ISOLATION OF PROBIOTIC MICROORGANISMS FROM HOMEMADE CHEESE AND STUDY OF ITS ANTAGONISTIC POTENTIAL

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Abstract

Probiotics are live microorganisms that, when administered in sufficient quantities, enhance the health of the host. The probiotic microorganism is also frequently associated with the production of bacteriocins, siderophores, lysozymes, proteases, and hydrogen peroxides that are often responsible for limiting the growth of pathogenic microbes. The present work focusses on isolation of probiotic microbes from homemade cheese products, followed by its characterization and evaluation of its potential application towards aquaculture industry. The microorganism was isolated employing MRS medium and was found to be gram negative coccobacilli in their morphological features. The isolate was determined to be *Acinetobacter indicus* through 16srRNA analysis. This was also confirmed through the biochemical characterization that exhibited the features of *Acinetobacter indicus*. The organism was deposited in GenBank as *Acinetobacter indicus strain CR1* with the accession number OQ736577.1. The organism upon evaluation for probiotic characteristics revealed an auto aggregation activity of 15.4% and cell surface hydrophobicity of 35 %. Further, it didn't exhibit any Hemolytic and DNase activity, while displaying tolerance towards lower pH values and phenol. Also, the organism exhibited considerable antimicrobial activity towards *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia.* The *Acinetobacter indicus strain CR1* displayed substantial antibiotic resistance towards Nalidixic acid and Amphotericin, intermediate susceptibility to vancomycin and found to be sensitive to tetracycline, streptomycin, kanamycin, and ampicillin.

Keyword: Acinetobacter indicus, Gram-negative coccobacillary, 16srRNA, Cell surface hydrophobicity, auto aggregation assays.

Introduction

Probiotics are living organisms that are used as food additives with beneficial effects on the healthy body by setting microbial balance in gastrointestinal tract. [(1)] For at least the past 10 years, probiotic microorganisms have been used continuously for health benefits in both humans and animals. [(2)] Because microorganisms derived from food fermentation has been considered safe and is generally referred to recognize as Safe (GRAS) microorganisms, the majority of lactic acid bacteria (LAB) and *Bifidobacterium* can be generally considered as safe asgenerally for use. [(3)] Food probiotic products because of their nutritional value and health sector over the past parallel to the therapeutic effects are taken into consideration. [(4, 5)] Nevertheless, probiotics, including newly isolated strains, need to be characterized in terms of more safety aspects to ensure they are safe for human consumption (Huys et al., 2013). Lactic acid bacteria (LAB) as protective cultures are common probiotic organisms that are considered safe due to having specific characteristics. These bacteria cause reduction of gastrointestinal diseases by increasing benefit microorganisms' growth and reducing pathogens' population mechanisms. LAB is widely distributed in the environment that can prevent the growth of pathogenic microorganisms by producing substances. [(6)] Main genera of LAB are *lactobacillus, Enterococcus, Lactococcus, Bifidobacterium, Leuconostoc, Pediacoccus and Streptococcus.*

One kind of lactic acid bacteria called *lactobacilli* is regarded as harmless bacteria. These microorganisms are gram-positive, non-spore-forming, catalase-negative, and typically stationary (7). Numerous bacteria, including pathogens and species that cause food spoilage, are highly hostile to *lactobacilli*. The main mechanisms for food preservation involve the creation of secondary metabolites, lactic acid, and the subsequent drop in pH. Some strains can also produceother inhibitors, like bacteriocins, which can help preserve fermented foods (8). During lactic fermentation, lactobacilli create a variety of substances, including organic acids and hydrogen peroxide, which can have an inhibiting effect on a variety of bacteria (9).

A potential probiotic microbe must possess several new characteristics. It comprises surviving the host's stress, staying safe, colonizing the host, engaging in antimicrobial action, andgaining additional health advantages. By evaluating the probiotics tolerance to phenol and low pH, it is possible to measure its ability to endure stress in the host. Cell surface hydrophobicity assays and auto aggregation assays can be used to evaluate a probiotics ability to cling to a host.

Testing for Hemolytic activity, antibiotic resistance, and mammalian cell adhesion can be used to determine safety. By examining the synthesis of antimicrobial compounds and using a co-aggregation assay, antimicrobial activity can be evaluated. These are the prerequisite qualities that bacteria must have in order to be taken into consideration as a possible probiotic organism (bindiya & Bhat, 2017).

The primary probiotic mechanisms of action also include immune system modulation, enhanced epithelial barrier function, increased adhesion to intestinal mucosa, concurrent inhibition of pathogen adhesion, competitive exclusion of pathogenic microorganisms, and production of antimicrobial substances. It depends on the probiotic microorganism's tolerance to acid and bile whether it can survive during passage through the upper gastrointestinal tract, especially in an acidic condition of the stomach (kavitha et al., 2018).

Cheese is a dairy product made from the coagulation of milk protein casein. It comes in a broad variety of flavors, textures, and forms. It contains milk fat and proteins. Cheese has played a significant role in a balanced diet for humans for hundreds of years. It includes all the necessary macronutrients and micronutrients. Due to its high-water content, alkaline pH, and variety of chemicals, cheese makes a good culture media for microorganism growth (settani etal., 2012). In comparison to other dairy products, homemade cheese prepared from raw milk hasa higher genetic

variety and a more intricate microbial habitat. [10] In order to make the casein coagulate, milk is often acidified during manufacture and either rennet enzymes or bacterial enzymes with a comparable activity are added.

In the presence of lactic acid produced by bacteria, casein is coagulated into cheese by the renin enzyme or other enzymes that are comparable to it (11). The liquid whey is subsequently separated from the firm curds, which are finally pressed to create finished cheese.

Unpigmented, oxidase-negative, coccobacillus-shaped microbes make up the Acinetobacter genus. The name of the genus (derived from the Latin "bacter" and the Greek "acinetus," which both indicate "which does not move") derives from this final trait. This genus currently has 63 identified species, the majority of which are non-pathogenic environmental organisms. Initially, this genus's microorganisms were thought to be opportunistic commensals, or low virulent and clinically insignificant. The organism *Acinetobacter* is most used in marine environment as a probiotic such as fish feed etc. mostly this organism adheres to the mid gut of the intestine of fishes which serves as a microflora organism. Some studies suggest that proteolytic and lipolytic enzyme produced by Acinetobacter spp. And other bacteria from dairy foods could beneficially contribute to the taste, odor, or texture of the product. Like other organisms these bacteria are also capable of producing antimicrobial peptides, which act in a competitive niche against their competitors. These compounds can be purified and subsequently used by the food industry as tools to protect against bacteria that cause spoilage in their products, thereby extending their shelf life and maintaining product characteristics without changing the sensory properties of foods. These substances are essential for the food industry particularly since antibiotics cannot be used in these products.

Acinetobacter species produce medicinally and commercially important diverse group of molecules such as Extracellular polysaccharides (ECP). ECP are the alternative class of biothickerners and proved to have good emulsifying property apart from the texture promoting property in various foods.

In this study *Acinetobacter indicus* isolated from the homemade cheese had good property of probiotic function which can be used as a probiotic supplement for the marine animals and humans, and had good antimicrobial properties against pathogenic bacteria.

Materials required

Collection of samples

In this investigation, homemade cheese sample were collected from homemade dairy product shop at Coimbatore. The samples were transported to microbiology lab and maintained there at 4 °C until the experiment started.

Sample preparation and initial culture

Samples were homogenized in a mortar piston and serially diluted up to a concentration of 10^{-6} in distilled water. Then, 1 ml of the 10^{-6} and 10^{-5} sample was distributed on an MRS agar plate (Merck, Germany) for first isolation (de MAN, ROGOSA, and SHARPE). The samples were kept in an anaerobic jar at 37 °C for 24 to 48 hours. The streak plate method was then applied to the samples to produce a single colony, which was cultured on MRS agar for 37–48 hours at 37 °C.

Morphology study

Each colony was first subjected to the Gram's procedure before being viewed and noted using a 100x optical microscope.

Biochemical characterization

Acinetobacter indicus was identified using the Oxidase, catalase, indole, urease, MR, VP,and citrate assays according to *Cappuccino & Welsh, 2017* and Bergey's Manual.

Carbohydrate fermentation

Tests for the fermentation of carbohydrates reveal whether bacteria can do so. 0.2 mg of phenol red was used to create 20 ml of peptone broth. Use an inoculating needle or loop to aseptically dispense the test microorganism into each tube. To avoid bubbles, place Durham's tube into each test tube after adding 0.05 g of lactose to each test tube containing 5 ml of the broth. Incubate tubes for 18 to 24 hours at 35 to 37 °C. To confirm a negative result, additional incubation times may be needed. Positive lactose is indicated by the observation of a colour change from red to yellow.

Molecular identification and sequencing

By using a molecular approach, polymerase chain reaction (PCR) was utilized to identify and confirm the bacterium. The bacterial sample was initially processed to extract DNA in accordance with the instructions provided by the Helini pure fast DNA bacterial genomic DNA mini spin prep kit (Helini biomolecules, Chennai, India). Finally, sequencing of the PCR result was transmitted to applied biosystem, Chennai.

Probiotic characterization screening of isolation

Tolerance to low pH

Selected isolates were cultured in both regular nutrient broth and nutrient broth with a pH range of 2 to 5. To gauge the isolates' low pH tolerance, optical density was evaluated at 0 hours,2 hours, and 24 hours. (Azat et al., 2016)

Auto aggregation activity

Using a spectrophotometer, the auto-aggregation capacities of the isolated culture were assessed in order to assess the adhesion potential to intestinal epithelial cells. Fresh cultures were briefly centrifuged at 4500 rpm for 10 minutes to collect the pelleted cells. They were then cleaned twice with sterile 1 PBS and adjusted to 108 CFU/ml in the same buffer. The adjusted cell suspension was then vortexed for 10 seconds and incubated at 37°C for 24 hours. Aspectrophotometer was used to measure the absorbance (600 nm) at 0, 3, 6, and 24 h in order to observe the auto-aggregation ability. Three technical replicates were used in each of the three times that each experiment was repeated. The following calculation was made to determine the mean value of the outcomes of the three separate experiments:

$$\text{Auto-Agglutination rate (\%)} = 1 - (A_T/A_O) \times 100$$

Were A_T stands for the absorbance at 3, 6, or 24 hours and A_O for the absorbance at zero hours.

Cell surface hydrophobicity

The cell surface hydrophobicity of the chosen isolates was assessed in order to assess their hydrocarbon adhesion capacity. First, a freshly isolated overnight culture was collected by centrifugation at 4,500 rpm for 10 minutes. The pelleted cells were then twice washed with sterile 1 PBS and then resuspended in the same buffer. After that, 2 ml of cell suspension and 2 ml of xylene (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) were combined. The solutions were then vortexed for 10 min. and then left to separate into two phases for 40 min. at 25°C. For the calculation of the cell surface hydrophobicity (%), the lower aqueous phase was carefully absorbed, and its absorbance was measured at 600 nm in triplicate. The formula is as follows:

$$\text{Cell surface hydrophobicity (\%)} = 1 - (A_F/A_O) \times 100$$

In which A_F represents final absorbance and A_O represents initial absorbance.

Phenol tolerance

In nutritional broth with phenol concentrations ranging from 0% to 0.5%, chosen isolates were cultured. To gauge the isolates' phenol tolerance, optical density was evaluated at 0, 2, and 24 hours. (Padmavathi et al., 2018)

Safety assessment

Hemolytic activity

The Hemolytic activity of a bacterium or molecule is its ability to destroy red blood cells, leading to the release of haemoglobin. The chosen isolates underwent further Hemolytic activity testing. On blood agar plates containing 5% (v/v) human blood, all the isolates were streaked andthen incubated for 24 hours at 37 °C. The plates were tested to see if they had haemolysis, non- haemolysis, or neither. [Mathialagan and Others 2018]

DNase activity

DNase agar is a selective medium used to assess an organism's capacity to manufacture deoxyribonuclease, also known as DNase, an exoenzyme that hydrolyzes DNA. DNA, nutrients for the bacteria and an indicator called methyl green are all present in DNA agar. The negatively charged DNA is attracted to the cation methyl green. No zone was visible when colonies were streaked on a DNase agar plate and cultured for 24 hours.

Antimicrobial activity- Broth dilution assay

Antibacterial activity was determined by broth dilution assay. In this method, 5 sterile test tubes with 3ml sterile Luria broth were taken. Different concentrations of samples (50ul, 75ul, 100ul) were added and 30ul inoculum was also added to 4 test tubes. A positive control was also prepared containing Luria broth with 30ul inoculums to indicate the growth promotion capacity of the media. A drop of sterile oil was added to the test tube to maintain an anaerobic condition. Test samples were incubated at 37°C for 24 hours. Calculate the OD value for using UV spectrometer in 600 nm.

Antibiotic Resistance

The following antibiotics were utilized in this study: Vancomycin, Kanamycin, Streptomycin, Nalidixic acid, Ampicillin, and Tetracycline. The targeted strain was first cultivated on Muller Hinton Agar medium with a density of 0.5 McFarland to determine the isolate's susceptibility to the antibiotics. Then, 4 cm apart, antibiotic discs with a particular density were placed on the plate's surface. Following that, the plate was incubated for 24 hours at 37 °C. Following this time, the antibiotic sensitivity and resistance were assessed, along with the diameter of the inhibitory zone surrounding the disc. The positive control strain was additionally cultured on Muller Hinton Agar media.

Gummy Probiotic production

Gummy probiotic manufacturing was changed from Lele et al. (2018). Bovine gelatin (11g), sugar (20 g), glucose syrup (10 g), water (20 g), and skim milk powder with L. plantarum Dad-13 (5 g) were the main ingredients in the formula. The initial cell counts were 7.58109 CFU/g. In a nutshell, the gelatin was bloomed in water for 15 minutes. The sugar solution was made by combining water, glucose syrup, and sucrose and diluting them at 115 to 120 degrees Celsius for 15 minutes. After that, the sugar solution and bloomed gelatin were thoroughly combined, and the mixture was then chilled to a temperature of 40°C. L. plantarum Dad-13- containing skim milk powder was dissolved in water and combined with a candy concoction before the probiotics were added. At the end of the procedure, 0.1

g of citric acid was added to the candy mixture. Agents for flavoring could be added in the desired quantity. The candy mixture was then put into a Mold, allowed to sit for 30 minutes at room temperature, and finishedchilling. After it was finished, the gummy probiotic was placed, sealed, and kept dry until the analysis. It was then packaged.

Results

Isolation of Bacteria from homemade cheese samples

The bacterial isolates obtained from the homemade cheese samples upon culturing in MRS agar is shown in the Fig. 1. A, B. The pure cultures further processed from these are provided in Fig. 1. C, D.

Morphological characterization

The gram staining technique revealed Gram negative coccobacillary shape as displayed in the Fig. 1E.

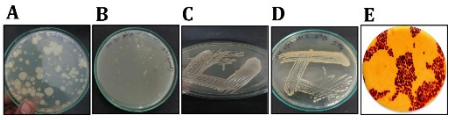

Fig 1. Isolation of bacteria from homemade cheese sample (A)10^{-5} dilution of cheese sample, (B)10^{-6} dilution of cheese sample streak plates, (C) MRS Streak plate (10^{-5}), (D) MRS Streak plate (10^{-6}) and (E) Gram staining of isolate.

Biochemical characterization

The biochemical characterization of the culture isolates demonstrated negative results for Catalase, oxidase, indole, MR, VP, citrate and urease assays (Table 1). Further, the carbohydrate fermentation analysis showed colour change from red to yellow, thus establishing the capability of the culture isolate to ferment all the four sugars.

Table. 1 Biochemical characterization of the isolate

S. No	Biochemical test	Result
1.	Indole	Negative
2.	Citrate	Negative
3.	MR	Negative
4.	VP	Negative
5.	Urease	Negative
6.	Oxidase	Negative
7.	Catalase	Negative

Molecular characterization

The molecular characterization of the isolate through 16srRNA sequencing and further, NCBI's BLAST examination was able to identify the organism as *Acinetobacter inidcus* strain CR1.The sequence of the isolate was then deposited in the gene bank repository of the NCBI with the accession numberofOQ736577.1. Thus, the isolate was deposited as a novel strain that can be referred as *Acinetobacter inidcus* CH1.

Probiotic characterization

The isolated *Acinetobacter inidcus* strain CR1 exhibited prominent tolerance towards lower pH levels of 2,3 & 4. The organism also displayed superior survival rate post 24 hrs incubation in the above-mentioned pH levels. The results are displayed in the Fig. 2a. The organism also exhibited significant

auto aggregation capacity of 15.4 % upon 24 hrs of incubation as displayed in Fig. 2b. Further, the cell surface hydrophobicity for the organism was found to be 35 % in the presence of xylene as depicted in Fig. 2c. The organism also exhibited predominant tolerance towards phenol at 0.5 % as represented in Fig. 2d.

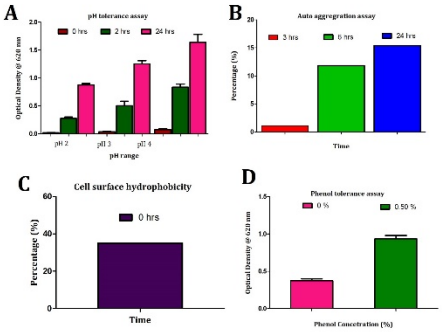

Fig. 2 Probiotic characterization of isolate (A)Tolerance to low pH, (B)Auto aggregation activity, (C)Cell surface hydrophobicity activity and (D)Phenol tolerance.

Safety assessment

The isolated *Acinetobacter inidcus* strain CR1 didn't demonstrate any hemolysis upon its growth in blood agar. Likewise, the organism also didn't show any zone of clearance in the DNase agar plate indicating the absence of DNase activity.

Antibacterial activity

The antibacterial activity of the isolated *Acinetobacter inidcus* strain CR1 against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa* by broth dilution assay. *Acinetobacter inidcus* strain CR1 showed considerable inhibition of the pathogens though not as prominent as the standard antibiotics. The growth inhibition percentage of the pathogens by *Acinetobacter inidcus* strain CR1 is provided in Fig. 3a.

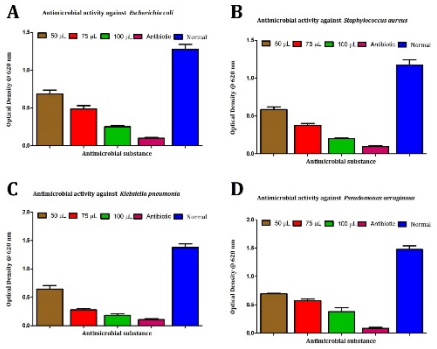

Fig.3 Antimicrobial activity of the isolate. (A) Activity against *Escherichia coli,* (B) Activity against *Staphylococcus aureus,* (C) Activity against *Klebsiella pneumoniae* and (D) Activity against *Pseudomonas aeruginosa.*

Antibiotic resistance activity

Acinetobacter inidcus strain CR1 showed substantial resistance towards amphotericin and nalidixic acid by disc diffusion assay. Likewise, the organism showed intermediate susceptibility for vancomycin and was found sensitive towards tetracycline, streptomycin, kanamycin and ampicillin (Fig 3b).

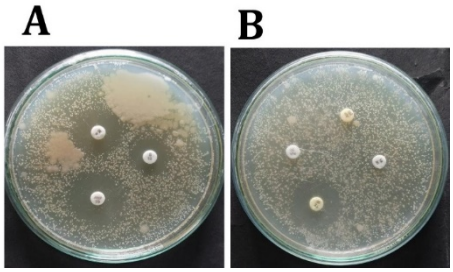

Fig 4. Screening of the isolate against various antibiotics. (A)ampicillin, streptomycin, kanamycin and (B)tetracycline, vancomycin, amphotericin and nalidixic acid

Discussion

Probiotics are living organisms that are added to food as food additives and have positive benefits on a healthy body by restoring gastrointestinal tract microbial equilibrium. Since there is such a wide variety of *Acinetobacter indicus* in food items and various geographic environments, there is also a complex and global distribution of this variety in dairy products. More research is needed in order to develop acceptable strains with particular functional traits. One of the many dairy products with a huge variety of varieties is cheese. One of the cheese varieties that has been researched is homemade cheese.

Acinetobacter indicus was identified morphologically and biochemically in the study's first section, and it was isolated from homemade cheese. The preliminary assessment of the isolate indicates that the coccobacillary-shaped bacteria, which have a variety of cell configurations, are members of the genus *Acinetobacter* in the family *Moraxellaceae* and are gram negative, catalase-negative, oxidase-negative, indole-negative, citrate-negative, MR-negative, VP-negative, and Urease-negative. The biochemical tests followed by validation by sequencing approach, wherein the isolate was identified to be *Acinetobacter indicus*.

Previous studies suggest that there has been considerable *Acinetobacter sp.,* isolation from cheese-oriented products. *Rafei et al.* (2015) used raw cheese to extract *Acinetobacter species* for their research. Acinetobacter species such as *A. baumannii, A. junii, A. haemolyticus, and A. calcoaceticus* were recovered from raw milk by *Fournier et al. in 2006*. From the Domiati cheese, *SAAD, N.M. et al.* (2017) isolated *Acinetobacter species*.

The probiotic characteristics of *A. indicus* were the subject of the second portion of this study. In the course of our research, it was found that the isolate was quite good at tolerating low pH and phenol. Hydrophobicity-induced colonisation and auto aggregation are crucial factors in the probiotic assessment of an isolate. A number of factors, including the charges of human and bacterial cells, their hydrophobicity, extracellular polysaccharides, and protein, come into play when bacteria attach to the human intestinal layer. The isolate has a cell surface hydrophobicity of 35% and an auto aggregation activity of 15.4%. According to the current adherence studies, hydrophobicity and auto aggregation should be at least 15% and 30%, respectively. Thus, from our study it can be concluded that the adherence capability of the isolate was efficient than the result which are already available from other earlier investigation.

The third section of this research focused on the antibacterial activity and antibiotic susceptibility of *A. indicus*. Different bacterial samples, including *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia*, and *Pseudomonas aeruginosa*, were used to test the broth dilution procedure. Thus, the isolate could also prevent illnesses brought on by such common pathogens of the gastrointestinal tract. Also, there are wide reports indicating the capability of *A. indicus* in production of antimicrobials, including bacteriocin, antimicrobial peptides and anti-biofilm substance production.

The present study has shown that the isolated *Acinetobacter inidcus* strain CR1 produces varying susceptibility towards different classes of antibiotics. Previous reports also suggest such pattern, *Gurunget al*. discovered that the majority of *Acinetobacter* isolates isolated from bulk milk tank samples were antibiotic-susceptible. Alternatively, a 2016 study by *Fernando DM et al*., *A. indicus* is resistant to amphotericin, vancomycin, and cefoperazone. Thus, it's difficult to ascertain a uniform antimicrobial characteristic common to that of the organism.

The probiotic utility of the *Acinetobacter indicus* is widely distributed in the aquaculture, wherein several studies have reported its potential activity against common pathogens like *V. harvey* (Caipang et al., 2023). The use of such probiotic supplementations effectively in aquaculture diets to reduce antibiotic adverse effects and increase the productive performance while safeguarding public health.

Conclusion

The newly isolated strain *Acinetobacter inidcus* strain CR1 was found to exhibit potential probiotic activity. The different strain of the same species is not very popularly regarded as probiotics due to the existence of the pathogenic variants. However, some of the variants of the same organisms are also used in aquaculture as prominent probiotics. *Acinetobacter inidcus* strain CR1 was also found to exhibits inhibition of routine pathogens as evident from the in vitro assays. Additionally, the considerable evidence arising recently regarding the beneficial effects of substances produced by *Acinetobacter inidcus* doesn't totally exclude them from the category of beneficial microorganism. Thus, *Acinetobacter inidcus* strain CR1 could be recommended towards potential application, both as probiotic supplements for humans or in aquaculture, though additional validation is necessary to provide definitive conclusion.

Reference

1. Ahamdi S, Khamiri M, Khosroshahi A, Kashani Negad M. Isolation and identification of Lacic acid bacteria from Lighvan cheeses. J Agric Nat Resour. 2008;3(16):80-91.
2. Azizi F, Habibi Najafi MB, Edalatian Dovom MR. The biodiversity of Lactobacillusspp. from Iranian raw milk Motal cheese and antibacterial evaluation based on bacteriocin-encoding genes. AMB Express. 2017;7(1):176.
3. Bindiya, E. S., & Bhat, S. G. (2017).*Bacteriocins BaCf3 and BpSl14 with anticancer and antibiofilm potential from probiotic Bacillus amyloliquefaciens BTSS3 and Bacillus pumilus SDG14 isolated from gut of marine fishes: Enhanced production, Purification and Characterization*[PhD Thesis]. Cochin University of Science and Technology.
4. Bomko, T. V., Nosalskaya, T. N., Kabluchko, T. V., Lisnyak, Y. V., & Martynov, A. V. (2017). Immunotropic aspect of the Bacillus coagulans probiotic action. *Journal of Pharmacy and Pharmacology*,*69*(8), 1033–1040.
5. Caglar E, Kargul B, Tanboga I. Bacteriotherapy and probiotics' role on oral health.Oral Dis. 2005;11(3):131-7.
6. Chikindas ML, Weeks R, Drider D, Chistyakov VA, Dicks LM. Functions and emerging applications of bacteriocins. *Curr Opin Biotechnol.* 2018;49:23–8. doi: 10.1016/j.copbio.2017.07.011.
7. Christopher Marlowe Caipang; Kathleen Mae Trebol; Indra Suharman; Rolando Jr. Pakingking; Joel Jr. Deocampo, et al. ISOLATION OF POTENTIAL PROBIONTS FROM BRACKISHWATER ENRICHED WITH HIGH LEVELS OF CARBON SOURCE 2023-08-01| DOI: 10.55251/jmbfs.9819
8. Conceição CS, Souza BV, Vieira JMBD, Nascimento JS. Pathogen killing pathogen: antimicrobial substance from *Acinetobacter* active against foodborne pathogens. *J Infect Dev Ctries.* 2018;12:297–304. doi: 10.3855/jidc.9894.
9. De Roos NM, Katan MB. Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. Am J Clin Nutr.

2000;71(2):405-11. [DOI:10.1093/ajcn/71.2.405] [PMID]
10. Fernando DM, Khan IUH, Patidar R, Lapen DR, Talbot G, Topp E, et al. Isolation and characterization of *Acinetobacter baumannii* recovered from *Campylobacter* selective medium. *Front. Microbiol.* 2016;7:1871–1871. doi: 10.3389/fmicb.2016.01871. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]
11. FAO/WHO. Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria: Report of a Joint FAO/WHO Expert Consultation 2006; 25 November, 2008.
12. Gurung, M.; Nam, H.M.; Tamang, M.D.; Chae, M.H.; Jang, G.C.; Jung, S.C.; Lim, S.K. Prevalence and Antimicrobial Susceptibility of Acinetobacter from Raw Bulk Tank Milk in Korea. *J. Dairy Sci.* **2013**, *96*, 1997–2002. [Google Scholar] [CrossRef] [PubMed]
13. Hawaz E. Isolation and identification of probiotic lactic acid bacteria from curd and in vitro evaluation of its growth inhibition activities against pathogenic bacteria. Afr J Microbiol Res 2014;8:1919-425.
14. Hojjati M, Behabahani BA, Falah F. Aggregation, adherence, anti-adhesion and antagonistic activity properties relating to surface charge of probiotic Lactobacillus brevis gp104 against Staphylococcus aureus. Microb Pathog.2020;147:104420.
15. Holzapfel et al., 1998; Leroy and Vuyst, 2004; Wessels et al., 2004
16. Ilayaraja R. Antimicrobial activity and characterization of Lactobacillus reuteri isolated from human milk. Indian J Med Sci. 2010;3(1/2):27-33.
17. Islam T, Sabrin F, Islam E, Billah M, Islam Didarul KM. Analysis of antimicrobial activity of Lactobacillus paracasei ssp paracasei-1 isolated from regional yogurt. J Microbiol Biotechnol Food Sci 2012;7:80-9.
18. J Anim Sci Technol. 2016; 58: 26.Published online 2016 Jul 19. doi: 10.1186/s40781-016-0108-2 PMCID: PMC4949924 PMID: 27437119Probiotic isolates from unconventional sources: a review
19. Kavitha, M., Raja, M., & Perumal, P. (2018). Evaluation of probiotic potential of Bacillus spp. Isolated from the digestive tract of freshwater fish Labeo calbasu(Hamilton, 1822).*Aquaculture Reports*,*11*.
20. Koohi MV, Bari MR, Mehrnoosh F, Abad MAK. A research on existence and special activities of *Acinetobacter* in different cheese. *Int J Adv Biol Biomed Res.* 2014;2:517– 25. [Google Scholar] [Ref list]
21. LPSN - List of prokaryotic names with standing in nomenclature. *Genus Acinetobacter.* 2019. [Accessed on: 12 March 2020]. Available at: http://www.bacterio.net/Acinetobacter.html. [Ref list]
22. Muthukumar, P., &Kandeepan, C. (2015). Isolation, identification and characterization of probiotic organisms from intestine of fresh water fishes. *Int J Curr Microbiol Appl Sci*,*4*, 607–616.
23. Nacef M, Lelièvre-Desmas M, Drider D, Flahaut C, Chollet S. Artisanal and industrial Maroilles cheeses: Are they different? Comparison using sensory, physico-chemical and microbiological Approaches. International Dairy Journal. 2019; 89:42-52
24. Pangallo D, Šaková N, Koreňová J, et al. Microbial diversity and dynamics during the production of May bryndza cheese. *Int J Food Microbiol.* 2014;170:38–43. doi: 10.1016/j.ijfoodmicro.2013.10.015. [PubMed] [CrossRef] [Google Scholar] [Ref list]
25. Shokraei SS, Rahimifard N, Shad MM. Comparison Of Three Methods For Evaluation Of The Antimicrobial Activity Of Lactobacillus Acidophilus Bacteria Againstescherichia Coli And Salmonella Enterica Serotype Enteritidis. Iran J PublicHealth. 2014;43(2):167.
26. Siso MG. The biotechnological utilization of cheese whey: a review. Bioresour Technol. 1996;57(1):1-11. [DOI:10.1016/0960-8524(96)00036-3]
27. Smid EJ, van Enckevort FJ, Wegkamp A, Boekhorst J, Molenaar D, Hugenholtz J, et al. Metabolic models for rational improvement of lactic acid bacteria as cell factories. J Appl Microbiol 2005;98:1326-31.

28. Thankappan, B., Ramesh, D., Ramkumar, S., Natarajaseenivasan, K., &Anbarasu, K. (2015). Characterization of *Bacillus spp*. From the gastrointestinal tract of Labeorohita— Towards to identify novel probiotics against fish pathogens. *Applied Biochemistry and Biotechnology*,*175*(1), 340–353.
29. Vancanneyt M, Naser SM, Engelbeen K, De Wachter M, Van der Meulen R, CleenwerckI, et al. Reclassification of Lactobacillus brevis strains LMG 11494 and LMG 11984 as Lactobacillus parabrevis sp. nov. Int J Syst Evol Microbiol. 2006;56(7):1553-7. [DOI:10.1099/ijs.0.64215-0] [PMID]
30. Vijayaram, S., Kannan, S., &Muthukumar, S. (2016). Isolation and characterization of probiotic bacteria isolated from diverse fish fauna of the trodden Vaigai river at Theni district. *Journal of Chemical and Pharmaceutical Research*,*8*(7), 883–889.