Electrophoresis on acrylamide gel in the presence of SDS

Determination of the optimum pH for the stability of the product peptide

Figure (9) shows the stability of the JKD5 peptide towards different pH ranging from (3-11) by calculating its residual inhibitory activity (%) of the peptide activity and calculating its residual inhibitory activity as a percentage of the peptide's activity as a control treatment against test B bacteria. Cereus. Figure No. (10) shows the stability of the peptide produced from the isolate of yogurt JKD5 towards different pH ranging from (3-11) by calculating its residual inhibitory activity as a percentage of the effectiveness of the peptide as a control treatment against the *E. coli* test bacteria. It can be seen from the figure that the peptide produced in this study was characterized by high resistance to acidic pH, as it retained its activity at pH 3 and 5. The activity at pH 3 of the test bacteria B. cereus reached 240 units/ml and the remaining effectiveness was 75%, while At the test *E. coli* bacteria for the same pH reached 280 units/ml and the remaining effectiveness was 82.35%, while at the pH 5 it reached 200 units/ml and the remaining effectiveness was 62.5% against the test bacteria B. cereus, as for the test *E.coli* bacteria The effectiveness reached 180 units/ml, and the remaining 52.94% was that its effectiveness began to gradually decrease when treated with pH 7 and more, and the activity was in the test bacterium B. cereus. With pH 7, 9 and 11, it was (180, 120 and 40) units/ml, and the remaining yield was (56.25, 37.5 and 12.5) %, respectively. As for the effectiveness of the same pH of the test bacteria, *E. coli* was (120, 80 and 0) units/ml and the remaining yield was (35.29, 23.52 and 0) %, respectively. There was no sense of any activity of the peptide at pH 11 against the test *E.coli* bacteria. This means that the peptide produced from the yogurt isolate JKD5 in this study is highly stable towards acidic pH but loses a high percentage of its activity at neutral and basal pH.

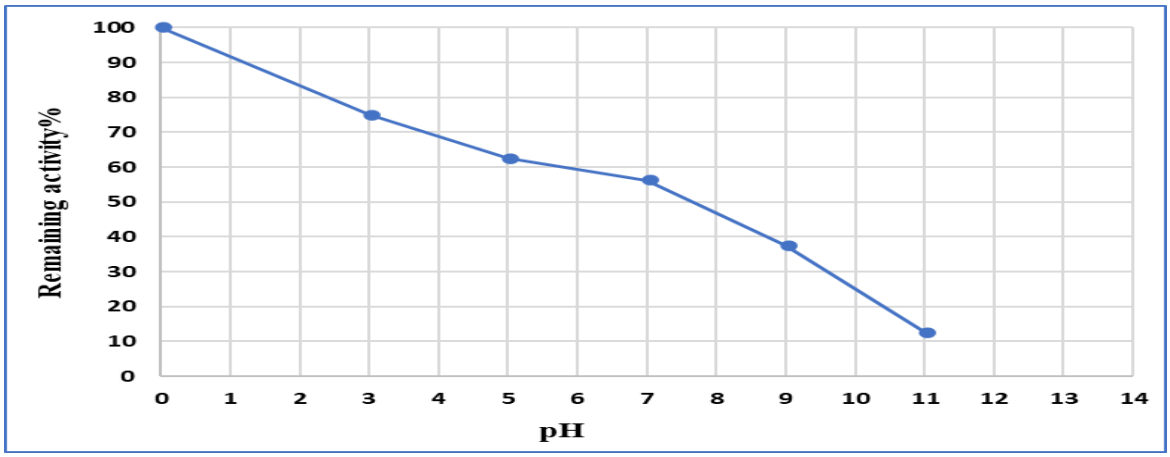

Figure No. (9) The optimum pH of the peptide produced from isolated JKD5 against the test bacterium *B. cereus.*

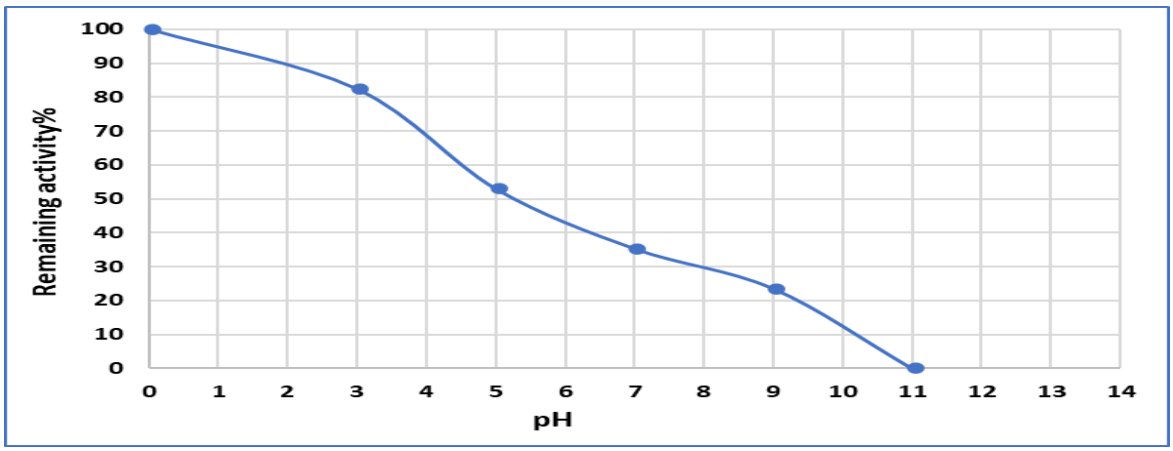

Figure No. (10) The optimum pH of the peptide produced from isolated JKD5 against test *E. coli* bacteria.

The results reached by the researchers regarding the resistance of peptides to pH varied, and the results were close to what was found by Laukova *et al*. (1998) that the bacteriocin produced by *Ent. faecium V24 bacteria* retained its full activity at pH between (4-7).

The results were similar to Qahar (2004) that the produced bacteriocin was characterized by high resistance towards acidic pH, as it retained its full activity at pH less than 6, and it is worth noting that bacteriocin treated with pH numbers less than 4.5 for one hour in this study gained additional effectiveness At pH 3, the efficacy of bacteriocin in the control treatment. The efficacy of bacteriocin gradually decreased when treated with pH 6.5 and above. While sensitization was not effective for bacteriocin at pH 8.5, 9, 9.5 and 10.

Determines the optimum stability temperature for the produced peptide

Figure No. (11) and Figure (12) show the thermal stability of the peptide produced from isolated JKD5 by testing its inhibitory activity against two types of test *E. coli* bacteria. *and B. Cereus* after treating it at

different temperatures for an hour. It is noted from the figure that the peptide retained its effectiveness when treated at 60 and 80 degrees C, while it was not observed when treated at 100- and 120-degrees C.

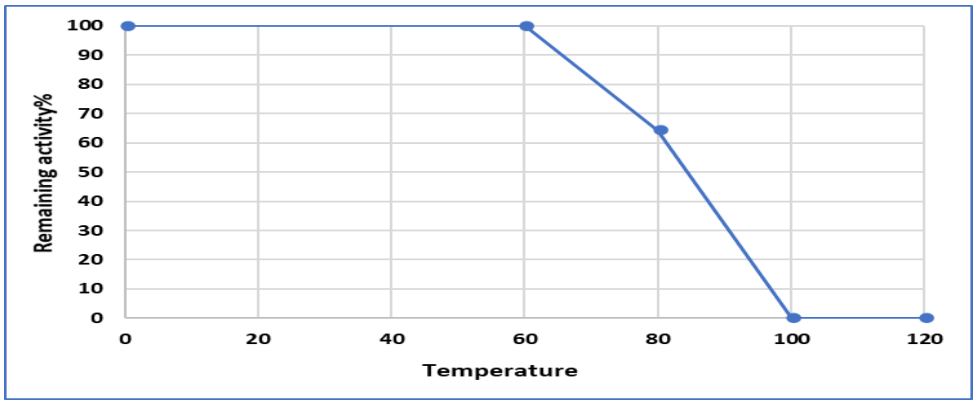

Figure No. (11) Temperature stability of the peptide produced from isolated JKD5 against the test bacteria *B. cereus*.

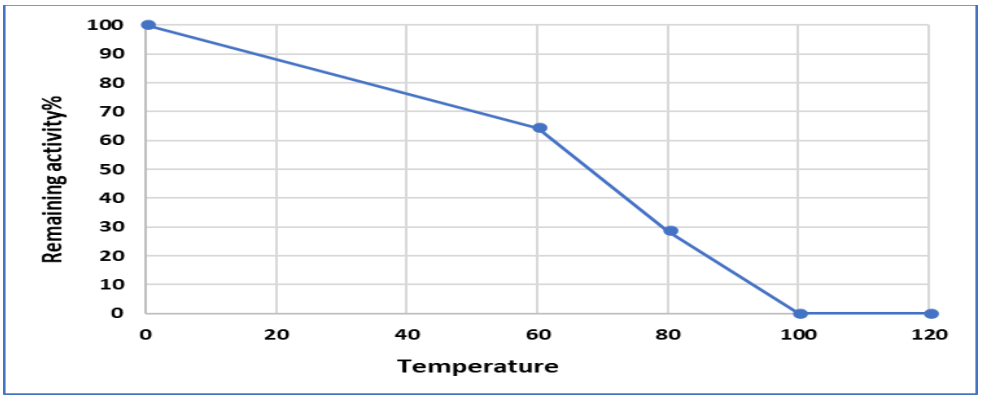

Figure No. (12) is the temperature stability of the peptide produced from isolate JKD5 against the test *E. coli* bacteria

These results are in agreement with the results of many studies that confirm that most of the peptides from lactic acid bacteria have high thermal stability. The result is consistent with what was found by Qahar (2004) that Lacticin G, by testing its inhibitory activity against three types of bacteria, retained its effectiveness when treated at 50 °C for 60 minutes, and when treated at boiling temperature for 15 minutes, while no notice of any Effective when treated in sterilizer at 121°C for 15 minutes.

Conclusions

Lactobacillus delbrueckii is the main bacteria used in yogurt production. It also plays an important role in the maturation of some cheeses as well as in other processes that involve naturally fermented products. It is defined as homologous lactic acid bacteria due to lactic acid being the only end product of carbohydrate digestion and it is also considered a probiotic. Its scientific classification is a bacillus of the lactobacilli family of the genus *Lactobacillus delbrueckii* which is Gram-positive and may appear long and filamentous in shape, immobile and non-sporulating, and it is not pathogenic. They are considered acidic or acidophilic, as they require a low pH of about (5.4- 6.5) to grow effectively. In addition to being anaerobic, as it grows on raw dairy, it creates and maintains the acidic environment it needs to thrive through its production of

lactic acid. In addition, they grow optimally at temperatures (35-40°C) under anaerobic conditions and have complex nutritional requirements that vary according to the environment. It includes carbohydrates, unsaturated fatty acids, amino acids and vitamins.

Studies have proven that it kills unwanted bacteria, it is very important because it is effective in the fermentation process as well as it effectively preserves food products from spoilage Due to its usefulness in natural fermentation processes and specifically how it produces fermented food products from cow's milk, it has great economic importance. The peptides were extracted and purified by two HPLC ÄKTA pure apparatuses to reach total purification. A characterization study was also conducted for the produced peptide, and the study proved that the peptide produced from yogurt isolate JKD5 has high stability towards acidic pH, but loses a high percentage of its effectiveness at neutral and basic pH. And it showed that it has a molecular weight of 6.5 kDa and was stable at 60 and 80 ° C for 60 minutes, which indicates that the peptides can withstand high temperatures in the sterilization process, which is one of the very important processes in food production.

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