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Evaluation of Probiotic Properties of Yeasts Isolated from Sidra - An Ethnic Fermented Fish Product of North East India

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A B S T R A C T

Sidra is a traditional sun-dried fish product of the North Eastern India. A total of 13 probiotic yeasts were isolated and and evaluated on the basis of morphological and biochemical characteristics and were examined for their antagonistic activity against various food borne pathogens i.e., *Staphylococcus aureus, Leuconostoc mesenteroides, Bacillus cereus, Pectobacterium carotovorum, Escherichia coli, Pseudomonas syringae, Enterococcus faecalis, Listeria monocytogenes,* and *Clostridium perfringens.* Based on their maximum antagonistic potential against food borne pathogens two isolates i.e F3 and F6 were characterized using ITS gene sequencing and the isolates were identified as *Debaroymyces hensanii* F3 |MF588674|and *Candida psychrophila* F6 |MF616363|. These two isolates were further screened for their probiotic potential i.e acid and bile tolerane, autoaggregation and coaggregation, hydrophobicity, had been performed. This study affirms the potential of selected probiotic yeast strains as excellent probiotic candidates with exceptional therapeutic effect.

Introduction

Geographically North East (NE) India is located within the Eastern Himalayas and Purvanchal Himalayas.

The Eastern Himalayan region lies between the latitudes 26° 40'-29° 30' North and longitudes 88° 5' - 97° 5' East and covers a total area of 93,988 km2 comprising two North East states, viz. Sikkim and Arunachal Pradesh, besides eastern Nepal, Darjeeling hills in India, Bhutan and Tibetan Autonomous Region in China (Tamang, 2010). Fermented foods have heterogeneousness of traditions and cultural preferences found in the different geographical areas, where they are produced. They have been consumed since ancient times due to their prolonged shelf life, reduced volume, shorter cooking times and superior nutritive value as compared to the non-fermented ingredients. Fermentation processes are considered as developed in order to preserve food (Rolle and Satin, 2002). Ethnic fermented foods and alcoholic beverages and drinks have been consumed by the ethnic people of North East India for more than 2500 years old as per the historical records (Tamang, 2010).

Seafood is atypical to North East India, people catch the available fishes from the various sources and preserve them traditionally (Tamang, 2010). The ethnic people use their indigenous knowledge to preserve the fish, these methods have still being used to preserve fishes which are located near water bodies with plenty of freshwater fishes. These fish products are generally consumed in the daily diet of the people.

These traditionally preserved fish are smoked and sun dried by the traditional people of North East India. *Sidra* is an ethnic fermented sun-dried fish product commonly consumed in the North East India, its pickle is a popular dish, the microbial composition of *sidra* includes bacteria (*L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, *L. plantarum*, *L. mesenteroides*, *E. faecium*, *E. faecalis*, *P. pentosaceus*, and *Weissella confusa*) and yeasts (*Candida chiropterorum*, *C. bombicola*, and *Saccharomycopsis* spp.). Preservation of perishable fishes by the indigenous people residing near the coastal regions is done through fermentation, sun drying, smoking, and salting without refrigeration, and are consumed as seasoning, condiments, and side dishes (Salampessy *et al.*, 2010).

The aim of present work has been to identify the yeast species occurring during fermentation of sidra and to characterize new yeast strains with probiotic potential. The isolated yeasts strains were further tested against various food borne pathogens and their ability to survive gastrointestinal conditions such as high bile salt concentration and low pH. Furthermore their probiotic attributes were also assessed.

Materials and Methods

The current research was divided in some steps, as follows: a) yeast isolation from fermented fish product sidra; b) phenotypic and genotypic characterization; c) Analysis to select the most promising strains, by assessing their probiotic attributes i.e., acid and bile tolerance, autoaggregation and coaggregation, hydrophobicity.

All the chemicals used in the study were purchased from Hi-Media.

Yeast isolation

In total, 31 yeast isolates were isolated from sidra. The fermentation of sidra was conducted by the indigenous people household scale.1gm of sample was diluted with 9 ml of a sterile saline solution (0.9% NaCl) and homogenized for 60 s. The homogenates were serially diluted in saline solution and plated onto the Yeast Universal Medium (YM) (Yeast Extract, 3.0 g/L; Malt Extract, 3.0 g/L; Peptone, 5.0 g/L; Glucose, 10.0 g/L; agar, 15 g/L), incubated at 25 ºC for 2-4 days for the evaluation of yeasts. Plates were incubated for 72 h at 28 °C. Yeasts, showing the typical appearance of *Saccharomyces* and *Candida* (white-to-yellow colonies) and the typical cell appearance at the microscope, were randomly selected and labeled with code names. The selected yeast strains were further purified by successive streaking on YM media. All isolates were maintained at −80 °C in 20% (v/v) glycerol (Hi-Media). Isolates were propagated in YM broth

Identification of sidra yeast isolates to species level by genotypic and phenotypic tests

Phenotypic and biochemical characterization

All the selected yeast isolates were phenotypically characterized according to Kurtzman *et al.*, (2011b), and further biochemical tests were carried out i.e catalase reaction, carbohydrate fermentation, hydrogen

sulphide formation, urease activity and growth at different temperature

Biochemical characterization

Catalase activity

Catalase activity of yeasts was evaluated by adding 3% (v/v) of hydrogen peroxide onto the cultured colonies, according to the Whittenbury method (1964).

The results were expressed as positive (+) or negative (-).

Carbohydrate fermentation

Carbohydrate fermentation was performed for the yeasts isolates following the method of Aneja (2003).

Hydrogen sulphide production

Hydrogen sulphide production was evaluated following the method of Aneja (2003).

Urease activity

This test was performed in the Christensen's urea agar (Oxoid), containing phenol red as pH indicator. After yeast inoculum, plates were incubated at 25º C for 2-7 days; color turning to purple highlighted urea hydrolysis and pH increase.

Growth at different temperature

Effect of different temperature on viability of yeasts isolates was examined by inoculating them in YEDA (yeast extract dextrose agar) broth, and incubating at 20, 25, 30, 35, 40 and 45ºC.

After the incubation period of 24 h, growth was examined for thermo-tolerance of the isolates.

Genotypic tests

Isolation of genomic yeast DNA was performed according to the method described by Cocolin *et al.,* (2000).

Primary screening of probiotic properties of yeasts

Tolerance to low pH

The survival studies under gastrointestinal conditions were determined following the method of Conway *et al.,* 1987

Bile salt tolerance

Tolerance to bile salt of the yeast isolates was determined following the method of Walker and Gilliland, 1993.

Antimicrobial activity

The antimicrobial activity was assessed by bit/disk method.

Spoilage causing microorganisms i.e viz *Staphylococcus aureus* IGMC, *Leuconostoc mesenteroides* MTCC 107,

Bacillus cereus CRI, *Pectobacterium carotovorum* MTCC 1428, *Escherichia coli* IGMC, *Pseudomonas syringae* IGMC *Enterococcus faecalis* MTCC 2729, *Listeria monocytogens* MTCC 839 and *Clostridium perfringens* MTCC 1739, were used to check antagonistic activity of the yeast isolates.

Secondary screening of probiotic yeast isolates

Cell surface hydrophobicity (Mishra and Prasad, 2005)

Assessment of hydrophobicity of cell surface was done based on MATS (Microbial

Adhesion to Solvent), following the method of Mishra and Prasad, 2005.

Cell surface hydrophobicity was calculated following the equation:

$$\text{Hydrophobicity \%} = \frac{[(A-A_0)]}{A} \times 100$$

Where A and A_0, were the absorbance before and after hydrocarbons (xylene, ethyl acetate and chloroform) extraction respectively.

Autoaggregation (Del Re *et al.*, 2000)

Autoaggregation assay was performed as described by Del Re *et al.*, (2000)

$$\text{Autoaggregation \%} = \frac{1- A_t}{A_0} \times 100$$

Where A_t represents the absorbance at time t=1, 2, 3, 4, 5 h and

A_0 the absorbance at t = 0 h (i.e. 0.5)

Co-aggregation (Del Re *et al.*, 2000)

Coaggregation ability of each isolate was determined by following the method described by Del Re *et al.*, (2000)

Co-aggregation % was calculated

$$\text{Coaggregation (\%)} = \frac{[\text{APath+ALAB/2}] - \text{AMix}}{[\text{APath-ALAB/2}]} \times 100$$

Estimation of Siderophore production

Screened probiotic isolates were checked for siderophore production by following the method of Schwyn and Neilands (1987).

Estimation of Exopolysaccharide (EPS) production

Exopolysaccharide (EPS) production was evaluated as described by Mora *et al.*, (2002).

Estimation of β-Galactosidase production (Lin *et al.*, 1989)

β-galactosidase activity was evaluated following the method of Lin *et al.*, 1989

Safety profile of yeast isolates

Antibiotic-resistance

Antibiotic-resistance was carried out with the KirbyeBauer method according to the NCCLS protocol (National Committee for Clinical Laboratory Standard, 1993). The antibiotics used were, Tetracycline (30 µg), Ciprofloxacin (10 µg), Penicillin-G (10 µg), Gentamycin (10 µg), Ofloxacin (10 µg), Erythromycin (15 µg), Clindamycin (10 µg), Fluconazole (25 µg) and Ketoconazole (10 µg)

Haemolytic activity (Marakoudakis *et al.*, 2009)

The haemolytic activities of potential probiotic isolates were determined according to the method of Marakoudakis *et al.*, (2009)

DNase production (Gupta and Malik, 2007)

DNase enzyme production of isolates was determined by following the method of Gupta and Malik (2007).

Gelatinase production (Harrigan and McCance, 1990)

Gelatinase enzyme production of isolates was determined by following the method of Harrigan and McCane (1990).

Results and Discussion

Phenotypic and biochemical characterization

Physiological and Biochemical characterization of the yeasts isolates had been done and their characteristics were noted down in Table 1-3.

Pedersen *et al.,* (2012), reported yeast isolates from Fura, a spontaneously fermented pearl millet product consumed in West Africa, and these were identified by pheno- and genotypic methods. Perricone (2014), isolated probiotic yeasts from cereal based food and beverages following technological and biochemical characterization.

Antimicrobial activity

Probiotic yeast strains isolated from sidra were tested for their antagonistic activity against selected food borne/spoilage causing microorganisms viz. *Staphylococcus aureus* IGMC; *Leuconostoc mesenteroides* MTCC 107; *Bacillus cereus* CRI; *Pectobacterium carotovorum* MTCC 1428; *Escherichia coli* IGMC; *Pseudomonas syringae* IGMC; *Enterococcus faecalis* MTCC 2729; *Listeria monocytogens* MTCC 839; *Clostridium perfringens* MTCC 1739. The data on inhibitory spectrum of probiotic yeast by bit/disc method had been shown in Table 4.

Genotypic tests

The best selected yeast isolates on the basis of their broadest antagonism against pathogenic bacteria were identified at genomic level by using ITS gene technique. Genomic DNA of two best yeast isolates was isolated by following the method of Hashem *et al.,* (2010).

Primary screening of probiotic properties of yeasts

Tolerance to low pH

To resist acidic pH and remain viable under gastric juices is an important characteristic of probiotic microorganisms. Acid tolerance of the screened potential probiotic isolates was studied by suspending bacterial cells in phosphate buffer saline of different pH 1.0, 2.0 and 3.0 followed by their incubation for 0, 60,120 and 180 min.

It was observed in this experiment that cells of all the isolates could tolerate an incubation of 60 to 120 min at pH 1.0 to 3.0. (Table 5).

Fig.1 Neighbor-joining with 1000 bootstrap values in MEGA 6.0 showing phylogenetic relationship of *Debaryomyces hansenii* F3 based on a distance matrix analysis of 16S rRNA sequences

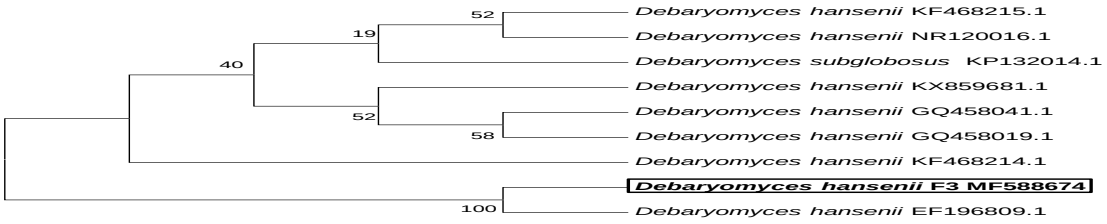

Fig.2 Neighbor-joining with 1000 bootstrap values in MEGA 6.0 showing phylogenetic relationship of *Candida psychrophila* F6 based on a distance matrix analysis of 16S rRNA sequences

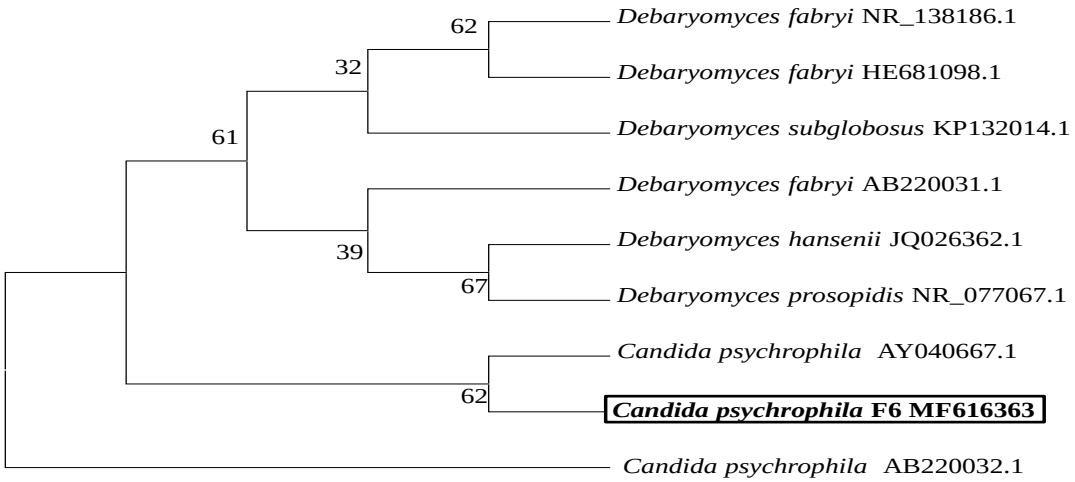

Table.1 Morphological characteristics of probiotic yeasts isolates

S. No	Isolate	Source	Color	Surface	Margin	Elevation	Cell-shape
1	YS	Sidra	Cream	Round	Undulate	Convex	Round
2	YS1	Sidra	Cream	Round	Undulate	Convex	Ellipsoid
3	Y5	Sidra	Cream	Round	Entire	Convex	Oval
4	Y5(A)	Sidra	Cream	Round	Entire	Raised	Ellipsoid
5	Y4	Sidra	Cream	Smooth	Entire	Raised	Ellipsoid
6	F1	Sidra	Cream	Wrinkled	Entire	Convex	Rough
7	F2	Sidra	White	Smooth	Undulate	Convex	Rough
8	F3	Sidra	White	Rough	Undulate	Raised	Ellipsoid
9	F4	Sidra	Yellow	Rough	Etire	Raised	Ellipsoid
10	F5	Sidra	Yellow	Smooth	Entire	Convex	Rough
11.	F6	Sidra	Cream	Wrinkled	Entire	Flat	Ellipsoid
12.	F7	Sidra	Cream	Wrinkled	Entire	Raised	Ellipsoid
13.	F8	Sidra	Yellow	Rough	Undulate	Convex	Ellipsoid

Table.2 Biochemical characteristics of probiotic yeasts isolates

Sr. No.	Isolate	Catalase test	Carbohydrate utilization	H_2S production	Urease test
1.	YS	-ve	AG^-	-ve	-ve
2.	YS1	+ve	AG	-ve	-ve
3.	Y5	-ve	AG^-	-ve	-ve
4.	Y5(A)	-ve	AG	-ve	-ve
5.	Y4	-ve	AG^-	-ve	-ve
6.	F1	+ve	AG^-	-ve	-ve
7.	F2	+ve	AG	-ve	-ve
8.	F3	-ve	AG^-	-ve	-ve
9.	F4	-ve	AG	-ve	-ve
10.	F5	+ve	AG^-	-ve	-ve
11.	F6	-ve	AG^-	-ve	-ve
12.	F7	+ve	AG	-ve	-ve
13.	F8	+ve	AG^-	-ve	-ve

Table.3 Growth characteristics of probiotic yeasts isolates from traditional fermented food matrices of North-East India

S.No	Isolate	Growth temperature				
		25ºC	30 ºC	35 ºC	37 ºC	40 ºC
1.	YU	+	+	+	-	-
2.	YU1	+	+	+	-	-
3.	YU2	+	+	+	-	-
4.	U1	+	+	+	-	-
5.	U2	+	+	+	-	-
6.	YS	+	+	+	+	-
7.	YS1	+	-	-	-	-
8.	Y5	+	+	+	+	-
9.	Y5(A)	+	+	+	-	-
10.	Y4	+	-	-	-	-
11.	F1	+	+	+	+	-
12.	F2	+	+	+	-	-
13.	F3	+	+	+	-	-
14.	F4	+	-	-	-	-
15.	F5	+	+	+	+	-
16.	F6	+	+	+	+	-
17.	F7	+	+	+	-	-
18.	F8	+	+	+	+	-

Table.4 Preliminary screening of probiotic yeasts isolated traditional fermented food matrices of North-East India on the basis of their antagonistic pattern against test indicators by bit/disc method

Sr. No.	Name of isolate	Source	*S. aureus* (mm)	*L. mesenteroides* (mm)	*B. cereus*(mm)	*P. carotovarum* (mm)	*E. coli* (mm)	*P. syringia* (mm)	*E. feacalis* (mm)	*L. monocytogens* (mm)	*C. perfringenes* (mm)	Mean	Percent Inhibition (%)
1.	YS	Sidra	-	-	-	-	-	-	-	-	-	-	-
2.	YS1	Sidra	-	-	-	-	-	-	-	-	-	-	-
3.	Y5	Sidra	-	-	9	-	-	-	-	-	-	1	11.11%
4.	Y5(A)	Sidra	-	-	-	-	-	-	-	-	-	-	-
5.	Y4	Sidra	14	-	-	-	-	9	-	-	-	2.55	22.22%
6.	F1	Sidra	12	-	-	-	-	10	-	-	-	2.44	22.22%
7.	F2	Sidra	-	-	-	-	-	-	-	-	-	-	-
8.	F3	Sidra	15	10	-	-	-	20	17	15	16	10.33	66.66
9.	F4	Sidra	9	-	-	9	18	9	-	-	-	5	44.44%
10.	F5	Sidra	9	-	-	-	17	-	-	-	-	2.88	22.22%
11.	F6	Sidra	17	18	11	20	-	-	15	18	20	13.22	77.77
12.	F7	Sidra	-	-	-	-	-	-	-	-	-	-	-
13.	F8	Sidra	-	-	-	-	-	-	-	-	-	-	-

Table.5 Acidity tolerance of screened potential probiotic isolates

***1. Debaryomyces hansenii* F3**

pH	Incubation time (h)								
	Cell survival (log CFU/ml)*					**% Cell survival			
	0	60	120	180	Mean	60	120	180	Mean
1.0	0.00	0.00	0.00	0.00	0.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
2.0	6.23	4.07	0.00	0.00	2.57	44.67 (41.94)	0.00 (0.00)	0.00 (0.00)	14.89 (13.98)
3.0	7.30	5.62	5.00	5.00	5.73	61.69 (51.76)	54.94 (47.83)	55.55 (48.18)	57.39 (49.25)
Control	9.23	9.11	9.10	9.00	9.11	100 (90)	100 (90)	100 (90)	100 (90)
Mean	5.69	4.7	3.52	3.5		51.59	38.73	38.88	
$CD_{0.05}$	Treatment (0.09) Incubation Time (0.09) TxI (0.17)					Treatment (0.05) Incubation Time (0.06) TxI (0.10)			

*log CFU/ml: Mean of results from three separate experiments
**% Survivability = (log cfu pH $_{1.2.3/}$ log cfu $pH_{.65}$) × 100
# Transformed values (Arcsign transformation)

2. *Candida pyschrophila* F6

pH	Incubation time (h)								
	Cell survival (log CFU/ml)*					**% Cell survival			
	0	60	120	180	Mean	60	120	180	Mean
1.0	**8.19**	**4.94**	**0.00**	**0.00**	**3.28**	**50.40 (45.22)**	**0.00 (0.00)**	**0.00 (0.00)**	**16.8 (15.07)**
2.0	**8.23**	**7.07**	**4.0**	**0.00**	**4.82**	**72.14 (58.14)**	**45.66 (42.51)**	**0.00 (0.00)**	**87.36 (33.55**
3.0	**8.30**	**7.11**	**7.00**	**0.00**	**5.60**	**72.55 (58.40)**	**79.90 (63.36)**	**0.00 (0.00)**	**99.18 (40.58)**
Control	**9.81**	**9.80**	**8.76**	**8.55**	**9.23**	**100 (90)**	**100 (90)**	**100 (90)**	**100 (90)**
Mean	**8.63**	**7.23**	**4.94**	**2.13**		**73.77**	**56.39**	**25**	
$CD_{0.05}$	**Treatment (0.09) Incubation Time (0.09) TxI (0.18)**					**Treatment (0.05) Incubation Time (0.06) TxI (0.10)**			

*log CFU/ml: Mean of results from three separate experiments
**% Survivability = (log cfu pH $_{1.2.3/}$ log cfu $pH_{.65}$) × 100
# Transformed values (Arcsign transformation)

Table.6 Bile salt tolerance of potential probiotic microorganisms

***1. Debaryomyces hensanii* F3**

Bile salt concentration	Incubation time						
	Cell survival (log CFU/ml)*				Cell survival (%)**		
	0	4	8	Mean	4	8	Mean
0.3	6.66	6.64	6.29	6.53	72.01 (58.05)	68.44 (55.82)	70.22
0.5	6.20	6.19	6.15	6.18	67.13 (55.01)	66.92 (54.88)	67.02
1.0	6.09	6.06	6.00	6.05	65.72 (54.16)	65.28 (53.89)	65.5
1.5	6.00	6.00	0	4	65.07 (53.77)	0.00 (0.00)	32.53
2.0	0	0	0	0.00	0.00 (0.00)	0.00 (0.00)	0.00
Control	9.45	9.22	9.19	9.28	100 (90)	100 (90)	100
Mean	5.73	5.68	4.60		61.65 (51.83)	50.10 (42.43)	
$CD_{0.05}$	Treatment (0.09) Incubation Time (0.12) TxI (0.21)				Treatment (0.05) Incubation Time (0.08) TxI (0.12)		

*Log cfu/ml: Mean of results from three separate experiments
**% Survivability = (log cfu/ml 0.3,1,2%bile salt/ log cfu /ml0%bile salt) × 100
#Transformed values (Square root transformation)

2. *Candida psychrophila* F6

Bile salt concentration	Incubation time						
	Cell survival (log CFU/ml)*				Cell survival (%)**		
	0	4	8	Mean	4	8	Mean
0.3	8.22	8.12	8.12	8.15	79.60 (63.14)	80.07 (63.48)	79.83 (63.31)
0.5	8.05	8.00	8.00	8.00	78.43 (62.32)	78.89 (62.64)	78.66 (62.48)
1.0	8.03	8.00	8.01	8.01	78.43 (62.32)	78.99 (62.71)	78.71 (62.51)
1.5	7.56	7.55	7.44	7.51	74.01 (59.34)	73.37 (58.93)	73.69 (59.13)
2.0	7.27	7.22	7.00	7.16	70.78 (57.27)	69.03 (56.18)	69.90 (56.72)
Control	10.34	10.20	10.14	10.22	100 (90)	100 (90)	100 (90)
Mean	8.24	8.18	8.11		80.20 (65.73)	80.05 (65.65)	
$CD_{0.05}$	Treatment (N/A) Incubation Time (0.16) TxI (N/A)				Treatment (N/A) Incubation Time (0.12) TxI (0.18)		

*Log cfu/ml: Mean of results from three separate experiments
**% Survivability = (log cfu/ml 0.3,1,2%bile salt/ log cfu /ml0%bile salt) × 100
#Transformed values (Square root transformation)

Table.7 Adhesion of probiotic microorganisms to different hydrocarbons

Isolates	OD_{600}*				% Hydrophobicity**			
	Xylene	Chloroform	Ethyl acetate	Mean	Xylene	Chloroform	Ethyl acetate	Mean
D. hansenii F3	0.22	0.22	0.13	0.19	78	78	87	81
C. psychrophila F6	0.19	0.27	0.22	0.22	81	73	78	77.33
Mean	0.12	0.29	0.20		88	70.11	79.22	
$CD_{0.05}$	T (0.06) I (0.10) TxI (0.18)				T (0.79) I (1.36) TxI (2.35)			

*OD: Mean of results from three different experiments
**Hydrophobicity % = $[(A-A_0)/A] \times 100$

Table.8 Estimation of autoaggregation potential of screened probiotic isolates

Isolates	OD_{600}#						**Autoaggregation (%)					
	1h	2h	3h	4h	5h	Mean	1h	2h	3h	4h	5h	Mean
D. hansenii F3	0.04	0.03	0.03	0.03	0.03	0.03	96.0	97.0	97.0	97.0	97.0	96.8
C. psychrophila F6	0.03	0.05	0.05	0.03	0.03	0.03	97	95.0	95.0	97.0	97.0	96.2
Mean	0.05	0.04	0.03	0.02	0.02		95	95.88	96.88	98	98	96.75
$CD_{0.05}$	I(0.007) T(0.010)) IxT (0.022)						I (0.736) T (0.987) IxT (2.208)					

*Autoaggregation in terms of sedimentation rate
#OD_{600} = Mean of the results from three separate experiments
**Autoaggregation % = $1-(A_{t=1,2,3,4 \text{ and } 5h}/A_{0h}) \times 100$

Table.9 Evaluation of Co-aggregation ability of potential probiotic isolates with test indicators

	Co aggregation (%)			
Isolates	***C. perfringens***	***L. monocytogenes***	***B. cereus***	**Mean**
***D. hansenii* F3**	29.23	23.3	46.96	33.16
***C.psychrophila* F6**	45	52.72	21.31	39.67
Mean	34.15	30.70	34.23	

Bile salt tolerance

Resistance to bile salt (oxbile) concentration of screened potential isolates was also studied at different concentrations i.e., 0.3,0.5, 1.0, 1.5 and 2.0 % by viable cell count method during the gastrointestinal transit (at 4^{th} h and 8^{th} h) as the residence time of food in our body is of about 4 h Table 6.

Secondary screening of probiotic yeast isolates

Cell surface hydrophobicity

Adherence of cells is usually related to their cell surface characteristics. Cell surface hydrophobicity is a nonspecific interaction between microbial cells and host. The initial interaction may be weak, followed by adhesion processes that are mediated by more specific mechanisms involving cell surface proteins and lipoteichoic acids (Rojas *et al.,* 2002; Ross and Jonsson, 2002) (Table 7).

Autoaggregation

Autoaggregation ability determines the capacity of the probiotic strain to interact with itself in a nonspecific way. Aggregation between cells of the same strain (autoaggregation) or between different species and strains (coaggregation) is of considerable importance and is one of the key factors to check the ability of the probiotic strain to adhere to the oral cavity and gastrointestinal tract, where probiotics are to be active and provide their beneficial effects (Nikolic *et al.,* 2010) (Table 8).

Autoaggregation assay was performed as described by Del Re *et al.,* (2000)

Co-aggregation (Del Re *et al.,* 2000)

Probiotic and pathogenic microorganisms have been reported to form joint aggregate and the process is known as coaggregation (Surono, 2004). Probiotics are able to achieve coaggregation with pathogenic microorganisms and in turn effectively inhibit and kill them by secreting antimicrobial compounds that act directly on the cells of pathogenic bacetria (Bao *et al.,* 2010) (Table 89).

In the present study, 18 yeast strains were isolated, and characterized from sidra based on their morphological and biochemical characteristics and among them, two i.e., F6 and F3 were potential producers of antimicrobial substances against various food borne pathogens. The selected strains exhibited outstanding production of antimicrobial activity. The above mentioned results support the use of native probiotic yeasts strains in starter cultures or as preservative agents which can be achieved by inhibition of contamination causing food borne pathogens.

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