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The Antibacterial Effectiveness of Bacteriocin Output *via* *Streptococcus thermophilus* Versus Viral Pathogens and Spores

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ABSTRACT

Background: Probiotics are the fully being encouraging growth while microbes that display a valuable impact on the strength of humanitarian existence *via* ameliorative the abdominal infectious cap ability**.** The purpose of this investigation was stratifying output and Segregation approaches of Cell liberate supernatant (CLS) that detached of *Streptococcus thermophilus* for recognition the efficacy of defiant-pathogenic microbial development.

Methods: CLS gained as of the *streptococcus* insulate displayed restrained vigour versus *Bacillus, Pseudomonas, Staphylococcus* and *E. coli*.

Result: The restrained influence was presumed to be owed to various in gredient such as bacteriocin, biological acidulous in Augmentation H_2O which own corroding impact on microbial cells that devastate the elementary molecular construction of cell proteins. Research in this topic is promoted not only *via* the need to develop alternatives to chemical preservative that own display non-desirable impacts, but also to the capacity of several bacteriocin impact on diet pathogens. *Streptococcus thermophilus* was intelligent to output bacteriocin which antiseptic effectiveness was resolve *via* agar fully dissemination checking trial versus pointer creatures. The uncontaminated protein bacteriocin was description grounded on susceptibility to hotness, various pH values and sensitivity to NaCl. SDS-Page analysis revealed that thermophilic has a molecular mass of 66.9 kD for crumbs. This detached displays antibacterial effectiveness that may be beneficial for probiotic culture to be a good bio preservative as alternative from chemical preservative.

Key words: Bacteriocin, Lactic acidulent bacteria, Probiotics, *Streptococcus thermophilus*.

Abbreviations: CLS: Cell liberate supernatant, CRS: Cell release supernatant, LAB: Lactic acidulent bacteria, M.H. agar: Mueller-Hinton agar, MRS: De Man-Rogose-Sharpe, SDS-page: Sodium dodecyl sulfate-polyacry lamide gel electrophoresis, SDS: Sodium dodecyl sulphate, UTI: Uric region contamination.

INTRODUCTION

Lactic Acidulent Bacteria (LAB) refers to a collection of grams-positive bacteria inclusive several genera (Mohsen *et al.,* 2013). These times LAB is utilized as an (complete) portion of obstacle expertise utilize they are in collection *via* another protective procedure can impact measure deterioration bacteria and exam pathogens and can suppress the efficiency of a varied series of creatures inclusive intrinsically renitent Gram adverse and plus bacteria (Ibrahim *et al.,* 2021). The effectiveness of those bacteria is recognized to obstruct a huge quantity of bacterial pathogens owing to their probable positively possessions as probiotics. Probiotics are reanimating infectious nutrition complements comprising actually bacteria who own plus influence versus their steward strength *via* beneficent its abdominal infective cap ability (Abbas *et al.,* 2016). *Streptococcus* is deeming to be single of the great LAB types and their disinfectant efficiency had been in composite in several revisions (Pasolli *et al.,* 2020).

The utilize of LAB goods to monitoring confident contagion has commence earning approval, the disturbing increase of unsuitable antibiotic utilize and disinfectant durability, length wise *via* regenerated concern in environmental or dinary procedures to avoid pollutions CRS (Cell release Supernatant: harvests of LAB) an exact exciting domain for exploration (Dejene *et al.,* 2021) and bacteriocins are one of them, the bacteriocin are ribosomally synthesized disinfectant peptides widely distributed in nature., this peptide biodiversity is supported *via* several differences in their constructs (Dejene *et al.,* 2021). Bacteriocins of gram-plus creatures, for instance lactic acidulent bacteria (LAB), own concerned numerous courtesies and take been the topic of dense search as a result of their comprehensive combination for example bio-Augmentatives constituents inside typical nutrients mostly

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in the dairy industry (Abbas *et al.,* 2016; McHugh *et al.,* 2017). There is a ration of exploration around is in fectant vigour of LAB, for instance a penetrable education round the outcome of CLS versus pathogens detached from superficial, from inter change transcripts in Baghdad\ Iraq, from UTI (uric region contamination) (Mohsen *et al.,* 2013).

Thus, the purpose of present research was to concentrate on the antibacterial vigour of the CFS (cell-free supernatant) *Streptococcus* counter to microbes and development roughly of pathogenic bacteria attributable to shortage in this investigation scope and occasion to utilize it as manufacturing normal factor *via* nutriment *via* outside preserving procedure to minimize their pollution *via* durability the microbes.

MATERIALS AND METHODS

Origin of bacterial pathogens

Reference microbial strains including *E. coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtaind from Central Health Laboratory / Ministry of Health, Baghdad, Iraq from and *Bacillus* was isolated from food source from Environment and Water Research and Technology Directorate / Ministry of Science and Technology, Baghdad, Iraq. All identified by the chemical test and the stock were growth on nutrient agar and kept in refrigerator at 4°C until use.

Bacterial holdup making ready

Particular convenient inclined medium was utilized to stimulate the bacterial segregates to be examined *via* whereby of antiseptic process and vaccinated it inside the congruent fluid medium. It was possessed as pertypicalsol next wards culturing in the persistent incubator at the generality convenient temperature for 16-18 hrs.

Bacterial hold upon compassing bacteria of near *via* 10^6 cells per ml was contagious through disinfected biological salty for extra utilize (Mohsen *et al.,* 2013).

Spore creating bacteria capability exam

It was relying on Gomasin stain that dyed the spore (whether it is originate) *via* red paint. Stain was augmentation to the bacterial holdup and incubated in 95°C for ten minute. Next chilling, microscopical checking was complete and the spores were representing whether they were creating *via* red dye (McHugh *et al.,* 2017).

Source of streptococci

The samples of Streptococcus detach was obtained from Mustansiriyah university/ science collage / biology department /high studies laboratories.

Making ready of cell release supernatant (crs) of *streptococcus*

The detached was incubated in MRS broth (De Man - Rogose - Sharpe) soup for twent four hours, at 37°C (That isolated from food source). Bacterial cells were exception *via* discard the medium culture at 8000 xg for five minute., there after refined *via* 0.22 µm refinery sheet and stored at 4°C further trial (Aminnezhad *et al.,* 2015).

Definition of restrained efficiency of crs versus development of exam bacteria

Agar fully spread evaluate was synthetic to define the disinfectant effectiveness of CFS versus examined bacteria *via* whizzing of Muller Hinton agar medium *via* 0.1 µl of bacterial inoculums, double bores were synthetic *via* utilize bar perforator on extremely dish and loaded by CRS the monitoring (solely media). These dishes were incubated at 37°C for (18-24) h. The outcome was determined by *via* calculating the repression sector by mm (Baqer *et al.,* 2014).

Definition of restrained effectiveness of CRS versus spores creating

The restrained effectiveness of CFS versus spore creating was estimated *via* the utilization (blending by culture media) process. The exam was achieved *via* augmentation CFS of Streptococcus (10%) to specific media (M.H. agar). CFS was Augmentation to flasks gently and lightly to avoid the creation of foams, blended fully by whirlpool, decant the dish and leftward to chilled and stiffen. Extremely dish was incubated by exam bacteria detached that grew up effectiveness and incubated at 37°C for 24 h. Next, it had to understood if the detached were attended to monitor their possess and the capability to spores creating *via* microscopic checking. Medium lacking CRS was utilized as a minus monitoring and the medium by CRS lacking inoculum was utilized as a plus monitoring (Al-Jassani *et al.,* 2006).

Streptococcus bacteriocin output capability exam

MRS medium was ready for *Streptococcus* detached and disinfected *via* autoclaving at 121°C for 15 min. Next cooling 0.1% inoculums was Augmented to the soup and incubated for 24 hr. at 37°C. Next incubation the culture was centrifuged at 1000 rpm, 4°C for 10 min. The CFS was adjusted to pH 6.5 to 7.0 by 1 N NaOH to exclude all antibacterial organic acidulent. The rudimentary bacteriocin effectiveness was examined *via* utilize Agar fully spread process and the indicator strains utilized to check the bacteriocin effectiveness were examined bacteria in the study, if CFS display repression zone versus the target microorganism. This is meaning that this streptococcus is manufacture to bacteriocin (El-Mokhtar *et al.,* 2020).

Indicator detached

The indicator detached utilized to check the bacteriocin effectiveness were *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus,* all the cultures were preserved in Nutrient Agar slant at 4°C and except *E. Coli* was preserved in specific medium at tryptic Soy Agar slant medium at 4°C (Bradford *et al.,* 1976).

Bacteriocin refining

The partial refining of bacteriocin was conducted *via* 80% ammonium sulphate sedimentation and centrifuged at 10,000 x*g* at 4°C for 10 min to detach remnant and supernatants as of rudimentary accelerate separate; the supernatants were stored at 4°C in 100 mL disinfected flask (pH; 8.0). Dialysis was pursuing in a cylindrical cellulose

integument (1000 cut off) versus 2 L filtered aqueous for 24 h, from which the bacteriocin criteria was achieved. Protein concentricity of the bacteriocin in supernatant was specific *via* the Bradford process of protein assessment (Bradford *et al.,* 1976), utilize bovine plasma albumin as the criterion, the protein was refined in the following steps in detail.

Making ready of CLS

Soup culture (1000) ml by inoculate was incubated to gain a huge collection of bacteriocin at 37°C and pH: 5.0 for 18 h. Next the incubation Cell liberate supernatant (CLS) was gained *via* centrifugation of media at 12.000 ×g for 30 min., the CLS was utilized moreover for ammonium sulphate sedimentation.

Partial refining of bacteriocin

The partial refining of bacteriocin was conducted *via* 80% $[(NH_4)_2SO_4)]$ sedimentation (51.6 g from ammonium sulphate) it was Augmented quietly to avoid the aggregate by constant moving., next that centrifuged at 10.000 ×g and 4°C for 10 min. to detach remnant and supernatants from rudimentary accelerate separate; the supernatant was standard at 4°C in 100 ml disinfected flask (pH: 8.0).

Limited the bacteriocin titer *via* dialysis

To refined the bacteriocin; Dialysis was achieved follow up the partial refining in a cylindrical cellulose integument (1000 cut off) versus 2 L Filtered aqueous for 24 hr., as of who the bacteriocin criteria was achieved. Protein concentricity of the bacteriocin in supernatant was specific *via* utilize bovine plasma albumin as the criterion.

Definition of protein concentricity

Protein concentricity was executed utilize the Bradford process (1976) for example pursue: Augment 20 µl of specimen (rudimentary or refined) was blended by 50 µl of 1M NaOH (3.2.2.1.B) by constant trembling for (2-3minutes) subsequently (1ml) of Coomasie Resplendent Blue G-250 as in (3.2.2.1.C) was Augmented by trembling. Absorbance was recorded at 595 nm *via* a spectrophotometer. A criterion detour of bovine plasma albumin was executed utilize various concentricity (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg / ml) as display in (3.2.2.1.D). Extremely was pipetted in repeat in disinfected exam pipe, then permeability was intrigue versus the identical concentricity of bovine plasma albumin Fig 1.

Bovine plasma albumin (BSA) (20 µg/ml)

It was ready *via* resolve 0.025 g of BSA in 25 ml of filtered aqueous. From this standard sol the concentricity 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml were ready *via* diluting the standard in filtered aqueous.

Lotionpurification and Ion interchange chromatography

The dialyzed specimens were refined *via* lotion purification utilize Sephadex G-200). The rinsing of specimen was achieved *via* 10 mM Tris-HCL bumper (pH 8.0). Thecrumbs by ultimate protein insides were gather outside and practical for disinfectant examination. The vigorous portion gained of the lotion purification were more over refined *via* ion interchange chromatography. DEAE-Sephadex was utilized as resin in ion interchange chromatography pole balance by the 10 mM phosphate bumper (pH: 7.0). Rinsing was achieved *via* the inclination of 10 mM phosphate bumper and 0.1-1M NaCl (pH: 7.0). The specimen of partially refined excerption was then subjected to antibacterial examination. Dialysis, Lotion purification and ion interchange chromatography were achieved according (Hasson *et al.,* 2021).

Description of bacteriocin

Streptococcus thermophilus- The Description of the bacteriocin (peptides / proteins) was achieved *via* SDS - page (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) which executed of the rudimentary and refined protein specimens owning antibacterial effectiveness, the testing was executed on small-protein, tiny columnar electrophoresis device, utilize the 12% lotion. The specimens were ready *via* blending the protein 2:1 ratio by SDS specimen bumper of sigma. The lotion was stained

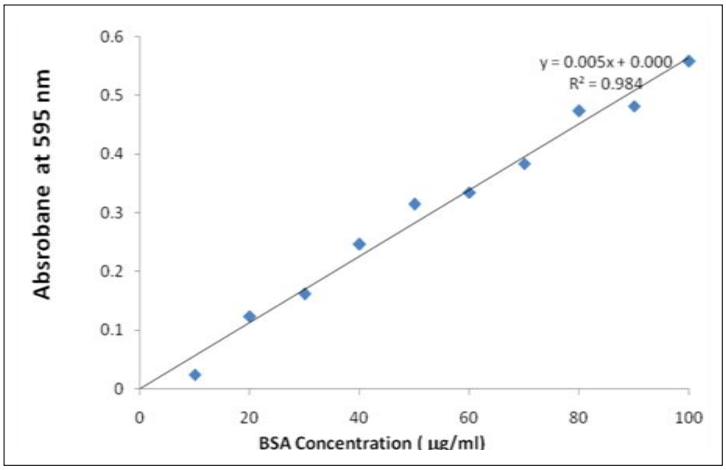


Fig 1: A criterion detour of bovine plasma albumin.

for protein by coomassie resplendent blue, sideways by the sign proteins, the conspicuous molecular enormous of the specimen was studied *via* differentiation by the movement of the criterion indicators (Kechagia *et al.,* 2013).

RESULTS AND DISCUSSION

CLS gained from the *S. thermophiles* detached display restrained effectiveness versus *B. cereus, P. aeruginosa, S. aureus* and *Escherichia coli*. The grade of repression was specified as extremely robust repression (30 mm) versus *E. coli,* mild repression (22 and23) mm, versus *P. aeruginosa, S. aureus* respectively and minimal repression (18 mm) versus. *Cereus* Table 1.

The potential impact of CLS on the spore creating was estimated and the education of CLS decrease the capability of spore creating may evidence the advantage of utilize a CLS condensed the output of spores, therefore it can prohibit the maliciousness pathogenicity of bacteria and diet carried illness. Screening and output of bacteriocin and its relation by effectiveness the zones of repression display the existence of bacterial effectiveness versus exam bacteria in primary inspection. Bacteriocin output was robustly subordinate on pH, period and temperature. Cell release supernatant display the bacteriocin effectiveness as 2600 AU/ml gained next centrifugation and as follows:

Bacteriocin retrieval was achieved *via* partial refining steps, ammonium sedimentation and dialysis. The effectiveness of bacteriocin was retained next refining process and effectiveness was found to increase by increased refining. Bacteriocins were insoluble in aqueous and therefore, the impurities which were soluble in aqueous were easily extracted (Zhang *et al.,* 2018).

Table 1: The effectiveness of (CLS) produced by *S. thermophiles* versus exam pathogenic bacteria.

Examined isolate	Zone of inhibition (mm)
B. cereus	18
P. aeruginosa	22
Escherichia coli	30
S. aureus	23

In refining of bacteriocin, protein regain was 26-foldnext Ammonium sulphate sedimentation. Dialysis extracted the salts and intensified the protein by 5 millilitres magnitude. Dialyzed protein specimen was the portion *via* Lotion purification and ion-interchange chromatography, utilize DEAE-Sephadex. Lotion Purification and ion-interchange chromatography are shown in Fig 1 and 2.

In the Fig 2, the blue line (y-axis) represents the protein concentration, while the orange line (y-axis) represents the diameter of the inhibition zone indicating antibacterial activity. To evaluate the protein's effectiveness, it was observed at fraction 21, peaking at fraction 25 at the maximum protein concentration of 0.32 mg/ml and gradually declining. Regarding antibacterial activity, the diameter of the inhibition zone appeared at fraction 23 and reached its maximum at fraction 25, approximately 13 mm, before declining after fraction 27.

The results of the strong overlap between protein concentration and inhibition zone diameter indicate the strength of the extracted protein complex. This pattern confirms that the purification strategy succeeded in isolating the bioactive component, which is primarily concentrated between fractions 23 and 27. These results demonstrate the success of isolating effective antimicrobial agents through protein purification.

Fig 3 shows a typical chromatogram for protein separation using ion exchange chromatography, plotted at a wavelength of 280 nm versus the number of eluted fractions. This is a standard method for monitoring protein extraction, as aromatic amino acids (especially tyrosine and tryptophan) absorb strongly at this wavelength, indicating the presence of protein.

The first major peak, from fractions 5-13, represents early eluted protein fractions, which are weakly bound to the ion exchange matrix. The sharp rise and plateau indicate a high protein concentration, as evidenced by an absorbance value approaching or exceeding 3.0. The intermediate region, from fractions 14-27, exhibits a gradual decline in absorbance, with little protein content. This extended low plateau indicates a smaller number of proteins. The second peak (fractions 28-39) is visible from

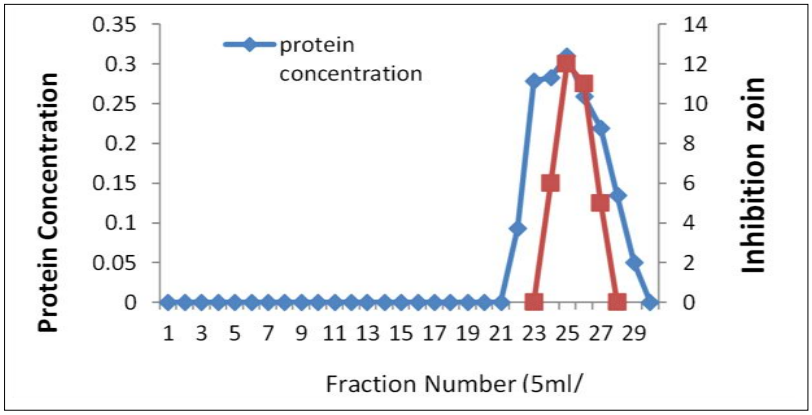


Fig 2: The correlations between protein concentricity and Repression zone (In Lotion Purification).

Table 2: Influence of initial pH and Temp. on bacteriocin output.

Detached	Influence of growth conditions									
	pH			Temp.				NaCl		
Streptococcus	4.5	5.5	6.5	4°C	65°C	80°C	100°C	1	2	3
thermophilus	+	++	+++	+	++	+	+	++	++	+

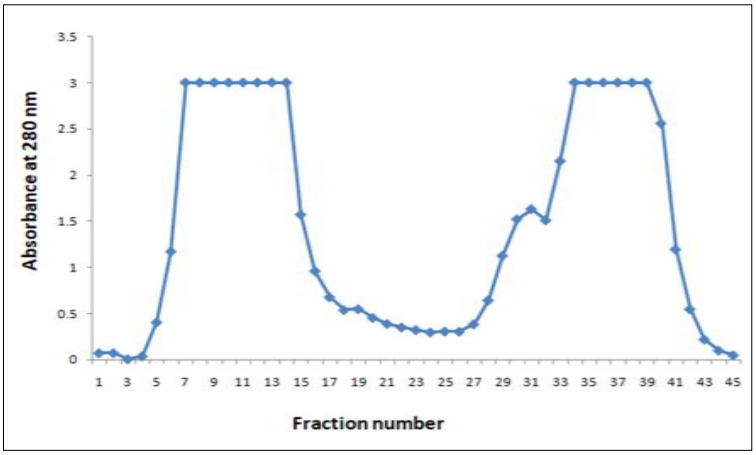
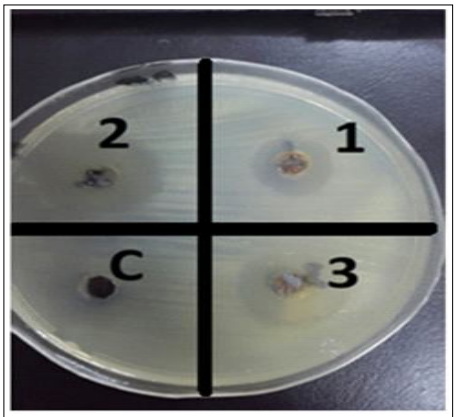


Fig 3: The absorbance of protein crumbs in ion interchange chromatography.



1. Cell Release Supernatant versus *Bacillus;* 2. Cell Release Supernatant versus *E. coli*; 3. Cell Release Supernatant versus *Staphylococcus aureus*; C. Monitoring.

Fig 4: Antibacterial effectiveness of cell release supernatant *streptococcus* versus exam bacteria.

the graph and this peak likely corresponds to proteins that required higher ionic strength.

The results above support (Fig 4) the antibacterial effectiveness of the cell-free supernatant (CFS) obtained from Streptococcus spp. culture is examined in this work against a subset of harmful bacteria, including *Staphylococcus aureus, Bacillus spp*. and *Escherichia coli*. The agar well diffusion technique was used for evaluation. Zone of inhibition (ZOI) studies highlighted the potential of streptococcal-derived metabolites in antimicrobial approaches, which demonstrated variable antibacterial responses (Razni *et al.,* 2024; Geetha *et al.,* 2015).

Description of bacteriocin

Sensitivity of bacteriocin to pH, temperature

The optimal initial pH for higher output of the restrained factor was found to be 6.5 and Temp. was vigorous in (65, 80 and 100) C°, Table 1.

The main environmental factors affecting bacteriocin production by *Streptococcus thermophilus,* such as temperature, pH and sodium chloride concentration, were studied using a semi-quantitative assay. Based on the observed effect, activity was divided into three levels: weak (+), moderate (++) and strong (+++).

The Table 2. shows that the effect of pH on bacteriocin activity was highest at pH 6.5, while the effect of temperature was greatest at 65°C. as foe sodium choride concentration, bacteriocin activity was moderate (++) at concentration of 1% and 2%, but decreased to weak (+) at 3%. This indecates that *Streptococcus thermophilus* can maintain antibacterial activity under moderate osmatic pressure, but esperinces metabolic activity problem as salt concentration increases, possiblydue to decreaesed enzyme effecincy or changes in membrane integrity.

Molecular- weighing definition

Molecular- weighing of the bacteriocin was specific *via* SDS-PAGE. The molecular weighing of the refined bacteriocin effectiveness was 66.9 KDa (Fig 5).

The results showed that the bacteriocin produced by *Streptococcus thermophilus* has antibacterial properties due

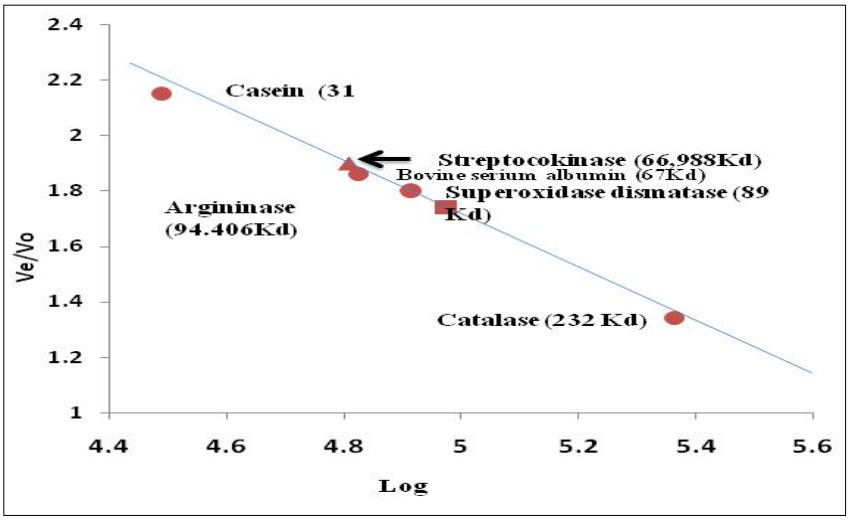


Fig 5: Definition of molecular weighing of refined protein.

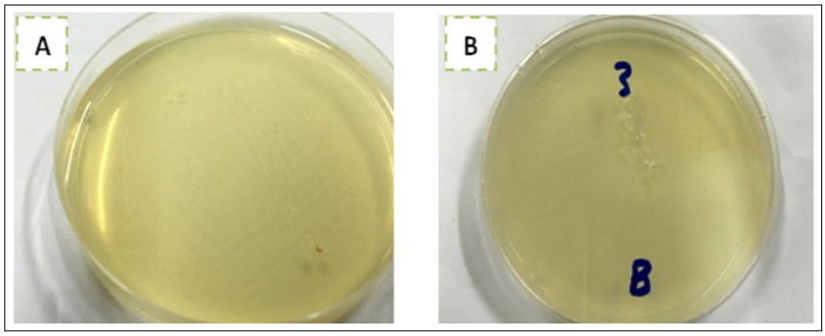
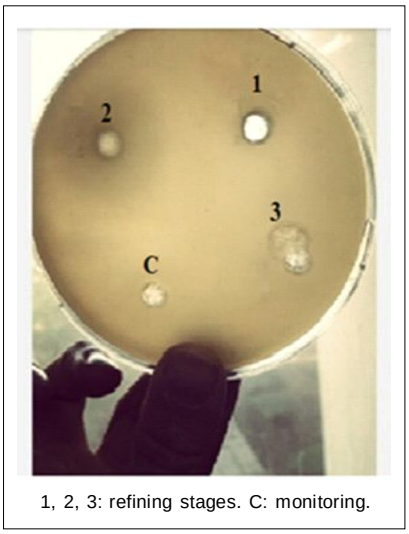


Fig 6: (A) Inchoate dish, (B) *B. cereus* next insinuation to (CFS) for feat of spores.



1, 2, 3: refining stages. C: monitoring.

Fig 7: The restrained impact of bacteriocin during the purification stages next Dialysis.

to its specific types against different pathogens, Gram-positive and Gram-negative bacteria *(Escherichia Coli, Pseudomonas aeruginosa, Staphylococcus aureus*) and this was shown in our study Table 1, (Fig 6,7), these antimicrobial bacteriocins are attributed to the protein compounds, which were stable in different levels of pH and temperature (Sonbol *et al.,* 2020). Bacteriocins are also able to control spore-forming bacteria (Fig 6) which pose challenges in food safety due to their flexibility (Hamad *et al.,* 2025). The effective effect of bacteriocins was demonstrated by their mechanism of action, where there are biological acids and water that have a corrosive effect on bacterial cells that destroy the basic molecular structure of proteins in the cell. They form holes in the sensitive cell membranes and exhaust the possibility of membrane penetration or pH gradient, which leads to leakage of internal components (Sharma *et al.,* 2020; Sharma *et al.,* 2021).

These results highlight the dual role of bacteriocins in treating both pathogenic bacteria and germ-related issues and present them as promising alternatives in combating antimicrobial resistance. Their applications extend beyond food preservation to include roles in veterinary medicine and nanotechnology, where genetically modified bacteria can enhance therapeutic efficacy through targeted delivery systems (Cesa-Luna *et al.,* 2021; Miglani *et al.,* 2023).

In general, bacteriocins produced from *Streptococcus thermophilus* were a valuable resource because they are a safe source of probiotics that promote the overall survival

of LAB in the complex ecosystem and focus on inhibiting the growth of bacteria as an anti-infective drug. Thus, it inhibits the growth and adhesion of harmful microbes through secreted products that change the optimal pH, these secretory components that act as antibacterial production *via* LAB have a broad spectrum of efficacy against bacteria (Girma *et al.,* 2021).

CONCLUSION

These outcomes display the possibility utility of these bacteriocins excuse an additional in-deepness examination for those consistency and implementation as diet bio preservatives. Nevertheless, more overlabour is necessary to completely realize the molecular technique, construct-task associations and exploration of application.

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Conflict of interest

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