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ARTICLE

Screening of health beneficial microbes with potential probiotic characteristics from the traditional rice-based alcoholic beverage, *haria*

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ABSTRACT Fermented foods are natural habitats of various food-grade microorganisms which not only fortify the food material with bioactive molecules, but they could directly exert health beneficial effect to the consumers. The present study aimed to screen the microbial consortium of *haria* (a traditional alcoholic rice beverage), for therapeutic potentiality. Twenty-nine fermented beverage samples were collected from different areas of Bankura District (West Bengal, India). Initially, 45 dominant bacterial isolates were purified from the collected samples. From these, 3 microorganisms were screened out based on growth and acidification kinetics: these proved to be *Bifidobacterium* sp. (MKK4), *Pediococcus lolli* (MKK21), and *Lactobacillus* sp. (MKK37) isolates. Finally, based on a cumulative probiotic score, MKK4 (*Bifidobacterium* sp.) was selected for further studies. The ubiquitous presence of this strain in the collected samples was confirmed through PCR-DGGE fingerprinting. This strain was considerably stable in simulated acid and bile solutions; it also exhibited strong auto-aggregation and cell surface hydrophobicity of 82% and 53%, respectively. Under conditions of nutrient depletion, the isolate was capable to form biofilm (66.3%). The selected bacterium showed strong antimicrobial activities against *Shigella dysenteriae* MB14, *Salmonella typhi* E 1590, *Micrococcus luteus* ATCC 9341, *Staphyloccus aureus* MB13, *Vibrio cholerae* K510, and *Escherichia coli* ATCC 25922 isolates. These results suggest that the food-borne *Bifidobacterium* sp. MKK4 can be used as potent probiotic agent.
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Introduction

Fermented foods are the products of artisanal food culture which is prevailed even today among the native people. These foods are prepared particularly by the rural women by using simple utensils and locally available plant/animal raw materials. From the very beginning, food fermentation has applied for preservation of locally available excess raw food during harvesting (Ray et al. 2016). Nowadays, scientific exploration established that these foods are nutritive as well as exert medicinal value compared to the raw food. The complex consort of microorganisms and their metabolic interplay result the changes in flavour, aroma, texture, nutritional quality as well as the self-life of the raw foods (Mondal et al. 2016). The acceptance of these fermented foods is increasing but their scientific as well as technological upgradation till now in nascent stage to commercially exploit these advantages, particularly in developing countries. These indigenous fermented foods are now subjected to global attention to explore their hidden resources (nutrient and therapeutic components) as these are experienced by the native people from generation to generation as safe and effective to different ailments (Ghosh 2016).

The prevalent microorganisms in these foods are mainly lactic acid-producing bacteria and yeasts which either come from the environment or from a starter culture. In both cases, microbes participate in food fermentation either singly or as part of a complex microbial population. They remain biochemically active in the intestine and these are historically referred as "healthy microorganisms", and now considered as probiotic microorganisms (Ray et al. 2016). According to the World Health Organization (WHO 2002), probiotics are defined as live microorganisms that when administered in adequate amounts, confer health benefits on the host. Food-originated probiotic bacteria are becoming popular because these are generally regarded as safe (GRAS), biochemically active, secrets different bioactive molecules, do not show pathogenicity and survive from the environmental stresses

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in respect to faecal originated probiotics. Considering the multifaceted health benefits of probiotics, their global market was $27.9 billion in 2011 and it will reach $44.9 billion in 2018 (Starling 2013). Indian probiotic market is valued at $12 million in 2011, is expected to witness a CAGR (Compound Annual Growth Rate) of 11% by 2016 (Sharma et al. 2013). The stability and growth of organism in intestinal lumen are the prerequisite for any probiotic organisms and that can be assessed by many *in vitro* tests like tolerance to the harsh environment (gastric juices or bile), the ability to adhere to the intestinal surface, production of antimicrobial substances, biofilm formation, and the ability to modulate the immune response in the host.

Rice-based fermented foods and beverages are part of the cultural heritage, particularly in Asia. In general, it is believed that the fermented rice is nutritionally more enriched than the raw rice and confers more health benefits over dairy-based products (Ray et al. 2017). Ghosh and co-workers (2014) reported *haria*, an ethnic rice beverage, very popular in East-Central India, has ethnomedicinal importance in certain degenerative and infectious diseases and many gastrointestinal ailments, particularly dysentery, diarrhoea, amoebiasis, acidity, and vomiting. Earlier, a group of food-grade microbes like *Lactobacillus* spp., *Bifidobacterium* spp., *Lactococcus* spp., *Bacillus* spp., and *Saccharomyces* spp. were reported from *haria* (Ghosh et al. 2015a). One isolate from this beverage, *Lactobacillus fermentum* KKL1, has been characterised as a potent probiotic organism (Ghosh et al. 2015b). The role of other dominant microbes in this beverage, as well as their health beneficial impacts is now determining to establish this as a national ethnic drink. The exploitation and consumption of bifidobacteria from this beverage was postulated as safe by the long historical consumption of this fermented product and the growing knowledge about bifidobacterial taxonomy, physiology and health beneficial impacts (Schell et al. 2002). Considering the indigenous microbes from traditional fermented food as natural biological resource for health beneficial impacts, this study aimed to explore some potent food-grade probiotic that can be helpful for the formulation of probiotic food preparation as well as proprietary probiotic medicine.

Materials and Methods

Sample collection and microbiological exploration

Fresh fermented rice beverage (*haria*) samples (n = 29) were collected from different villages of Bankura District, West Bengal, India (23.1645°N, 87.0624°E) in sterile conical flasks with cotton plugs. They were immediately transported into the laboratory under chilled condition. Samples were serially diluted in peptone water and inoculated into sterile selective MRS agar (HiMedia, India) and incubated under slightly anaerobic conditions (78.18% N_2, 19.85% O_2, 0.85% Ar, and 5.04% CO_2) at 37 °C for at least 48 h. Microbial colonies developed into the matrix of the poured solid medium were enumerated on the basis of colony forming units per gram of wet solid substrate (cfu/gwss), and some of them selected according to their larger colony size. These colonies were purified until homogeneity by repeated sub-culturing. The isogenic microbial isolates were preserved in glycerol stock (20% v/v) at -20 °C for further analysis.

Screening and selection of potent isolate

Forty-five different dominant colonies were selected from the collected samples. In a two-step screening procedure, ten and finally three isolates were selected out from these. In the first round, 10 isolates were selected based on growth rate and colony size, and from these, 3 isolates were screened out based on their growth at different temperature and pH, acidic conditions, catalase, microscopic appearance and gram reaction. Finally, one potent isolate was selected based on different probiotic properties, like the growth at simulated salivary, gastric, intestinal fluid, bile salt tolerance capacity, synthesis of α-amylase and glucoamylase, acidification capability. The composition of all the tested fluids and procedure for tolerance study were done according to the description and methodology of Vizoso Pinto et al. (2006). The activity of α-amylase and glucoamylase were measured as per the method of Ghosh et al. (2015a).

Calculation of probiotic potential

The cumulative probiotic potential of the MKK4 isolate was calculated by using standard score card. Cumulative probiotic potential is the sum of score of growth at acidic condition and physiological temperature, production of hydrolytic enzymes, tolerance to saliva, gastric, intestinal, bile, antibacterial and antibiotic resistance profile. Probiotic potential was calculated as observed score divided by maximum score and multiplied by hundred.

$$\text{Probiotic potential} = (\text{Observed score}/\text{Maximum score}) \times 100$$

Genomic DNA isolation and PCR-DGGE fingerprinting

The probiotic isolate MKK4 was selected and identified previously (Ray et al. 2017) based on 16S rRNA sequencing and its occurrence within the collected sample diversity was checked through PCR-DGGE fingerprinting. Initially total genomic DNA was isolated from representative food

samples of four different zones (East, West, North and South zones of Bankura District, West Bengal) by using Hipura Bacterial Genomic DNA purification Kit (HiMedia, India). The extracted genomic DNA was used as a template for PCR amplification by specific GC clamp primer sets, GC clamp was added in the 5' end of the reverse sequence (Bif164-f, 5'-GGGTGGTAATGCCGGATG-3' and Bif662-GC-r, 5'-CGCCCGCCGCGCGCGGCGGGCGGGGCG-GGGGCACGGGGGGCCACCGTTACACCGGGAA-3'). The PCR mixture for 25 µl reaction volume was prepared by using 2X PCR master mix for required number of reactions. In brief, each reaction mixture containing 12.5 µl of PCR master mix, 1 µl of each primer (20 pmol), 1 µl of DNA template (50 ng/µl) and 9.5 µl of sterile Milli-Q water. Samples were amplified in a thermocycler using the following protocol: initial denaturation at 95 °C for 5 min, 10 cycles of denaturation at 94 °C for 30 sec, annealing at 66-56 °C for 30 sec, extension at 72 °C for 30 sec, another 20 cycles of denaturation at 94° C for 20 sec, annealing at 56 °C for 30 sec, final extension at 72 °C for 30 sec, final extension at 72 °C for 7 min, and store at 4 °C). The PCR product of samples was subjected to DGGE analysis following the method of Satokari et al. (2001). DGGE was performed with a DCode electrophoresis system (Bio-Rad) and gels measuring 16 cm × 16 cm × 1 mm. PCR products were loaded onto a 45 to 55% gradient of urea and formamide in a polyacrylamide gel (8%) and electrophoresed at a constant temperature of 60 °C and a constant voltage of 85 V for 16 h. A 100% denaturant corresponds to 7 M urea and 40% (v/v) formamide. Gels were stained with ethidium bromide (0.5 mg/l) in TAE buffer for 20 min, destained in sterile deionized water for 10 min and viewed by UV transillumination.

In vitro probiotic characteristics

Autoaggregation and hydrophobicity assay

The autoaggregation pattern of the isolate MKK4 was measured according to the method of Rahman et al. (2008). For that, cells of target bacterium were inoculated and incubated for 24 h, the selected young cells were mixed vigorously by vortexing for 15 s and allowed to stand at 37 °C. Changes in absorbance were monitored at 660 nm after 4 h. Autoaggregation ability was expressed as relative percentage of autoaggregation (AAg%) and calculated using

Relative percentage of autoaggregation (%) =

$$(A_0 - A/A) \times 100$$

Where, A_0 and A are the absorbance of cultured media at 0 h and after 4 h of intervals, respectively. From the relative percentage of autoaggregation (%) value, organisms are classified into three major groups, *viz*., high autoaggregation (>70%), medium autoaggregation (20-70%) and low autoaggregation (<20%) able strains.

The cell surface hydrophobicity of the isolate MKK4 was determined following the method described by Thapa et al. (2004). Briefly, overnight cell pellet was suspended in 10 ml of Ringer solution (6% NaCl, 0.0075% KCl, 0.01% $CaCl_2$, and 0.01% $NaHCO_3$) and initial absorbance was taken at 600 nm (A^i_{600}). Next, equal volume of n-hexadecane was added to the cell suspension and mixed thoroughly by vortexing for 2 min. The two immiscible phases were separated by 30 min of standby, and absorbance of the lower phase was recorded at the same wavelength (A^f_{600}). The percentage of hydrophobicity of the strain adhering to hexadecane was calculated using the equation:

Hydrophobicity (%) = $(A^i_{600} - A^f_{600}) / A^i_{600} \times 100$

Antibacterial activity

Supernatant of the *Bifidobacterium* sp. MKK4 culture was used to determine the antibacterial activity against enteric pathogens (Badji et al. 2007). The test isolates were *Shigella dysenteriae* MB14, *Salmonella typhi* E 1590, *Micrococcus luteus* ATCC 9341, *Staphyloccus aureus* MB13, *Vibrio cholerae* K510, and *Escherichia coli* ATCC 25922. The antibacterial activity was evaluated by agar well diffusion method. Samples of clear (filtered) culture supernatant (50 µl) were transferred into the wells in the agar plates previously inoculated with the test microorganisms. Ciprofloxacin (20 µg/disc) was used as positive control. The diameters of inhibition zones were measured after an incubation at 37 °C for 24 h.

Biofilm formation and confocal microscopy

To evaluate biofilm formation by the isolate MKK4, young cells were washed with phosphate buffer saline (PBS) and inoculated (1×10^7 cells/ml) in the microtiter plate wells (12 well) containing 2 ml of minimal MRS broth (1/2 strength), and incubated for 90 min at 37 °C (adherence phase). Non-adherent cells were removed by gentle washing with 4 ml PBS (pH 7.0). Next, the well was again filled by fresh minimal MRS broth and incubated anaerobically (5% CO_2) for 48 h at 37 °C. Biofilms were quantified using a slightly modified crystal violate assay procedure as described previously by Stepanović et al. (2000). After staining, the adsorbed crystal violate was eluted using 1 ml of 70% v/v ethanol. The amount of dye solubilised was measured colorimetrically at 590 nm and expressed as directly proportional to biofilm size. The fresh and washed MKK4 biofilm were stained with acridine orange for imaging by confocal microscopy (Olympus BX51, Japan).

Statistical analysis

Collected data were presented as the arithmetic mean of three

Table 1. Primary screening among different dominant isolates present in the rice-based fermented beverage, *Haria*.

Isolate ID	Gram reaction	Morphology	Catalase	Growth at different pH			Growth at different temperature			Length/ diameter (µm)	Acidification capacity (ml)
				pH 3.0	pH 5.0	pH 7.0	10 °C	25 °C	40 °C		
MKK2	+	Rod shaped	–	<0.1	1.0-2.0	1.0-2.0	<0.1	1.0-2.0	>2.0	2.83 ± 0.02/ 1.36 ± 0.03	9.81 ± 0.68
MKK4	+	Slightly irregular rods	–	1.0-2.0	>2.0	>2.0	<0.1	>2.0	>2.0	3.01 ± 0.07/ 1.33 ± 0.04	18.26 ± 0.52
MKK12	+	Slightly irregular rods	–	1.0-2.0	>2.0	>2.0	<0.1	1.0-2.0	>2.0	1.79 ± 0.03/ 0.98 ± 0.02	8.69 ± 0.70
MKK14	+	Irregular rods	–	>2.0	>2.0	>2.0	<0.1	1.0-2.0	1.0-2.0	1.66 ± 0.03/ 1.02 ± 0.01	11.43 ± 0.57
MKK19	+	Slightly irregular rods	–	0.1-1.0	1.0-2.0	1.0-2.0	<0.1	1.0-2.0	1.0-2.0	0.83 ± 0.01/ 0.69 ± 0.02	9.82 ± 0.48
MKK21	+	Coccus	–	1.0-2.0	>2.0	>2.0	0.1-1.0	>2.0	>2.0	1.21 ± 0.04/ 1.18 ± 0.03	15.25 ± 0.73
MKK26	+	Rod shaped	–	<0.1	<0.1	1.0-2.0	<0.1	1.0-2.0	1.0-2.0	2.71 ± 0.04/ 0.76 ± 0.03	10.23 ± 0.82
MKK28	+	Rod shaped	+	1.0-2.0	>2.0	>2.0	1.0-2.0	1.0-2.0	>2.0	0.97 ± 0.04/ 0.63 ± 0.00	13.27 ± 1.04
MKK33	+	Rod shaped	+	<0.1	<0.1	1.0-2.0	1.0-2.0	1.0-2.0	1.0-2.0	2.97 ± 0.05/ 0.71 ± 0.03	15.18 ± 0.29
MKK37	+	Rod shaped	–	1.0-2.0	>2.0	>2.0	<0.1	1.0-2.0	>2.0	1.47 ± 0.02/ 0.82 ± 0.02	16.03 ± 0.91

The screening included the main characteristics like growth at different pH and temperature, acidification value (ml) is the average increase of titratable acidity equivalent to production of lactic acid over the control sample (uninoculated sample), Microbial growth was measured after 48 h as the absorbance of the culture at 600 nm. The growth ability (GA) was categorized on the basis of OD value as - GA < 0.1: no growth ability; 0.1 < GA < 1.0: weak growth ability; 1.0 < GA < 2.0: moderate growth ability, and GA > 2.0: Strong growth ability.

Table 2. Secondary screening of three potent dominant isolates, *viz*., MKK4, MKK21 and MKK37 selected from primary level screening.

	Enzyme production (U/ml)		Tolerance to simulated juices*				Acidification capacity (ml)
	α-Amylase	Glucoamylase	Saliva	Gastric	Intestinal	Bile	
MKK4	23.49 ± 0.62	11.24 ± 0.93	+++++	++++	++++	+++	16.62 ± 0.94
MKK21	14.03 ± 0.58	5.91 ± 0.69	+++++	+++	++++	+++	11.79 ± 0.72
MKK37	27.16 ± 0.91	2.62 ± 0.11	+++++	+++	+++	++++	14.31 ± 0.77

*+++++ = heavy growth (OD>2.00); ++++ = moderate growth (OD 1.5-2.0); +++ = less growth (OD 1.0-1.5)

replicates (mean ± SD). Significant variation was accepted at the level of 5% (*i.e.* $p<0.05$) was measured by student *t*-test using Sigmastat 11.0 (USA) statistical software.

Results and Discussion

Sample collection

Twenty-nine *haria* samples were collected from various parts of Bankura District of West Bengal, India. The principal constituent of this traditional drink is only rice (*Oryza sativa* L., *Poaceae*), that fermented by a group of indigenous microbes originated from the plant based starter. This is healthy drink consumed by the tribal people of rural West Bengal and contains numerous food-grade microorganisms as described by Ghosh et al. (2014).

Screening for some dominant organisms

Forty-five diverse types of colonies were isolated from the 29 *haria* samples using MRS broth medium (HiMedia, India). Among them, 10 isolate (Table 1) were selected primarily according to their larger colony size (>3 mm) after 2 days of growth. Out of these 10 isolates, 3 isolates (Table 2) were selected according to the growth at wide range of temperature and pH values, catalase, and acidification test. These 3

Table 3. Calculation of cumulative probiotic effect of MKK4, MKK21, and MKK37.

Test parameters	Score limits	MKK4	MKK21	MKK37
Growth at acidic condition (pH 3.0)	Heavy growth (>2.0) = 2 Moderate growth (1.0-2.0) = 1 No/less growth (<1.0) = 0	1	1	1
Growth at temperature (25 °C)	Heavy growth (>2.0) = 2 Moderate growth (1.0-2.0) = 1 No/less growth (<1.0) = 0	2	2	1
Acidification value	≥15.0 ml = 1 <14.0 ml = 0	1	0	1
α-Amylase	≥25 U/ml = 2 24-20 U/ml = 1 ≤19 U/ml = 0	1	0	1
Glucoamylase	≥10 U/ml = 2 9-5 U/ml = 1 ≤4 U/ml = 0	2	1	0
Saliva juice tolerance	≥+++++ = 2 ++++-+++ = 1 ≤++ = 0	2	2	2
Gastric	≥+++++ = 2 ++++-+++ = 1 ≤++ = 0	1	1	1
Intestinal	≥+++++ = 2 ++++-+++ = 1 ≤++ = 0	1	1	1
Bile	≥+++++ = 2 ++++-+++ = 1 ≤++ = 0	1	1	1
Sensitivity to tetracycline	≥23 = 3 15-22 = 2 ≤14 = 1	3	3	2
Sensitivity to amoxicillin	≥23 = 3 15-22 = 2 ≤14 = 1	3	3	3
Sensitivity to methicillin	≥23 = 3 15-22 = 2 ≤14 = 1	3	3	3
Antimicrobial against *S. typhi* B3274	≥10 = 3 6-9 = 2 ≤5 = 1	3	3	3
Antimicrobial against *S. faecalis*	≥10 = 3 6-9 = 2 ≤5 = 1	3	3	3
Observed score		27	24	23
Percent of maximum possible score (= 32)		84.37	75.0	71.87

isolates were further characterised according to their tolerance to saliva, gastric and intestinal juice, tolerance to bile salt, α-amylase and glucoamylase production, as well as their acidification capability. The selected three strains were identified on the basis of 16S rDNA sequence as *Pediococcus lolli* MKK21, *Lactobacillus* sp. MKK37, and *Bifidobacterium* sp. MKK4 (Ray et al. 2017). Among them, one potent strain (MKK4) was finally selected based on these enzymatic, probiotic characteristics and acidification capability.

Cumulative Probiotic Score of strain MKK4

The probiotic potential of any bacterial strain is based upon the cumulative probiotic score. Probiotic potential of the selected isolate MKK4 was calculated in the present study and the probiotic potential for MKK4 was 81.82%. Thus, MKK4 qualify the score to be recommended as commercial probiotics (Table 3). The commercially available probiotic preparations have probiotic score in a range of 75-85% (Gautam and Sharma 2015). The present study revealed that MKK4 follows the criteria of FAO/WHO.

PCR-DGGE profiling

Isolation of specific group of organisms by using selective media can almost excluded many members of the microbial

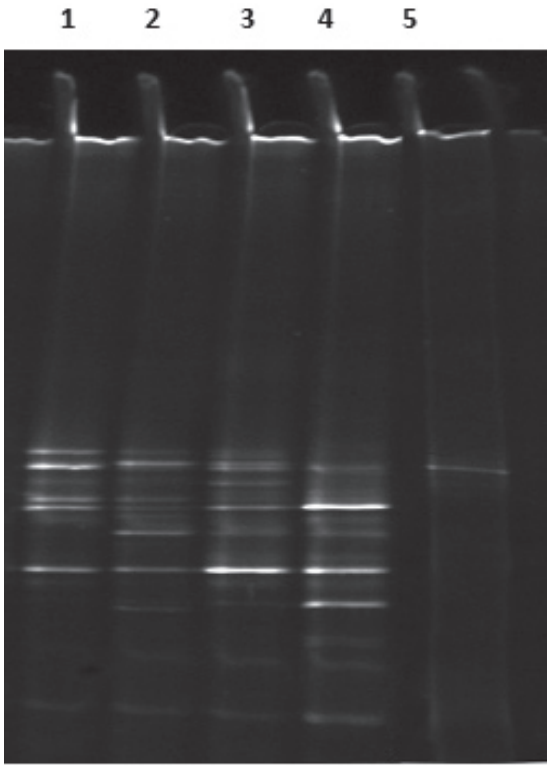

Figure 1. DGGE profiling of fermented beverages (lane 1-4) and isolated strain MKK4 (lane 5).

community (Chen et al. 2008) as, most of the microbes are till now uncultivable. PCR-DGGE based method is a preferred tool for rapid analysis of specific group of cultivable as well as uncultivable microbial community in an ecological niche. To determine the diversity of bifidobacterial strains and occurrence of our isolate in the collected samples, a PCR-DGGE profiling was made using bifidobacterial species specific primers. The DGGE profile showed the isolated organism (MKK4) ubiquitously present in all the collected samples (Fig. 1).

In vitro evaluation of probiotic characteristics

After initial selection and identification, some important properties of the selected strain *Bifidobacterium* sp. MKK4 was assessed *in vitro* for evaluation of its effective probiotic characteristics.

Hydrophobicity and autoaggregation

The relative auto-aggregation of *Bifidobacterium sp.* MKK4 was found to be 82% with the relative cell surface hydrophobicity of 53% with hexadecane. Autoaggregation and hydrophobicity of strain are the two independent but both traits are related with the adhesion of the cell to the intestinal wall (Rahman et al. 2008). These two manners could be used for the preliminary screening to identify potentially adher-

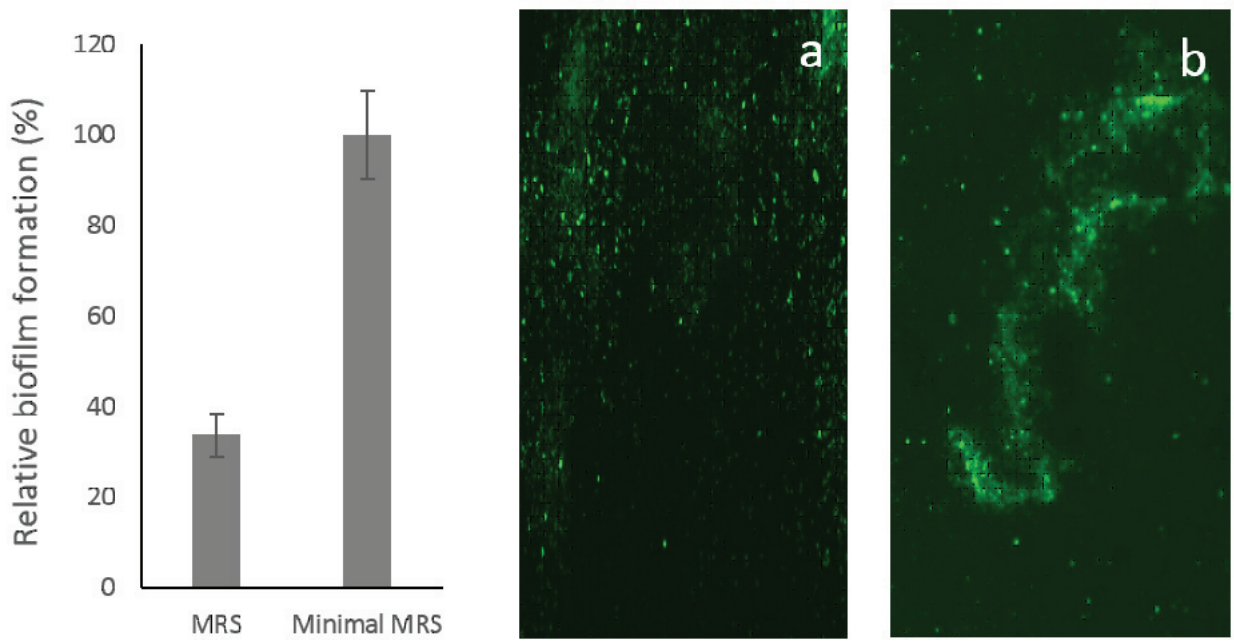

Figure 2. The biofilm formation ability of *Bifidobacterium* sp. MKK4 under minimal nutritional condition (minimal MRS broth) and the planktonic appearance (a) of the isolate compared to the sessile appearance (b) under minimal nutrition (details described in the Materials and Methods section).

Table 4. Antimicrobial activities of *Bifidobacterium* sp. MKK4 against several human pathogens (type strains and indigenous isolates).

Pathogenic bacteria	Zone of inhibition (mm)
Shigella dysenteriae MB14	3.68
Salmonella typhi E 1590	8.21
Micrococcus luteus ATCC 9341	4.51
Staphyloccus aureus MB13	3.94
Vibrio cholerae K510	4.26
Escherichia coli ATCC 25922	5.84

ent isolates (Del Re et al. 2000). Cellular aggregation is an important feature for probiotics as it is related to inter and intra-species microbial interaction as well as interaction with host epithelial cells. Probiotic bacterial autoaggregation can also prevent the pathogen colonization along the intestinal epithelial surfaces. The higher auto-aggregation of the isolate MKK4 led the organisms to form biofilm. Cell surface hydrophobicity of the organism measures the bacterial adhesion to a certain substratum not measure the intrinsic microbial cell surface hydrophobicity (Busscher and Van der Mei 1995).

Biofilm formation

The relative biofilm formation of *Bifidobacterium* sp. MKK4 was tested and found to be induced by 66.3% under nutrient depletion. Microbial biofilm formation seems to be a major mechanism for pathogen exclusion and it is possibly dependent upon the probiotic ability to reduce the environmental pH (Chapman et al. 2014). The relative biofilm formation ability of *Bifidobacterium* sp. MKK4 was comparable with the normal MRS medium and nutrient depletion medium by visualizing microscopic appearance of the cells. The planktonic cells were observed more in MRS broth, whereas, sessile growth was observed in the nutrient depleted condition with the visibly appeared exopolysaccharide (EPS) network (Fig. 2). The phenotypic expression of biofilm is an essential and functional probiotic characteristic generally adapted in unfavourable microenvironment and effective to adhere on mucosal layer.

Antibacterial activity

Lactic acid producing bacteria have inherent ability to fight against enteral pathogens by exerting a group of inhibitory metabolic substances like organic acids, free fatty acids, ammonia, diacetyl, hydrogen peroxide, and bacteriocins. The selected *Bifidobacterium* sp. MKK4 showed the strong antimicrobial activities against *Shigella dysenteriae* MB14, *Salmonella typhi* E 1590, *Micrococcus luteus* ATCC 9341, *Staphyloccus aureus* MB13, *Vibrio cholerae* K510, *Escherichia coli* ATCC 25922 (Table 4).

Conclusion

Ethnic fermented foods are wealth resources of health beneficial microbes and their metabolites. As these foods are consumed from ancient times and generation to generation, their microbial consortia can be generally considered as safe. Present days the probiotic concept derived considering this safety issues and health benefits of the food-grade microorganisms. This study demonstrated that microorganisms isolated from *haria* have variable probiotic activities and among them *Bifidobacterium* sp. MKK4 have been identified as a potential probiotic organism. This study for the first time highlighted the occurrence of probiotic *Bifidobacterium* and *Lactobacillus* in this type of rice-based alcoholic beverage and there is enough scope to exploit their bio-therapeutic potential in the future.

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