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RESEARCH ARTICLE - BIOLOGY

Isolation and identification of new species *Leuconostoc carnosum* from cauliflower and broccoli

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Abstract

A study was conducted on cauliflower and broccoli which were fermented to isolate and characterize lactic acid bacteria that are consumed in these fermented foods by people. One –hundred eighty isolates belong to the lactic acid bacteria were taken from 120 samples of different parts of cauliflower and broccoli were obtained from different markets of Baghdad city. Only 20 isolates were bacteriocin producers thus showed antibacterial activity against indicator bacterial isolates (*S. aureus, S. agalactiae, E. coli and P. aeruginosa*). The lactic acid bacteria (LabBr no. 28) isolate had the highest activity against indicator bacteria with inhibition zones of (22 mm) against *S. aureus*, (16.5 mm) against *S. agalactiae* ,(18mm) against *P. aeruginosa* and (19mm) against *E.coli*. This isolate was identified by using conventional methods then by VITEK®2 System .The identification was confirmed by16sRNA sequencing to characterize and describe the new species *Leuconostoc carnosum* .

The aim of this study: Recognition of lactic acid bacteria species that found in cauliflower and broccoli, which have ability to produce the bacteriocin by detection their ability to inhibit growth of pathogenic bacteria.

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Keywords: Lactic acid bacteria; *Leuconostoc carnosum;* bacteriocin; antibacterial activity; 16sRNA sequencing;

1. Introduction

Broccoli and cauliflower act as anti-proliferative effects on a wide variety of tumour cell lines due to have phytochemical compounds. It's fermentation seems to be an ideal processing step. As it improves organoleptic properties; increases the level of safety and extends shelf life. Lactic acid bacteria are able to give extra nutritional value to these raw vegetables and benefits of which constitute a portion of the microbiota. Raw vegetables may be subjected to spontaneous lactic acid fermentation When providing the positive conditions of anaerobiosis, water activity, salt concentration, and temperature [1],[2]. LAB are a kind of Gram- positive bacteria that can ferment carbohydrates lead to the acidification (lower than pH 4.0) due to the production lactic acid, and are currently widely used in the fermented food industry due to its useful effect dependent on its safety, nutritional, organoleptic and preservative properties [3],[4]. The genus *Leuconostoc* placed within the family of

Lactobacillacea. All species within this genus are heterofermentative. Which it was metabolizing the carbohydrates during pentose phosphate and phosphoketolase pathway; yielding lactic acid, CO2, ethanol and/or acetic acid [5]. The *Leuconostoc carnosum* frequently occurs in the microbiota of foods. Mostly meat-based products and have been isolated from processed vegetables. It have been able to produce of bacteriocins. [6], [7], [8]. Bacteriocins of LAB have significant applications of biotechnology, since they are simple to produce, constant at low pH, non-toxic to human, and susceptible to proteases [9]. The16s rRNA gene analysis is molecular method used for the detection of genetic polymorphism of lactobacilli at the strain level. Phenotypic characters (morphological and biochemical characters) of lactic acid bacteria is not appropriate to differentiate them properly. For this reason,molecular characterization based on genetic characterization is very important and also beneficial for tracing new strains[10].

2. Experimental work

Isolation of Lactic acid bacteria

One hundred twenty of small pieces were taken out of 30 samples of fresh Vegetables (cauliflower and broccoli) were collected randomly from Baghdad markets, Iraq during the period from November 2022 to January 2023 and transported in an box to the food microbiology lab., gently washed with distilled water and cut in to small pieces, 1.0 g of vegetable was suspended in tube containing 1.0 mL sterile saline; then incubated at room temperature for 24 hours. The Lactic acid bacteria were isolated by using tubes containing 9 ml of MRS broth were inoculated with 1 ml of the upper layer of saline containing samples separately and incubated anaerobically at 30°C for 48 hours .After incubation, serial dilutions were made for each culture by using physiological saline solution. Spread-plate method was used with 0.1 ml samples from appropriate dilutions were spread on MRS agar plates and incubated at 30°C for 48 hours under anaerobic conditions. After incubation ,large and small pure colonies were selected , transferred and purified by streaking method on MRS agar and incubated under the same incubation conditions above before being subjected to identification tests [11],[12].

Identification

-Macroscopic Examination of colonies

The appearance of colonies on solid media was studied with respect to form, color, size, opacity, and margin as described by [13].

-Microscopic Examination

The microscopic examination was made for all bacterial isolates .The bacterial cells were studied with respect to their morphological characteristics (Gram's staining, shape, arrangement, and spore formation) [13].

-Biochemical Tests

Catalase Test

On the clean glass microscopic slide the fresh bacterial colony have been placed by sterile wooden stick and rubbed, a drop of 3% hydrogen peroxide reagent was added to perform catalase test. A positive result occurs when release of gas bubbles within 5-10 sec seconds [14].

Oxidase Test

An 18-hours old bacterial colony transferred to the filter paper which was saturated with oxidase reagent, then rubbed onto the reagent with a sterile wooden stick. A positive result appearance during 30 seconds with purple colour [15].

Gelatinase Test

An 18-hours old bacterial isolates with 1% were cultured in tubes of gelatine medium (except control tubes) and incubated for 48 hours at 37°C. Then, all tubes were placed in refrigerator and positive result appearance during 30 minutes with liquefaction of medium [16].

Production of Ammonia from Arginine

An 24-hours old bacterial isolates with 1% were cultured in tubes of MRS-arginine broth medium (except control tubes) and incubated for 24 hours at 30°C. Then, 1 ml of Nessler's reagent was added to 1 ml of each bacterial isolate, the tubes were gently shaken. The development of orange-brown color indicates positive result [17].

Growth at Different Temperatures

Tubes of MRS broth were cultured (except control tubes) with 1% of fresh bacterial isolates and incubated for 24 hours at (10 and 45) °C. The development of turbidity indicates positive result [18].

Growth on Blood Agar

Blood agar plates were cultured (except control plates) with an fresh bacterial isolates and incubated for 24 hours at 30°C. Then, colony appearance was studied according to [19].

Production of CO2 from Glucose Fermentation

Tubes containing MRS broth supplement with durham tube were inoculated with 1% of 24 h old bacterial isolates and incubated at 30o C for 24 h [20].

-Detection of bacteriocin Production by Lactic acid bacteria

LAB was cultured in MRS medium for 24 hours at 30 °C. Cell-free supernatant extracted by centrifuging the cultures for 30 minutes at 4 °C / 4000 rpm. The supernatants were filtering through a 0.22 µm filter (Millipore filter) to be sterilized. The pH of supernatant was adjusted using 1N NaOH to 6.0 and used as crude bacteriocin [21]. The activity of bacteriocin produced by LAB was measured as antimicrobial against some pathogenic bacteria according to agar well diffusion method. Pathogenic isolates, including (*Escherichia coli, Pseudomonas aeruginosa, Streptococcus agalactiae, and Staphylococcus aureus*) were inoculated in nutrient broth, followed by incubation at 37°C overnight. The microbial concentration was adjusted to 0.5 McFarland standard and sub-cultured on Mueller Hinton agar when made a 6 mm diameter well by cutting a hole with sterile cork borer were filled with 100 µl of the culture supernatant. The plates were incubated at 37°C for 24 h. Inhibition was detected by a zone of clearing around the supernatant well [22], [23].The bacterial isolate that caused largest inhibition zone was chosen for further experiments.

-Detecting of bacteriocin producing isolates

One hundred eighty isolates of lactic acid bacteria were isolated and diagnosed then the most producer isolate of bacteriocin was selected after testing the ability of isolates to produce of bacteriocin by forming inhibition zone against pathogenic bacteria through agar well diffusion assay.

-Identification of the producer isolates by VITEK®2 System

Lactic acid bacterial isolates produced bacteriocin were identified at the species level using VITEK ® 2 systems. Identification-Gram positive bacteria (ID-GBB) cards were used according to manufacturer's instructions. The system contains of a printer, a computer and a VITEK® 2 instruments. The VITEK® 2 Compact system comes with software that includes analysis and data processing tools.

-Selection the most efficient producer isolate

The most efficient bacteriocin producer isolate was selected to study morphology, microscopically and biochemical characteristics, then identified by 16SrRNA sequencing.

-Identification the most efficient bacteriocin producer isolate by 16SrRNA sequencing

Genomic DNA Extraction.

DNA of selected isolate was extracted and purified using procedure of DNA extraction kit designed for the isolation of DNA from Gram positive bacteria as follows

•1 ml of overnight Gram positive bacteria was transferred to a 1.5 ml tube and centrifuge at 12000 rpm for 1 minute. The supernatant discarded and the bacteria resuspended with 500µl of 70% ethanol, incubated on ice for 20 minutes and then centrifuge at 10000rpm for 1 minute, the supernatant discarded.

•The bacteria re-suspended with 200µl of re-suspended buffer (containing 4mg lysozyme) and incubated at 37oC for at least 60 minutes then centrifuged at 12000 rpm for 1 minute, the supernatant discarded.

•The bacteria re-suspended with 100µl of lysis buffer and 20µl of proteinase k by vortex and Incubated at 55oC for 15 minutes for made clear solution.

•20µl of RNase added, mixed and incubated at room temperature for 2 minutes.

•Binding buffer was added with 400µl and vortex for 30 seconds , then transferred it to spin column and centrifuged at 12000 rpm for 30 sec, discarded the flow-through.

•500µl of clean buffer added and centrifuged at 12000 rpm for 30 sec, discarded the flow-through. Repeated this step once.

•500µl of wash buffer added and centrifuged at 12000 rpm for 30 sec, discarded the flow-through. Repeated this step 7 times.

•The spin column put in a1.5 sterilized tube of microcentrifuge and addition 50-200µl of elution buffer was of then incubated at room temperature for 2 minutes and centrifuged at 12000 rpm for 1 minute to elute genomic DNA. Repeated this step once. The isolated DNA stored at -20^{o}C.

Measurement Purity and Concentration of DNA:

The Purity of DNA was measured by using a nanodrop UV spectrophotometer, the optical density of DNA (1.5 µl) was measured at two wavelengths (260 and 280 nm). In most samples, DNA preparation gave A260/A280 ratio between 1.8 and 2.0, which is considered to be suitable for further analysis .The measurement of DNA concentration for the sample was in the range of (25-118) ηg/ml [24].

Agarose gel electrophoresis of DNA.

Electrophoresis was done to determine DNA segments following the extraction process. While the ladder is present in order to distinguish the size of bundle in the PCR interaction's result on the Agarose gel.Making an agarose gel usually has been made according to [25] as follow:

1. An amount of 100 ml with (1X) of tris acetate EDTA(TAE) or tris borate EDTA (TBE) buffer prepared from stock solution (10X) of TBE buffer by adding 10 ml from the stock to 90 ml of D.W.

2. In two separate flasks 1 gram and 2 grams of agarose powder respectively were added with amount of TBE or TAE buffer then the volume completed to 100ml of the same buffer.

3. The microwave was used for (1-3) minutes and preferably swirl the flask for each (30-45) second during the heating process and waited for it to boil.

4. At room temperature the gel has been left until it cools down and reaches about 50°C. Ethidium bromide dye was added in the amount of (2-3) µl per 100 ml of gel .the necessary precautions when using the dye, because it is neurotoxic. Then the gel was poured into a tray that previously had a comb fixed.

5.Casting of the Agarose Gel: The agarose solution was poured into the gel box (The gel has been poured gently to prevent air bubbles from forming) where the comb was placed and left for 30 minutes at room temperature until hardened. The carefully inserted comb was removed carefully and the tank used in the electrophoresis was contain the plate of gel installed to its stand in the Electrophoresis horizontal unit and the tank filled with (1X) of TBE buffer till reached 2-3 mm on the gel surface.

Preparation of sample

Amount of (3µl) of the processor loading buffer (TRANSGEN Biotech / China) has been mixed with (7µl) of the supposed DNA to be electrophoresis, after the mixing process, the (10µl) of loading mixture was done to the holes of the gel. An electric current of 70 volts has been exposed for 90 minute till the tincture has reached the other side of the gel. The gel has been tested by a source of the UV with a trans-illuminator and then photographed.

Amplification of 16S rRNA of selected Isolates using PCR

The PCR (polymerase chain reaction) was method an in vitro for forming large amounts of a specific fragment of DNA. It was defined length and sequence from small amounts of a complex template [26].The universal primer (27F: 5’ -AGA GTT TGA TCC TGG CTC A-3’ and 1492R 5’ -GGTTACCTTGTTACGACTT -3’) was lyophilized, it was liquefied in the nuclease-free water to give a final concentration of 100 pmol/µl as stock solution. The stock was kept at -20˚C, to prepare 10 pmol/µl concentration as work primer suspended 10 µl of the stock solution in 90 µl of the free deionized distilled water to reach a final volume of 100 µl. Fragments of the 16S rRNA genes of the producer bacterial isolate were amplified using the universal primer and other components of PCR reaction in the table 1.

Table 1. Reaction components for amplification

NO.	Components	Volume
1	2× Easy Taq PCR Super Mix	12.5 µl

2	Forward primer	1µl
3	Reverse primer	1µl
4	DNA template	5µl
5	Nuclease free water	5.5 µl
Final volume		25µl

The Easy Taq® PCR super Mix (+dye) components (dNTPs - DNA polymerase -optimized buffer - Gel loading buffer) and -20℃ was optimum temperature for it storage. The temperature program and the cycle of reactions were as initial denaturation step at 94◦C for 5 min., followed by 35 cycles of denaturation at 94◦ C for 60 sec, primer annealing at 58◦ C for 30 sec, and primer extension at 72◦ C for 60 sec with a final extension at 72◦ C for 5min. [27], [28]

Agarose gel electrophoresis of PCR products:

PCR products are transferred onto the agarose gel 2% and sample is loaded with a quantity (5µl) directly without adding a loading dye and finally it is relayed at a voltage of 70 for about an hour and a half. Then staining with Ethidium Bromide Stain, the PCR products were exposed to UV light (302 nm) for observation after being separated on a (2%) agarose gel electrophoresis.

Sequencing of PCR product

The primers and products of PCR used in this study were sent to the Macrogen, a South Korean company (dna.macrogen.com) for sequencing analysis using ABI (Applied Biosystem) automated DNA sequences. The sequencing results of the PCR products of the targeted fragments were edited, aligned, and analysed as long as with the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1.

The sequences were identified by searching for sequence homology between published reference sequences using the Basic Local Alignment Search Tool (BLAST) which is accessible online at (National Center for Biotechnology Information (NCBI), http://blast.ncbi.nlm.nih.gov/). Homologies with 99–100% a type strain were considered as excellent identifications [29], [30]

3. Results and discussion

Isolation and Identification of lactic acid bacteria from cauliflower and broccoli

One –hundred eighty isolates belong to the lactic acid bacteria were taken from 120 samples of various parts of cauliflower and broccoli were obtained from different markets of Baghdad city during period at 15 November 2022 to 15 February 2023. The microscopically investigating of lactic acid bacteria isolates was occurred under compound light microscope at 40X and 100X by using Gram stain examination, the Gram stain was appeared all isolates were positive to Gram stain and some isolates were rods, single , chain bacteria or in pairs and others were spherical, single or chain and all non-spore forming bacteria. These findings were agreement with the study of [31]. The cultural characteristics of some lactic acid bacterial colonies were large, raised, smooth, greyish, transparent, and others were white or milky color, round, large or small, opaque, flat or convex on MRS agar media. These results agreed with the result showed by [32], [33]. For characterization of lactic acid bacteria different biochemical tests were performed. The results were appeared that the bacterial isolates were

negative for oxidase, catalase, gelatinase, non-motile, anaerobic, and some isolate positive for gas production but other negative. These results agreed with the result presented by [34]

Detection of Bacteriocin Production by Lactic acid bacteria

Table (2) shows the differences of inhibitory spectra between Lactic acid bacteria isolates by the agar well diffusion method. Crude filtrate of 180 lactic acid bacterial isolates was tested to investigate the activity as antibacterial against indicator bacterial isolates (*S. aureus, S. agalactiae E. coli and P. aeruginosa*), As antibacterial activity showed with only 20 isolates at inhibition zone diameter between 8.5-22 mm. The three isolates of lactic acid bacteria (LabBr no. 26, 27and 28) were showed highest inhibition zone of antibacterial activity against all indicator bacteria isolates. The lactic acid bacteria (LabBr no. 28) had the highest activity against indicator bacteria with inhibition zones of (22 mm) against *S. aureus*, (16.5 mm) against *S. agalactiae* ,(18mm) against *P. aeruginosa* and (19mm) against *E.coli* . One study found that the antibacterial activity of lactic acid bacteria on *Listeria innocua* (resistance zone of 19.24 mm), *S. aureus* (resistance zone of 23.17 mm), and *E. coli O157* (resistance zone of 21.16 mm) [35]. Bacteriocins have been used in food preservation due to their antibacterial action against food-borne pathogenic and spoilage microorganisms [36].

Table 2: Inhibitory effect of bacteriocin producer isolates against indicator bacterial isolates

	Diameters of inhibition zone around (mm)			
Isolates	***S. aureus***	***S. agalactiae***	***P. aeruginosa***	***E.coli***
LabBr1	12mm	11mm	0	0
LabBr2	13mm	14mm	0	0
LabBr4	11.5mm	12mm	0	0
LabBr6	0	9mm	10mm	0
LabCa7	15.5mm	0	0	0
LabCa8	14mm	0	0	0
LabCa9	14.5mm	0	0	9mm
LabCa11	17.5mm	8.5mm	0	10mm
LabCa26	14mm	12mm	15mm	16.5mm
LabBr27	13mm	9mm	13.5mm	17mm
LabBr28	22mm	16.5mm	18mm	19mm
LabCa74	10mm	12mm	0	0
LabCa97	9mm	0	0	0
LabCa101	10mm	0	0	0
LabBr112	0	0	12.5mm	11mm
LabBr116	15mm	0	0	12.5mm
LabCa124	11mm	0	0	0
LabBr131	13.5mm	11mm	12mm	10
LabBr 137	11.5mm	10mm	11mm	12mm
LabCa148	12mm	-	-	9mm

-well diameter 6.0 mm, LabBr: lactic acid bacteria isolated from broccoli, LabCa: lactic acid bacteria isolated from cauliflower

-Identification of the producer isolates by VITEK®2 System

Biochemical assays of LAB isolates which produced the bacteriocin were investigated using the VITEK 2 Compact system. The isolates cultured on MRS agar and produced positive findings from biochemical testing were verified using the VITEK2 compact system.

Results of the identification isolates of the study recorded as 5 (25%) isolates of *Leuconostoc mesenteroides* ssp. *mesenteroides*, 4 (20%) isolates of *Leuconostoc mesenteroides* ssp *dextranicum*, 3(15%) isolates of *Lactococcus garvieae*, 7 (35%) isolates of *Leuconostoc citreum a*nd 1(5%) isolate of *Leuconostoc carnosum* as show in Fig.1. *Leuconostoc mesenteroides* ssp *mesenteroides* and *Leuconostoc mesenteroides* ssp *dextranicum* isolates were isolated from cauliflower while *Leuconostoc citreum*, *Leuconostoc carnosum* and *Lactococcus garvieae* were isolated from broccoli. The study reported the Lactococcus garvieae isolates from radish and broccoli sprouts [37]. Species belonging to genus *Leuconostoc (Lc. argentinum, Lc. carnosum, Lc. citreum, Lc. fallax, Lc.ficulneum, Lc. fructosum, Lc. gasicomitatum, Lc. gelidum, Lc. inhae, Lc. kimchii, Lc. lactis, Lc. mesenteroides, Lc. pseudomesenteroides)* can be found mainly in vegetables, cereals, silage, fruits, wine, fish, meat, and dairy products [38]. Figure -1 shows the results of the identification of the bacterial isolates by VITEK 2 System and ID GP card. The VITEK 2 system's GP card has biochemical assays to measure enzyme activity, carbohydrate consumption, and resistance to certain compounds, all of which can be used to identify GP, non-spore-forming bacteria [39].

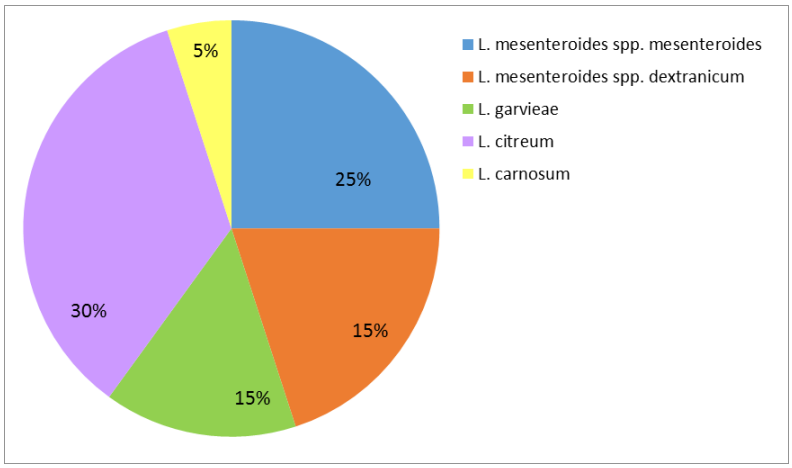
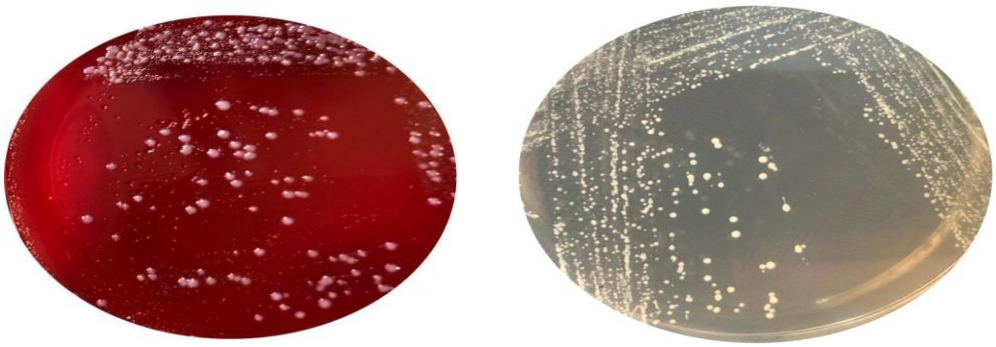


Fig. 1. Identification of LAB isolate by using VITEK 2 System

Selection the most efficient producer isolate

During the study, the *Leuconostoc carnosum* (LabBr28) was the highest bacteriocin producer among other LAB isolates and it was isolated for the first time from broccoli because it was not predominant in the broccoli while the genus Lactobacillus occupied the dominant position of the broccoli juice, (abundances of 79.0 %) as showed with search by [40]. *Lactobacillus curvatus*, *Leuconostoc carnosum*, and *Leuconostoc mesenteroides* were also identified as the main LAB in kimchi was explained by [41]whereas *L. carnosum* 4010, which was isolated from the surface of sliced vacuum-packed ham as reported with the study of [42]. Morphological characteristics of *L. carnosum* on MRS agar and blood agar showed creamy, round colonies, convex and large as shows in Fig.2 .The genus *Leuconostoc ssp*. round colonies have smooth edges, a convex surface, 1-4 mm in size, and a whitish or cream colour [43] .



A.Blood agar B. MRS agar

Fig. 2. Colony morphology of *Leuconostoc carnosum* (LabBr28) on A: Blood agar , B: MRS agar

The microscopic and biochemical characteristics of *L. carnosum* were summarized in Table (3) which appeared gram positive, ovoid cocci, arranged singly, in pairs or forming chains, negative for oxidase, catalase, gelatinase, production of CO_2 there for it was belonged to hetero fermentative lactic acid bacteria, non-producer of ammonia from arginine, non-motile, non-spore forming, and growth at 10^oC but not growth at 45^oC with anaerobic condition. These results agreed with the result presented by [44], [45], [46].

Table 3. Biochemical tests of the *L. carnosum* isolates

Biochemical and other tests	result
Gram stain	+
Catalase test	-
Oxidase test	-
gelatinase test	-
Gas from glucose	+
Producing ammonia from arginine	-
Growth at 10^oC	+
Growth at 45^oC	-
Spore forming	-
motility	-

Symbols: + = positive result; - = negative result

-Identification the *Leuconostoc carnosum* (LabBr28) by 16SrRNA sequencing

Genomic DNA Extraction

Genomic DNA was successfully extracted from LabBr28 isolate and analysed by agarose gel electrophoresis as shows in fig.3.

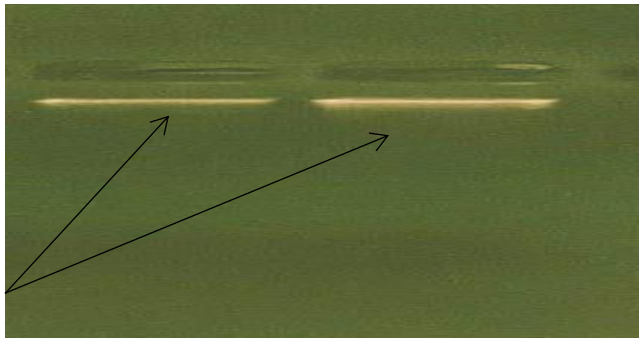


Fig.3. Gel electrophoresis for Extracted DNA, (Agarose 1%, at 100 volts, 60min.). Visualized under U.V light after staining with ethidium bromide.

Measurement of DNA Purity and Concentration:

The concentration and purity of DNA were measured by using Nano Drop spectrophotometer. DNA concentration showed (57.5 ngμ/l) at 260 nm wavelength which optimum to absorb of the nucleic acids. The ratio of absorption at 260 nm wavelength to absorption at 280 nm wavelength which represented protein contaminants appeared (~1.82) purity of DNA. The A260/A280 ratio must be within the range 1.7-1.9.

Amplification of 16S rRNA of selected Isolates using PCR

The identification of LabBr28 isolate to determine the isolate carried out based on the 16S rRNA gene. The amplification process to form more copies of the 16S rRNA gene for the next step using the PCR method and the PCR of the 16S rRNA gene of LabBr28 isolate provides an amplicon of about ~1507 bp as shows in Fig. 4 and this result agreed with [46].

Fig. 4. Agarose gel (2%) electrophoresis showing amplified 16S rRNA gene of LabBr28 isolate. A: PCR product~ 1507pb; B: DNA marker ladder (100–1500bp)

The Sequencing of Amplified Product

The sequencing reactions for LabBr28 isolate indicated the exact identity after performing NCBI blast for these PCR amplicons. The NCBI BLAST engine also showed 97% of sequences similarities between the sequenced sample and *Leuconostoc carnosum* reference sequences as showed in Fig.5. Depending on the morphological, microscopic and biochemical tests as well as molecular identification, the isolate was identified as *Leuconostoc carnosum* by using the basic local alignment search tool (BLAST) of the

GenBank. The nucleotide sequence of the 16S rRNA gene found in all microbes as housekeeping genetic marker for taxonomic and bacterial phylogeny analysis moreover, the 16S rRNA genes (1500 bp) are huge enough for informatics and genetic analysis purposes therefore it was one of the molecular detection techniques used to determine the kinship relationship between bacteria [31],[47].

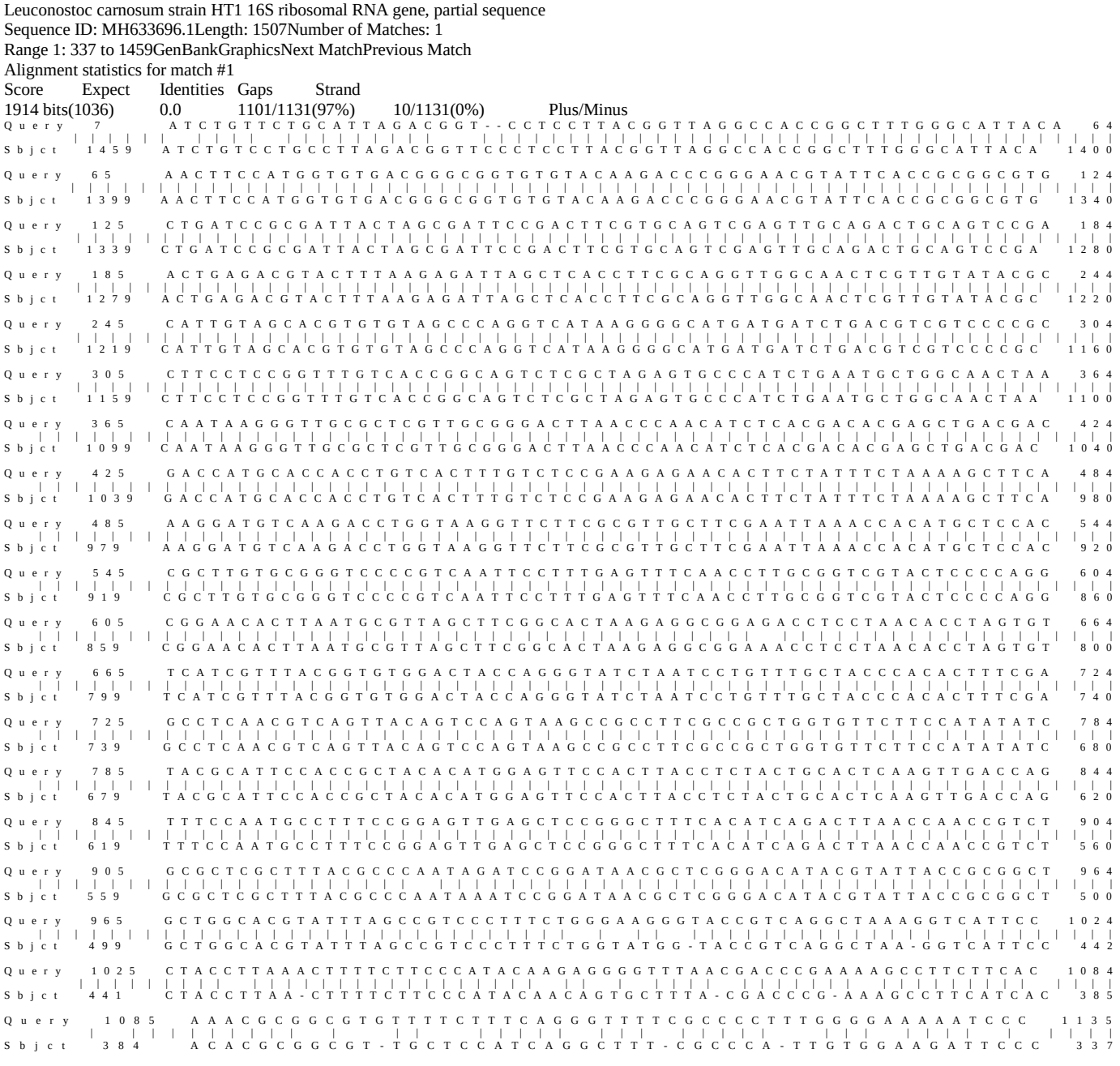


Fig. 5. Sequencing of 16sRNA gene of *Leuconostoc carnosum*

4. Conclusion

New species of lactic acid bacteria *Leuconostoc carnosum* was isolated for the first time in this study from broccoli and cauliflower. This species was diagnosed by classical and VITEK®2 system techniques and was able to produce antimicrobial peptides (bacteriocin) efficiently. 16sRNA sequencing has revealed 97% similar to *Leuconostoc carnosum* .

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References

[1] S. Paramithiotis, A. Doulgeraki, A. Vrelli, G.-J. Nychas, and E. Drosinos, "Evolution of the Microbial Community During Traditional Fermentation of Globe Artichoke Immature Inflorescence," *Int. J. Clin. Med. Microbiol.*, vol. 1, no. 2, pp. 1–5, 2016, doi: 10.15344/2456-4028/2016/117.

[2] D. Iga-Buitrón *et al.*, "Lactic Fermentation of Broccoli (Brassica oleracea var. italica) to Enhance the Antioxidant and Antiproliferative Activities," *Fermentation*, vol. 9, no. 2, 2023, doi: 10.3390/fermentation9020122.

[3] A. A. Otunba, A. A. Osuntoki, D. K. Olukoya, and B. A. Babalola, "Genomic, biochemical and microbial evaluation of probiotic potentials of bacterial isolates from fermented sorghum products," *Heliyon*, vol. 7, no. 12, 2021.

[4] Y. Wang *et al.*, "Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry," *Front. Bioeng. Biotechnol.*, vol. 9, p. 612285, 2021.

[5] F. Candeliere *et al.*, "Comparative genomics of Leuconostoc carnosum," *Front. Microbiol.*, vol. 11, p. 605127, 2021.

[6] M. E. Stiles, "Bacteriocins produced by Leuconostoc species," *J. Dairy Sci.*, vol. 77, no. 9, pp. 2718–2724, 1994.

[7] S. Raimondi *et al.*, "Phenotypic traits and immunomodulatory properties of Leuconostoc carnosum isolated from meat products," *Front. Microbiol.*, vol. 12, p. 730827, 2021.

[8] S. P. M. Silva, S. C. Ribeiro, J. A. Teixeira, and C. C. G. Silva, "Application of an alginate-based edible coating with bacteriocin-producing Lactococcus strains in fresh cheese preservation," *Lwt*, vol. 153, p. 112486, 2022.

[9] S. D. Todorov, B. D. G. de Melo Franco, and J. R. Tagg, "Bacteriocins of Gram-positive bacteria having activity spectra extending beyond closely-related species," *Benef. Microbes*, vol. 10, no. 3, pp. 315–328, 2019.

[10] K. M. Hossain, B. Mazumder, S. M. M. Rahman, and M. A. Hamid, "Genetic diversity analysis of lactic acid bacteria isolated from regional yogurt samples," *Bangladesh J. Livest. Res.*, pp. 55–63, 2020.

[11] O. B. Oyewole and S. A. Odunfa, "Characterization and distribution of lactic acid bacteria in cassava fermentation during fufu production," *J. Appl. Bacteriol.*, vol. 68, no. 2, pp. 145–152, 1990.

[12] M. Junnarkar, S. Pawar, S. Gaikwad, A. Mandal, J. Jass, and N. Nawani, "Probiotic potential of lactic acid bacteria from fresh vegetables: application in food preservation," *Indian J. Exp. Biol.*, vol. 57, pp. 825–838, 2019.

[13] S. Bennani, K. Mchiouer, Y. Rokni, and M. Meziane, "Characterization and identification of lactic acid bacteria isolated from Moroccan raw cow's milk," *Greener J. Biol. Sci.*, vol. 25, pp. 4934–4944, 2017.

[14] C. Winn Washington *et al.*, *Koneman's Color Atlas and Textbook of Diagnostic Microbiolgy*. Lippincott, Williams & Wilkins, 2006.

[15] H. J. Benson, *Microbiological applications: a laboratory manual in general microbiology*. [McGraw-Hill], 2002.

[16] B. A. Forbes, D. F. Sahm, and A. S. Weissfeld, *Diagnostic microbiology*. Mosby St Louis, 2007.

[17] M. A. Ismaili, J. Guilal, A. Hamama, B. Saidi, and M. Zah, "Identification de bactéries lactiques du lait cru de chamelle du sud du Maroc," *IJMS*, vol. 1, pp. 81–94, 2016.

[18] A. A. Onilude, O. Olaoye, I. F. Fadahunsi, A. Owoseni, E. O. Garuba, and T. Atoyebi, "Effects of cultural conditions on dextran production by Leuconostoc spp.," *Int. Food Res. J.*, vol. 20, no. 4, p. 1645, 2013.

[19] D. Borah, O. Gogoi, C. Adhikari, and B. B. Kakoti, "Isolation and characterization of the new indigenous Staphylococcus sp. DBOCP06 as a probiotic bacterium from traditionally fermented fish and meat products of Assam state," *Egypt. J. Basic Appl. Sci.*, vol. 3, no. 3, pp. 232–240, 2016.

[20] S. Lahtinen, A. C. Ouwehand, S. Salminen, and A. von Wright, *Lactic acid bacteria: microbiological and functional aspects*. Crc Press, 2011.

[21] T. F. Borgonovi *et al.*, "Dual role of probiotic lactic acid bacteria cultures for fermentation and control pathogenic bacteria in fruit-enriched fermented milk," *Probiotics Antimicrob. Proteins*, pp. 1–16, 2023.

[22] S. S. Manfi Al-Rawi *et al.*, "Antibacterial Activity of Bacteriocin - Isolated from Lactobacillus Spp. against Some Pathogenic Bacteria," *J. Med. Chem. Sci.*, vol. 6, no. 4, pp. 702–709, 2023, doi: 10.26655/JMCHEMSCI.2023.4.1.

[23] N. A. Sandi and S. I. O. Salasia, "Alternative antibiotics source from symbiont of lactid acid bacteria inside stomach of honeybees (Apis mellifera and apis dorsata) against multiresistant antibiotics pathogenic bacteria," *Res. J. Microbiol.*, vol. 11, no. 2–3, pp. 93–100, 2016.

[24] M. U. E. Sanam, A. I. R. Detha, and N. K. Rohi, "Detection of antibacterial activity of lactic acid bacteria, isolated from Sumba mare's milk, against Bacillus cereus, Staphylococcus aureus, and Escherichia coli," *J. Adv. Vet. Anim. Res.*, vol. 9, no. 1, p. 53, 2022.

[25] S. M. M. Nzilibili, M. K. H. Ekodiyanto, P. Hardjanto, and A. Yudianto, "Concentration and purity DNA spectrophotometer: sodium monofluorophosphate forensic impended effect," *Egypt. J. Forensic Sci.*, vol. 8, pp. 1–7, 2018.

[26] P. Y. Lee, J. Costumbrado, C.-Y. Hsu, and Y. H. Kim, "Agarose gel electrophoresis for the separation of DNA fragments," *JoVE (Journal Vis. Exp.*, no. 62, p. e3923, 2012.

[27] T. J. White, N. Arnheim, and H. A. Erlich, "The polymerase chain reaction," *Trends Genet.*, vol. 5, pp. 185–189, 1989.

[28] E. Madoroba, *Molecular characterization and population dynamics of lactic acid bacteria during the fermentation of sorghum*. University of Pretoria (South Africa), 2009.

[29] B. T. Tadesse, A. Tesfaye, D. Muleta, A. Bahiru, Z. Terefework, and G. Wessel, "Isolation and molecular identification of lactic acid bacteria using 16s rRNA genes from fermented Teff (Eragrostis tef (Zucc.)) dough," *Int. J. food Sci.*, vol. 2018, no. 1, p. 8510620, 2018.

[30] K. Tamura, D. Peterson, G. Stecher, M. Nei, and S. Kumar, "Phylogenetic and Molecular Evolutionary Analyses were Conducted Using MEGA ver 5," 2011.

[31] H. J. Lawalata, R. Mariana, and S. Utari, "Identification by 16S Ribosomal RNA Gene Sequencing of Lactic Acid Bacteria Producing Antibacterial Agents from Langsat Fruit (Lansium domesticum) in North Minahasa District, North Sulawesi," *J Bacteriol Parasitol. S*, vol. 5, 2020.

[32] M. Mathialagan, Y. Edward, P. David, M. Senthilkumar, M. Srinivasan, and S. Mohankumar, "Isolation, characterization and identification of probiotic lactic acid bacteria (LAB) from honey bees," *Int. J. Curr. Microbiol. Appl. Sci*, vol. 7, no. 04, pp. 894–906, 2018.

[33] A. Sameen, F. M. Anjum, N. Huma, and M. I. Khan, "Comparison of locally isolated culture from yoghurt (dahi) with commercial culture for the production of mozzarella cheese.," 2010.

[34] S. S. Huligere *et al.*, "Isolation and characterization of lactic acid bacteria with potential probiotic activity and further investigation of their activity by α-amylase and α-glucosidase inhibitions of fermented batters," *Front. Microbiol.*, vol. 13, p. 1042263, 2023.

[35] D. Shilpakar, "Isolation, Identification and Characterization of Lactic Acid Bacteria Found in 'Juju Dhau' from Bhaktapur, Nepal," *Curr. Trends Biomed. Eng. Biosci.*, vol. 20, no. 4, 2022, doi: 10.19080/ctbeb.2022.20.556044.

[36] D. Abdullah, S. Poddar, R. P. Rai, E. Purwati, N. P. Dewi, and Y. E. Pratama, "Molecular identification of lactic acid bacteria an approach to sustainable food security," *J. Public health Res.*, vol. 10, no. 2_suppl, p. jphr-2021, 2021.

[37] A. Galvez *et al.*, *Natural antimicrobials for food biopreservation*. Springer, 2014.

[38] M. Kawanishi *et al.*, "Characterization of Lactococcus garvieae isolated from radish and broccoli sprouts that exhibited a KG+ phenotype, lack of virulence and absence of a capsule," *Lett. Appl. Microbiol.*, vol. 44, no. 5, pp. 481–487, 2007.

[39] A. T. de Paula, A. B. Jeronymo-Ceneviva, S. D. Todorov, and A. L. B. Penna, "The two faces of Leuconostoc mesenteroides in food systems," *Food Rev. Int.*, vol. 31, no. 2, pp. 147–171, 2015.

[40] W. P. Lokapirnasari, A. M. Sahidu, K. Soepranianondo, A. Supriyanto, A. B. Yulianto, and A. Al Arif, "Potency of lactic acid bacteria isolated from balinese bovine (Bos sondaicus) intestinal waste from slaughterhouse to improve nutrient content of wheat pollard as animal feedstuff by fermentation process," *Vet. World*, vol. 11, no. 8, p. 1127, 2018.

[41] X. Xu, D. Luo, Y. Bao, X. Liao, and J. Wu, "Characterization of diversity and probiotic efficiency of the autochthonous lactic acid bacteria in the fermentation of selected raw fruit and vegetable juices," *Front. Microbiol.*, vol. 9, p. 2539, 2018.

[42] Y. Hong, H. Yang, J. Li, S. Han, H. Chang, and H. Kim, "Identification of lactic acid bacteria in salted Chinese cabbage by SDS-PAGE and PCR-DGGE," *J. Sci. Food Agric.*, vol. 94, no. 2, pp. 296–300, 2014.

[43] B. B. Budde, T. Hornbæk, T. Jacobsen, V. Barkholt, and A. G. Koch, "Leuconostoc carnosum 4010 has the potential for use as a protective culture for vacuum-packed meats: culture isolation, bacteriocin identification, and meat application experiments," *Int. J. Food Microbiol.*, vol. 83, no. 2, pp. 171–184, 2003.

[44] R. Cholakov, Z. Denkova, V. Yanakieva, Y. Tumbarski, Z. Urshev, and I. Dobrev, "Biochemical and molecular identification of a strain Leuconostoc lactis BT17, isolated from a spontaneously fermented cereal beverage, Boza," *Akad. Gıda*, vol. 14, no. 3, pp. 230–234, 2016.

[45] S. V. N. Vijayendra, G. Palanivel, S. Mahadevamma, and R. N. Tharanathan, "Physico-chemical characterization of an exopolysaccharide produced by a non-ropy strain of Leuconostoc sp. CFR 2181 isolated from dahi, an Indian traditional lactic fermented milk product," *Carbohydr. Polym.*, vol. 72, no. 2, pp. 300–307, 2008.

[46] N. Yuliana, D. Koesoemawardani, S. S, and Y. Kurniati, "Lactic acid bacteria during fish fermentation (rusip)," *MOJ Food Process. Technol.*, vol. 6, no. 2, pp. 211–216, 2018, doi: 10.15406/mojfpt.2018.06.00167.

[47] B. G. Mohammed, "The Relationship of Certain Physiological Parameters and VEGF with chronic kidney disease," Mustansiriyah Journal of Pure and Applied Sciences, vol. 2, no. 3, pp. 75–90, 2024.