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CHARACTERIZATION OF BACTERIOCIN PRODUCER "*LACTOBACILLUS BREVIS* UN" AS POTENTIALPROBIOTIC STRAIN

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ABSTRACT

Present study was carried out to classify a bacteriocin producer bacterial isolate, *Lactobacillus brevis* UN under the category of probiotics. *L. brevis*UN isolated from "*Dhulliachar*" which is a powdered mixture of seeds of *Cucurbita pepo* and *Sesamum indicum* consumed by the people of North East region of India as a condiment of food.Isolate was identified by conventional and molecular techniques. Isolated strain was tested for different probiotic attributes. Lactic acid production and auto aggregation capacity of *L. brevis* UN were 1.44 % and 40.54 % respectively. Isolate showed maximum adhesion of 42 % for xylene, survived at pH 1.0 after 180 minutes, exhibited some degree of bile salt tolerance, showed bacteriocin production potential and found sensitive to most of the antibiotics. Overall cumulative probiotic potential for *L. brevis* UN was 95.83 %. *L. brevis*UN fulfill the criteria of potential probiotic therefore, it can be used by food industries in probiotic preparation.

Keywords: Autoaggregation, Bacteriocin, Bile salt, Hydrophobicity, MRS, Probiotic

INTRODUCTION

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host **(FAO, 2002).**They are also called "friendly bacteria" or "good bacteria"can be used as complementary/alternative medicine and are not presently considered to be part of conventional medicine **(Saikhon and Jairath 2010).** In today's world overuse of antibiotics and lesser consumption of traditional fermented food products by human population have disrupted the beneficial microflora of gastrointestinal tract. Due to lack of beneficial bacteria, GI track is prone to the attack of pathogenic organisms which may cause immune dysfunctions such as **allergies, asthma, eczema, depression, bipolar disease, ADHD** etc. Therefore, probiotics are recommended by doctors and, more frequently by nutritionists, after a course of antibiotics to re-stimulate immune functions (**Green, 2010**). Ingestion of probiotics improves human immune function. Most commonly used probiotics are species of *Lactobacilli* and *Bifidobacteria*. Most probiotic products consist of one or more species of bacteria from one or both of these types.*L. brevis* is widely seen in nature and has been found in fermented foods of both plant and animal origin as well as in human intestinal flora (**Yakabe*et al*., 2009**) and can therefore, be used as a probiotic.
L. brevis has been patented several times. Though, several workers have claimed the effectiveness of *L. brevis*as a probiotic but many strains of *L. brevis* are still unrecognized, as different strains of a species exhibit differences in acid and bile resistance, ability to colonize the gastrointestinal (GI) tract, clinical efficacy, and the health benefits they confer. Therefore, in present investigation an effort has been made to place bacteriocin producing new isolate of "*Dhulliachar*" - *L. brevis* UN into a class of probiotics that could be utilized in dietary supplements.

MATERIAL AND METHODS

Isolation, screening and identification of lactic acid bacteria

Lactic acid bacterial strain was isolated from "*Dhulliachar*"which is being consumed by the people of North East region of India. Isolation was carried out on De Man Ragaosa Sharpe (MRS) agar (Hi-media make) under anaerobic conditions by standard spread plate method **(Aneja *et al*., 2003).** Screening of isolates was done on the basis of morphological, physiological, biochemical characteristics and antagonistic potential **(Gautam *et al*., 2014**; **Barefoot and Klaenhammer 1983**). Colour, form, margin, elevation and texture of each isolated strain were noted down. Gram staining, catalase test, oxidase test, citrate utilization test, gas production from glucose, casein hydrolysis, H_2S production and sugar fermentation were performed with isolated strains by standard microbiological techniques. Serious food borne pathogens/food spoilage bacteria i.e. *Listeria monocytogenes* MTCC 839 and *Leuconostoc mesenteroides* MTCC 107, *Enterococcus faecalis* MTCC 2729, *Lactobacillus plantarum* CRI, *Bacillus cereus* CRI, *Clostridium perfringens* MTCC 1739, *Pectobacterium caratovorum* MTCC 1428, *Escherichia coli* IGMC and *Staphylococcus aureus* IGMC, *Aeromonas hydrophila* IGMC were used to study antimicrobial potential of isolated strains. The test strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India, Central Research Institute, Kasuali, India and Indira Gandhi Medical College (IGMC), Shimla, India. All these test strains were revived twice for 24 h at 37^{0}C before performing the experiments, as all these indicators were preserved in 40 % glycerol at -20^{o}C.The bit disk method **(Gautam and Sharma 2009; Kimura, 1998; Sharma *et al*., 2009**) and well diffusion method **(Del *et al*., 2000)** were used to study antimicrobial potential of isolated strains. The gram positive, catalase-negative rods having maximum antagonistic activity were selected for further studies. Out of 12 isolates UN was selected. On the basis of 16S rRNA gene technique UN identified as *Lactobacillus brevis.* The sequences so obtained were submitted in National Centre for Biotechnology Information (NCBI) to get an accession numbers. *L. brevis* UN registered under the accession no. JX046150 **(Gautam *et al*., 2014).**

Probiotic attributes

Probiotic potential of *L. brevis* UN was studied by evaluating various factors viz., bacteriocin producing potential, lactic acid production, autoaggregation capacity, adhesion to solvents, bile salt tolerance and antibiotic sensitivity.

Bacteriocin production

L. brevis UN (10 %) was inoculated in MRS broth and incubated at 35^{0}C, at 120 rpm for 30 h in orbital shaker. The isolate with 1.99 OD was centrifuged at 18,000 rpm for 20 min at 4^{o}C. The supernatant was filtered and collected in a sterilized test tube. This collected supernatant was neutralized to pH 7.0 (with sterilized 1N NaOH) and catalase was added (2 mg in 20 ml).Well diffusion method was repeated with this preparation against respective indicators. Further bacteriocin activity in cell free supernatants was determined by activity unit per milliliter (AU/ml). Activity unit per ml was determined as the inverse of the last

dilution at which growth inhibition was still detectable following the well diffusion **(Gautam *et al*., 2014; Gautam and Sharma 2009; Kimura, 1998)**

Lactic Acid production

Inoculum preparation

Twenty four h old active culture of *L. brevis* UN was inoculated (1 % v/v) into 10 % sterile reconstituted skimmed milk and incubated at 35 ^{0}C for 72 h. Samples were withdrawn every 24, 48, 72 h interval of incubation period. The pH of cultured reconstituted skimmed milk was measured using pH meter and acidity was determined by titrating cultured reconstituted skim milk against 0.1 N NaOH as given below:

Acidity in terms of lactic acid (Gotcheva *et al*., 2002; Ranganna, 1997)

An aliquot of the sample prepared was diluted with recently boiled distilled water. 2-3 drops of 1% phenolphthalein solution was used as an indicator and titration was done with 0.1 N NaOH. Titre value was noted and calculations were done as percent anhydrous lactic acid.

$$\text{Titratable acidity (\%)} = \frac{\text{Titre x Normality of alkali x Volume made up x Equivalent weight}}{\text{Volume of sample taken x Volume of aliquot taken x 1000}} \text{X } 100$$

Autoaggregation assay(Walker and Gilliland 1993)

The active bacterial culture *L. brevis* UN (25 ml) was centrifuged at 10,000 rpm for 10 min at 4^{0}C. Pellets were collected, washed twice in sterile phosphate buffer saline (PBS ,0.1 M phosphate buffer, 0.8 % NaCl, pH 7.2), re-suspended in 25 ml PBS and mixed by gentle vortexing for 10 s. Absorbance was measured at OD_{620} and incubated for 1h, 2h and 3h at 35^{0}C. Absorbance of upper suspension was measured after each interval. Autoaggregation % was measured as : A0-(At/A0) × 100, where At represents the absorbance at time t=1 h and A0 the absorbance at t = 0 h

Bacterial adhesion to solvents(Walker and Gilliland 1993)

Twenty four h old *L. brevis* UN (25 ml) were centrifuged at 10,000 rpm for 10 min at 4^{0}C and pellets were washed twice in sterile phosphate buffer saline (PBS ,0.1 M Phosphate buffer, 0.8 % NaCl, pH7.2), resuspended in 0.1 M KNO_3 (pH6.2).Initial absorbance was measured at OD $_{620}$ nm as A0. 3ml of cell suspension was taken in three test tubes and solvents viz. xylene, chloroform, ethyl acetate (1.0 ml) was added separately in each test tube. After pre-incubation for 10 min at room temperature, two phases were mixed by gentle vortexing for 2 min followed by further incubation for 20 min at room temperature. The aqueous phase so formed after 20 min was measured at OD_{620} as At. The percent of bacterial adhesion to solvent was calculated as A0- (At/A0) × 100. where At represents the absorbance at time t=1 h and A0 the absorbance at t =0 h.

Acid tolerance (Tambekar and Bhutada 2010)

Twenty four h old active culture of *L. brevis* UN was centrifuged at 10,000 rpm for 10 min at 4^{0}Cand pellets so collected were washed twice in sterile phosphate buffer saline (PBS, 0.1 M Phosphate buffer, 0.8 % NaCl, pH7.2) and re-suspended in PBS. Suspended bacterial cells in PBS were further diluted 1/100 in PBS at pH 1.0, 2.0 and 3.0 and incubated for 1, 2 and 3 h at 37^{0}C. Counts of surviving bacterial colonies were determined after plating the isolate on MRS agar with appropriate pH and incubating them anaerobically at 37 ^{0}C overnight. Control samples without acidification were also prepared and similarly handled.

Bile salt tolerance (Lindgren and Dobrogosz 1990)

L.brevis UN was evaluated for its ability to grow in the presence of bile salt (Hi-media make). 24 h old *L.brevis* UN (1 % v/v) was inoculated into MRS broth supplemented with 0.3 % bile salt and incubated at 37 ^{0}C for different time period i.e. 0 h to72 h. Absorbance was measured at 620 nm during the incubation an increase or decrease in absorbance was used to compare growth of cultures in presence of bile salt.

Antibiotic resistance

Twenty four h old active culture of *L. brevis* UN was seeded on MRS agar plates using swab. Antibiotic–impregnated discs (Hi-media, India) (Table 1) were placed on seeded plates and the zone of growth inhibition was measured after 24h of incubation at 37^{0}C to detect its sensitivity for them **(Halami*et al*., 1999)**

Table 1 Antibiotics used and their concentrations

S. No.	Antibiotic used	Concentration (µg)
1	Ampicillin	10
2	Gentamicin	10
3	Nalidixic	30
4	Chlorophenicol	30
5	Ceflazidine	30
6	Cefotaxime	30
7	Ciprofloxacin	5
8	Oloxacin	5
9	Tetracycline	30
10	Amoxyclov	30
11	Co-trimoxazole	25
12	Cefuroxime	30
13	Vancomycin	30
14	Methicillin	30

Cumulative probiotic potential (Bhakta*et al*., 2010)

The cumulative probiotic potential of *L. brevis* UN was calculated by using standard score card.

RESULTS AND DISCUSSION

Antagonistic potential of *L. brevis* UN

Inhibitory activity of *L. brevis* UN was tested against selected food borne/spoilage causing bacteria viz. i.e. *Listeria monocytogenes* MTCC 839 and *Leuconostocmesenteroides* MTCC 107, *Enterococcus faecalis* MTCC 2729, *Lactobacillus plantarum* CRI, *Bacillus cereus* CRI, *Clostridium perfringens* MTCC 1739, *Pectobacteriumcaratovorum* MTCC 1428, *Escherichia coli* IGMC, *Staphylococcus aureus* IGMC and *Aeromonashydrophila* IGMC. *L. brevis* UN exhibited 60% inhibition against test pathogens **(Gautam *et al*., 2014).** The data on inhibitory spectrum by bit/disc and well diffusion method is shown in Table 2.

Table 2 Antagonistic spectrum of *L. brevis*UN by Bit disc/well diffusion method in terms of zone size

Methods	*L. monocytogenes*	*L. mesenteroides*	*S. aureus*	*L. plantarum*	*B. cereus*	*C. perfringens*	*A. hydrophila*	*E. faecalis*	*E. coli*	*P. caratovorum*	% inhibition
Bit disc method	5 mm	3 mm	5 mm	3 mm	10 mm	1 mm	-	-	-	-	60
Well diffusion method	3 mm	-	8 mm	1 mm	-	3 mm	-	1mm	1 mm	-	60

Antagonistic activity in terms of inhibitory zone.

$$\text{*Percent Inhibition (\%)} = \frac{\text{No of inhibited indicators}}{\text{Total no. of indicators}} \text{X100}$$

Bacteriocin production

Bacteriocin production was estimated in terms of activity units of culture supernatant. The activity units were found to be 2×10^3 AU/ml.

Calculation of AU/ml for crude bacteriocin of *L. brevis* UN

100 µl = 200
1 µl = 200/100
1000 µl = 200/100 x 1000 = 2×10^3 AU/ml

*Arbitrary unit (reciprocal of highest dilution forming detectable zone of inhibition) expressed in the form of AU.

Lactic acid production

Production of lactic acid by LAB is most significant attribute for its use as probiotic strain. Lactic acid is a major end product of fermentation process. Maximum lactic acid is produced during stationary phase. Production of lactic acid during growth phase *L. brevis* UN has been presented in Table 3. The lactic acid production was measured by standard method as described by (**Gotcheva *et al*., 2002).** At 0h, lactic acid production was minimum i.e. 0.27 % and pH was maximum i.e. 5.99 which changes to 0.63 % and (pH 4.33), 1.08 % (pH 4.13) and 1.44 % (pH 4.01) after 24, 48 and 72 h of growth. Statistically correlation studies revealed that there was a negative relationship between the lactic acid concentration and pH during the growth phase (r = -0.8507 for *L. brevis* UN) i.e. lactic acid production is minimum when pH is highest and vice-versa.

Lactic acid bacteria are either homo-fermenters or hetero-fermenters depending upon metabolites they produce. *L. brevis* UN is hetero-fermenters as it produce lactic acid, hydrogen peroxide as well as ethanol **(Kandler, 1983).** Maximum lactic acid is produced during stationary phase. Lactic acid production by LAB's is one of the important criteria for its use as probiotic strain as this acid is a secondary metabolite which often plays an important role in defense mechanism by inhibiting the pathogenic bacteria and thus aids in colonization of LAB. One of the study showed that *Lactobacillus salivarius* capable of producing high amounts of lactic acid, which can inhibit the growth of *H. pylori* in vitro and it was concluded from the study that higher the level of lactic acid production by *Lactobacillus*, the more potent will be the effect on reducing *H. pylori's* urease activity **(Rayan *et al*., 2008).**

Table 3 Estimation of Lactic acid production by *L. brevis*UN

Time	Lactic acid (%)	pH
0 h	0.27	5.99
24 h	0.63	4.33
48 h	1.08	4.13
72 h	1.44	4.01

$$\text{Titratable acidity (\%)} = \frac{\text{Titre x Normality of Alkali x Volume made up x Equivalent weight}}{\text{Volume of sample taken x Volume of aliquot taken x 1000}} \text{X100}$$

Autoaggregation on the basis of sedimentation rate

Table 4 is depicting autoaggregation of *L. brevis* UN on the basis of sedimentation rate for 2 h. Autoaggregation capacity of bacterial isolate was measured by comparing the initial absorbance at 600 nm with absorbance of 1st h and 2nd h. The autoaggregation percentage was measured by A0- (At/A0) × 100. It was noticed that for the 1st h of incubation autoaggregation % for *L. brevis* UN was 27 % and for 2nd h of incubation it was 40.54 %. The autoaggregation ability exhibited by bacteria may contribute on its adhesion property. Such ability of microorganism provide resistance to peristaltic elimination by providing competitive advantage in ecosystem **(Ahire *et al*., 2011).** Above mentioned results showed that *L. brevis* UN exhibited a strong autoaggregating phenotype that is a required character for preparation of probiotic supplements.

Table 4 Autoaggregation* of *L. brevis* UN

Time	OD_{600}♦	**Autoaggregation (%)
0 h	1.85 ± 0	
1h	1.35 ± 0.008	27
2 h	1.10± 0.009	40.54

*Autoaggregation interms of sedimentation rate
♦OD_{600} = Mean (±Standard Deviation) of results from three separate experiments
****Autoaggregation %** =A0- (At /A0) × 100

Bacterial adhesion to solvents

The microbial adhesion to solvents was used to evaluate the hydrophobic/hydrophilic cell surface properties of *L. brevis* UN. The ability to adhere can give information about the possibility of probiotics to colonize and may modulate the host immune system. Cell hydrophobicity is one of factors that may contribute to adhesion of bacterial cells to host tissues. This property could indicate an advantage and importance for bacterial maintenance in the human gastrointestinal tract **(Klayraung *et al*., 2008).** The results in Table 5 indicated that *L. brevis* UN is fully hydrophobic, as it showed 42 % adhesion towards xylene, 22 % towards ethyl acetate while 13.75 % towards chloroform. Bacterial adhesion to xylene, chloroform and ethyl acetate was tested to assess the Lewis acid–base characteristics of the bacterial cell surfaces. *L. brevis* UN showed stronger affinity for xylene, which is a polar solvent, thus demonstrated hydrophobic cell surface of the strain which is highly desirable probiotic attributes. The three different solvents were tested in this study, Out of these xylene reflected cell surface hydrophobicity because it is a polar solvent whereas chloroform- a monopolar acidic solvent and ethyl acetate-a monopolar basic solvent were regarded as a measure of electron donor and electron acceptor. In literature it has been discussed that the presence of (glycol-) proteinaceous material at the cell surface results in higher hydrophobicity, whereas hydrophilic surfaces are associated with the presence of polysaccharides **(Kos *et al*., 2003).**Therefore, it could be said that there is a presence of (glycol-) proteinaceous material on the cell surfaces of *L. brevis* **(Pelletier *et al*., 1997).**

Table 5 Adhesion of *L. brevis* UN to different hydrocarbons

S.No.	Name of hydrocarbon	OD_{600}♦	% Hydrophobicity**
1	Chloroform	1.38± 0.45	13.75
2	Xylene	1.12± 0.012	42
3	Ethyl acetate	1.31 ±0.016	22

♦OD: Mean (±Standard Deviation) of resultsfrom three separate experiments
****Hydrophobicity**%:**A0- (At /A0)

Acid tolerance

To resist acidic pH of gastric juices is an important characteristic of probiotic lactic acid bacteria. Acid tolerance of isolate was studied by suspending bacterial cells in phosphate buffer saline of different pH 1.0, 2.0 and 3.0 following incubation for 60 min, 120 min and 180 min. It was observed from the following experiment that cells of *L. brevis* UN resisted an incubation period of 60 to 180 min at pH 1.0 to 3.0. Table 6 is depicting % survival of *L. brevis* UN at different pH for different time interval. Though, *L. brevis* UN survived at pH 1.0 after an incubation period of 3h but survival was slightly less i.e. 91.87% at pH 1.0 as compare to pH 2.0 and 3.0 where survival % was 91.98 and 94.5% with the same incubation period. In contrast to acidic pH, survival % was 100% in control after 180 min of incubation. So it could be said that isolate *L. brevis*could be possible probiotic candidate bearing acid tolerance properties.

The acid tolerance of LAB is dependent upon the pH profile of H^+-ATPase and the composition of the cytoplasmic membrane, which is largely influenced by the type of bacteria, type of growth media and growth conditions. In one of the study conducted by **Hoque *et al*., (2010),** it was found that a probiotic isolate i.e. *Lactobacillus*-2 was able to grow at low pH (2.2).

Table 6 Acid tolerance of *L. brevis* UN

	Log_{10}cfu/ml*				
S.No.	**0 min**	**60 min**	**120 min**	**180 min**	**% survival after 180 min.**
1	10.21±0.008	9.80±0.024	9.80±0.024	9.38±0.07	91.87
2	10.23±0.004	10.15±0.008	9.91±0.012	9.41±0.09	91.98
3	10.20±0.009	10.21±0.012	10.18±0.008	9.64±0.036	94.50
Control	10.24±0.008	10.26±0.0081	10.23±0.004	10.25±0.012	100

*Log cfu/ml : Mean (± standard deviation) of results from three separate experiments)

Bile salt tolerance

L. brevis UN was compared for its ability to grow in the presence of bile salt (Hi-media make). Twenty four h old, each bacterial culture (1% v/v) was inoculated into MRS broth supplemented with 0.3% bile salt and incubated at 37 ^{0}C for different time period i.e. 0h to 60h. Absorbance at 620nm and viable count was performed during the incubation period after every 4h. Increase or decrease in absorbance and viable count was used to compare growth of culture in presence of bile salt. *L. brevis* UN exhibited some degree of bile tolerance. For *L. brevis* UN OD and log cfu/ml for bile salt treated cells at 0h was 0.084 and 7.81 for control 0.08 and 7.82. It was noticed that after 4thh of incubation the cells growing in presence of bile salt were multiplying with almost at similar rate as that of control as OD and viable count for cells growing in presence of bile salt was 0.112 and 9.32, for control 0.12 and 9.34. At 8th h of incubation OD was 0.44 and cfu/ml was 9.52 for cells growing in presence of bile salt while for control OD was 0.55 and cfu/ml was 9.62. After 12th h of growth the OD for control was found to increase continuously, on 28th h of growth it became 1.99 with 10.18 logcfu/ml. OD of cells growing in presence of bile salt remain lower than control and was almost constant in between the range of 0.44 to 0.48 with 9.53 to 9.56 logcfu/ml upto 48th h of growth. After 56th h growth there was a decline in OD of bile salt treated cells was observed as OD came to 0.02 but for control OD was 1.99. Beside the strong acid media in the stomach, the probiotic microorganisms taken orally have to defend against the bile salt in the gastrointestinal tract. Hence, the bile salt is considered to be one of the most important properties required for high survival and a consequence for a probiotic activity. The physiological concentration of bile salt in the small intestine is between 0.2 and 0.3% (**Walker and Gilllliland, 1993**). Due to this fact, higher acid and bile salt tolerant strains of LAB would act as potential probiotics **Bhakta*et al*., (2010).**

Sensitivity to antibiotics of *L. brevis* UN

L. brevis UN was tested for antibiotic susceptibility / resistivity with antibiotic discs (Hi-media Make). Different antibiotic discs were used viz. ampicillin AMP (10µg), gentamicin GEN (10µg), nalidixic NA(30µg), chloramphinicol CH (30µg), ceftazidine CTZ (30µg), cefatoxime CTX (30µg), ciprofloxacin CFX (5µg), oloxacin OLO(10µg) tetracycline TET (30 µg), amoxyclov AMX (30 µg), co-trimoxazole CO-TRI (25 µg,) cefuroxime CEF (30 µg µg), vancomycin VAN (10 µg), methiocillin MET (10 µg). *L. brevis* UN showed sensitivity against most tested antibiotics and were found resistant against only few viz. nalidixic (30µg), ciprofloxacin (5µg) and oloxacin as shown in (Table 7). *Lactobacilli* are usually sensitive to inhibitors of protein synthesis and resistant to glycopeptides**(Coppola *et al*., 2005; Zhou *et al*., 2005)** but in our study *L. brevis* UN was found sensitive to protein inhibitors viz. ampicillin, chlormphenicol and tetracyclin as well as to glycopeptidesi.e.vancomycin. The antibiotic susceptibility of *L. brevis* UN make it very crucial for the safety point of view to their use as potential probiotics since bacteria used as probiotic may act as potential reservoirs for antimicrobial resistance genes, which can be transferred to pathogenic bacteria. According to world health organization WHO, 2001 and European Food Safety Authority-EFSA, 2008 bacteria used as probiotics for humans or animals should not carry any transferable antimicrobial resistance genes. Thus the susceptibility of our LAB isolate to the clinically important antimicrobials is beneficial as it minimizesthe chances of disseminating resistance genes to pathogens both in the food matrix and/or in the gastrointestinal tract.It could, thus, be concluded that *L. brevis* UN is not reservoirs of transferable resistance genes and can be used to prepare safe probiotic supplements.

Table 7 Antibiotic Sensitivity profile of selected LAB and selected pathogens

Bacterial strains	AMP	GEN	NA	CH	CTZ	CTX	CFX	OLO	TET	AMX	CO-TRI	CEF	VAN	MET
***L. brevis* UN**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	S	S

Cumulative probiotic score of *L. brevis* UN

The probiotic potential of bacterial strains is based upon cumulative probiotic score. Cumulative probiotic potential is the sum of score of acid, bile tolerance, autoaggregation capability, antibiotic resistance and antibacterial activity. Probiotic potential for the isolate was calculated by following formula:

$$\text{Probiotic potential} = \frac{\text{Observed score}}{\text{Maximum score}} \text{X } 100$$

In the present investigation probiotic potential for *L. brevis* UN was 95.83 % Table 8.The commercially available probiotic preparationshas probiotic score in a range of 75 to 85%. The present study revealed that *L. brevis* UN follows the criteria of **FAO/WHO (2002**). Hence this study affirm the use of *L. brevis* UN in the development of new pharmaceutical and functional foods for the betterment of the health of public as these two strains have been proved safe in present study.

Table 8 Calculation of cumulative probiotic effect of *L. brevis* UN

Probiotic characters	Indication	Score
Lactic acid production	Resistant = 1 Sensitive = 0	1
Acidity tolerance	Resistant = 1 Sensitive = 0	1
Autoaggregation capacity	Positive = 1 Negative = 0	1
Bile salt tolerance	Resistant = 1 Sensitive = 0	1
Antagonistic activity	< 3 = 0.25 3-5 = 0.50 5-20 = 0.75 20 = 1	0.75
Antibiotic sensitivity	Antibiotic sensitive =1 Antibiotic resistant =0	1
Total		5.75 /6.0

$$\text{**Probiotic potential} = \frac{\text{Observed score}}{\text{Maximum score}} \text{X100}$$

Probiotic potential = 95.83 %

CONCLUSION

In the present study bacteriocin producer *L. brevis* UN was isolated from rarely reported condiment "*Dhulliachar*. The strain was characterized for its probiotic potential and it showed all the desirable properties of a good probiotic strain viz., bacteriocin production, acid tolerance, bile salt tolerance, autoaggregation capacity, antibacterial activity and antibiotic sensitivity. The encouraging results of the present investigation make this strain a reliable one for obtaining new probiotic preparations.

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