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Full Length Research Paper

Screening of indigenous yeast isolates obtained from traditional fermented foods of Western Himalayas for probiotic attributes

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Twenty three indigenous isolates of yeast (*Saccharomyces cerevisiae*) obtained from various traditional fermented foods and traditional inocula of Western Himalayas (Himachal Pradesh) were subjected to *in vitro* probiotic tests. All the isolates were found to be intrinsically tolerant to upper gastrointestinal transit and this property was isolate dependent. Reduction in viability (in terms of log CFU/ml cells) was more in simulated gastric juice of pH 2 as compared to pH 3. These isolates were also investigated for surface hydrophobicity and autoaggregation abilities. Nine yeast isolates produced exopolysaccharide and four exhibited antioxidative activity using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay (in methanol and buffered methanol reaction systems). Interestingly, one indigenous yeast isolate (Sc15) was found positive for siderophore production, whereas none of the isolates was positive for bile salt deconjugation activity (towards glycine and taurine conjugated bile salts) and galactosidase enzyme production.

Key words: Probiotics, yeast isolates, simulated gastric and intestinal juices, Western Himalayas, antioxidant, exopolysaccharide.

INTRODUCTION

Fermented food products are essential components of diet in a number of developing countries and are consumed either as beverage, main dish or condiment, which contribute to one third the diet of people worldwide (Campbell-Platt, 1994). A variety of indigenous fermented food products and beverages of Western Himalayas (Himachal Pradesh) have been documented (Kanwar et al., 2007). Traditionally, fermented foods are processed through naturally occurring microorganisms; however, modern conventional methods of production generally exploit the use of defined starter culture to ensure consistency and the quality of the final product (Ross et al., 2002). Cultures and species involved in fermented foods do not pose any health risk, and thus are designated as 'GRAS' (generally recognized as safe) organisms (Adams, 1999; Nout, 2001; Hansen, 2002). Therefore, some of the species of these microorganisms because of their long history of safe use in food products can be implemented as protective cultures or probiotics. Fermented foods are rich sources of these probiotics or functional microorganisms which can antagonize the growth of some of the spoilage and pathogenic microorganisms present in these foods (El-Gazzar and Marth, 1992; Vignola et al., 1993; Chiang et al., 2000). Yeasts are the most common and important microorganisms associated with the fermented foods (Yarrow, 1998).

Mostly, selected strains of bacteria belonging to *Lactobacillus* and *Bifidobacterium* genera are used as probiotics (Prasad et al., 1998), however, species belonging to the genera *Lactococcus, Enterococcus,*

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Saccharomyces (Salminen and von Wright, 1998; Dunne et al., 1999; Sanders and in't Veld, 1999), and *Propionibacterium* (Grant and Salminen, 1998) are also considered as probiotic microorganisms. Some yeast strains such as *Saccharomyces cerevisiae* and *Saccharomyces boulardii* have also been used as probiotics in humans for many years because they exert some influence on the intestinal flora (Czerucka et al., 2007; Kumura et al., 2004). Food is the common delivery system for probiotic microorganisms. Probiotic microorganisms that are delivered through food systems have to first survive during the transit through the upper gastrointestinal tract, and then persist in the gut to provide beneficial effects for the host (Chou and Weimer, 1999). Given that fermented food products can contain probiotics, prebiotics or both, it is not surprising that their consumption has long been associated with good health.

In our laboratory, yeasts obtained from traditional fermented foods of Western Himalayas have already been characterized by using traditional and molecular tools (Pathania et al., 2010). However, these were not explored with respect to their probiotic diversity. The present study was therefore, conducted to screen these yeast isolates obtained from the traditional fermented foods of Western Himalayas, for various probiotic attributes.

MATERIALS AND METHODS

Twenty three indigenous isolates of yeast (Sc01 – Sc23) obtained from various traditional fermented foods (*chilra, babru, and bhaturu*), alcoholic beverages (*aara, chhang, chuli, faasur, lugari*) and traditional inocula (*phab, dhaeli, khameer*) of Western Himalayas, available with the Department of Microbiology, College of Basic Sciences, CSKHPKV, Palampur (H.P.) India, were used in the present investigation. These isolates were identified by employing traditional and molecular tools (Pathania et al., 2010).

Preparation of simulated gastric and small intestinal juices

Simulated gastric juice was prepared by dissolving pepsin (Merck Specialities Pvt. Ltd, Mumbai, India) in sterile saline (0.85% w/v) to a final concentration of 3 g/L. The pH of simulated gastric juice was adjusted to 2.0. Similarly, simulated intestinal juice was prepared by dissolving pancreatin from porcine pancrease, USP specifications (Sigma-Aldrich Inc. USA) to a final concentration of 1 g/L and its pH was adjusted to 8. These juices were then filter sterilized through millipore filter assembly using 0.22 µm Durapore membrane filter.

Preparation of washed cell suspension

The isolates were incubated in potato dextrose broth (PDB) at 37°C for 18 h and centrifuged at 2500 x g at 4°C for 10 min. The cell pellet obtained was washed three times in PBS buffer solution (pH 7.0). The collected cells were resuspended in sterile saline and viable count was determined by serial dilution method on PDA plates prior to the assay of transit tolerance.

Upper gastrointestinal transit tolerance assay

The tolerance of washed cell suspensions of yeast isolates to simulated gastric and small intestinal transit was determined by following the method of Charteris et al. (1998). For screening gastric transit tolerance, 0.1 ml aliquot was removed after every 30 min of intervals (upto 4 h) for determining the viable count. The small intestinal transit tolerance was evaluated by determining the viable count after 1, 4, and 8 h intervals.

Effect of ox bile on the growth rate

The effect of ox bile (HiMedia Laboratories, Pvt. Ltd., Mumbai, India) on the growth of yeast isolates was measured on the basis of time required to increase the absorbance at 620 nm by 0.3 units in PDB-thio broth with and without 0.3% ox bile (Walker and Gilliland, 1993). The difference in time (h) for attaining desirable absorbance between both culture media was considered as the lag time (LT) (Usman and Hosono, 1999).

Bile salt deconjugation activity

The ability of the yeast isolates to deconjugate bile salts was determined according to the method of Taranto et al. (1995) and Vinderola and Reinheimer (2003). Separate bile salt plates were prepared by adding 0.5% (w/v) of sodium salts (Calbiochem) of taurocholic acid (TC), taurodeoxycholic acid (TDC), glycocholic acid (GC) and glycodeoxycholic acid (GDC) to PDA, autoclaved (121°C, 15 min) and immediately used. The isolates were spot inoculated on the media and the plates were anaerobically (GasPak System-Hi Media) incubated at 37°C for 72 h. The presence of precipitated bile acid around colonies (opaque halo) was considered a positive result.

Microbial adhesion to hydrocarbon (MATH)

The test of adhesion to hydrocarbon n-hexadecane was adopted to screen yeast isolates for their cell surface hydrophobicity property. Microbial adhesion to hydrocarbon (MATH) in terms of the cell surface hydrophobicity (% H), was determined according to the method of Rosenberg et al. (1980) with slight modification as described by Vinderola and Reinheimer (2003).

Autoaggregation ability

Autoaggregation assay was performed as described by Collado et al. (2008) with minor modifications. Yeast isolates were grown at 37°C for 24 h in PDB. The cells were harvested by centrifugation and suspended in phosphate buffered saline (PBS) to 0.5 optical density (O.D.) units at 600 nm. 5 ml of this yeast suspension was incubated at 37°C for 20 h and then 3 ml of the upper suspension was carefully transferred to another tube and the O.D. was measured at 600 nm. Percent autoaggregation ability was calculated as:

1- (O.D. upper suspension/O.D. total bacterial suspension) × 100.

Production of exopolysaccharide

Exopolysaccharide (EPS) production was evaluated as reported by Mora et al. (2002). Overnight cultures were streaked on the surface of plates containing ruthenium red milk (10% w/v, skim milk powder, 1% w/v, sucrose and 0.08 g/L ruthenium red, 1.5% w/v agar). After incubation at 37C for 24 h, non-ropy strains gave red colonies due to the staining of the microbial cell wall, while ropy isolates appeared as white colonies.

Total antioxidative activity (TAA)

Preparation of whole cell extracts and intracellular cell free extracts

The yeast isolates were grown in PDB at 37°C for 24 h and harvested by centrifugation at 10000 × g at 5°C for 15 min. For the preparation of intact cells, cells were washed three times with phosphate buffered saline (PBS)/water and then were resuspended in the same. Total cell numbers were adjusted to 10^9 CFU/ml for subsequent preparation of whole cell and intracellular cell-free extracts. Ultrasonic disruption (B. Braun Biotech International, Germany) was performed for five times at 1-min intervals in an ice bath. The sonicated cell lysate was divided into two parts. In one part, cell debris was removed by centrifugation at 7800 × *g* for 10 min, and the resulting supernatant was the intracellular cell-free extract. In the second part, the cell debris was not removed and the resulting lysate was used as whole cell extract.

1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay

1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay (Sharma and Bhat, 2009) in methanolic and buffered methanol systems employing 200 µM of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) (Sigma-Aldrich Inc. USA) solution was used for screening yeast isolates for free radical scavenging (antioxidant) activity. Butylated hydroxytoluene (BHT) a standard antioxidant was used as positive control in all antioxidant assays. Protein concentration in the yeast extracts was estimated by using standard Lowry method (Lowry et al., 1951) with bovine serum albumin (BSA) as a standard. Dry weight of the yeast bacterial extracts used for antioxidant assays was also determined.

Galactosidase enzyme production

For qualitative assay of β-galactosidase, 100 mM solution of isopropyl β-D-1-thiogalactopyranoside (IPTG, Sigma) was prepared by dissolving 2.383 mg of IPTG in 100 ml of sterile double distilled water. Consequently 50 mg/ml solution of X-gal (Sigma) was also prepared in N, N' dimethyl formamide. Both the solutions were filter sterilized through 0.2 µm (Millipore) filters. For the detection of cultures producing this enzyme, 100 and 20 µl of IPTG and X-gal solutions, respectively, were spread plated on the surface of PDA plates aseptically. After that the test cultures were spot inoculated on these plates and incubated at 28°C for 24 to 48 h. Observations were then recorded as blue/white colored colonies.

Production of siderophore

Screening for siderophore production was done by the method of Schwyn and Neilands (1987). The glassware used for this experiment was acid rinsed and then three times rinsed with double distilled water. The plates prepared by adopting the aforementioned method, were spot inoculated with the test yeast cultures and incubated at 28°C for 48 h. Siderophore producing strain of *Pseudomonas aeruginosa*-MTCC 2581, was used as positive control for this trait. Formation of yellow-orange zone around the colony on blue colored medium plates was taken as positive result.

Statistical analysis

Statistical analysis of the obtained data was carried out by using WindoStat software version 8.0**.** The data were subjected to one way Analysis of Variance (ANOVA) at the significance level of 5%. The comparison of mean values was done with Duncan's Multiple Range test.

RESULTS AND DISCUSSION

Tolerance to simulated gastric and small intestinal juices

One procedure generally described concerning selection of probiotic microorganisms is the *in vitro* screening based on the capacity of microorganisms to survive in the simulated conditions of the digestive tract as it is an indispensable trait for a probiotic to act. The tolerance studies on 23 yeast isolates were conducted in simulated gastric juice of pH 2 (Table 1) and 3. At pH 2 of simulated gastric juice, the reduction in viability was more (2.71 to 4.12 log CFU/ml) as compared to reduction in pH 3 (0.88 to 3.06 log CFU/ml) after 240 min exposure. Lowest reduction in viability was noticed for Sc19 isolate indicating its high tolerance to gastric juice. All the yeast isolates exhibited more survival in the gastric juices as compared to bacterial isolates (Sourabh et al., 2010). This may be due to various factors like cell size, composition of cell wall etc. as reviewed by Czerucka et al. (2007). The tolerance of 23 yeast isolates in simulated intestinal juice of pH 8 is presented in Table 2. The overall reduction of 0.08 to 0.76 log CFU/ml in viability after 8 h exposure was noticed for all the indigenous yeast isolates, where lowest reduction was observed for Sc02 isolate and highest for Sc04 isolate. These food borne yeast isolates of *S. cerevisiae* demonstrated high tolerance to simulated human upper gastrointestinal tract juices, and thus they offer a relatively overlooked source of potential probiotics, apart from bacteria and *S. boulardii.* The tolerance to acidic pH has already been reported for *S. cerevisiae* strains (Psomas et al., 2001). Thus, indigenous yeast isolates used in the present study fulfill the preliminary *in vitro* selection criteria for being designated as potential probiotics.

Effect of ox bile on the growth rate of yeast isolates

The effect of ox bile on the growth rate of yeast isolates is shown in Table 3. Eight isolates showed a lag time of less than 0.5 h, indicating that they are fairly resistant to tested concentration (0.3%) of ox bile. In addition, the ability to withstand ox bile was noticed in all the indigenous yeast isolates though the lag time (except the aforementioned nine isolates) was more than 1 h. Several other workers have also reported the survival of yeast strains in the presence of bile (Psomas et al., 2001; van der Aa Kqhle et al., 2005). Gotcheva et al. (2002) similarly assessed probiotic potential of four lactic acid bacteria (LAB) and three yeast strains isolated from a traditional Bulgarian cereal-based fermented beverage

Table 1. Survival of indigenous yeast isolates in simulated gastric juice of pH 2.

S/N	Isolate	Viable count (log CFU/ml)						
		0 min	30 min	60 min	90 min	120 min	180 min	240 min
1	Sc01	10.93 (0.01)	10.87 (0.02)*	9.98 (0.01)**	9.48 (0.01)**	8.22 (0.02)**	7.67 (0.03)**	7.08 (0.02)**
2	Sc02	10.90 (0.02)	10.74 (0.02)**	9.48 (0.02)**	8.75 (0.03)**	8.21 (0.07)**	8.05 (0.04)**	7.73 (0.03)**
3	Sc03	10.79 (0.02)	9.99 (0.02)**	9.07 (0.02)**	8.83 (0.03)**	8.04 (0.03)**	7.66 (0.02)**	7.16 (0.03)**
4	Sc04	10.97(0.02)	10.23 (0.02)**	8.85 (0.04)**	8.55 (0.02)**	8.26 (0.03)**	7.86 (0.03)**	7.04 (0.03)**
5	Sc05	10.87 (0.02)	10.66 (0.02)**	9.89 (0.02)**	8.77 (0.01)**	8.25 (0.02)**	7.86 (0.03)**	7.06 (0.03)**
6	Sc06	10.88 (0.01)	10.68(0.03)**	9.82 (0.02)**	9.05 (0.01)**	8.89 (0.02)**	8.04 (0.02)**	7.26 (0.02)**
7	Sc07	10.94 (0.02)	10.89 (0.01)**	10.87 (0.01)*	9.66 (0.03)**	8.8 (0.01)**	7.76 (0.03)**	6.82 (0.06)**
8	Sc08	10.95 (0.01)	10.75 (0.02)	10.24 (0.01)**	10.02 (0.02)**	9.15 (0.03)**	8.04 (0.03)**	7.66 (0.02)**
9	Sc09	10.75 (0.05)	10.59 (0.02)**	10.04 (0.05)**	9.46 (0.05)**	9.14 (0.02)**	8.45 (0.02)**	7.29 (0.05)**
10	Sc10	10.81 (0.04)	10.12 (0.02)**	9.93 (0.02)**	8.8 (0.01)**	8.14 (0.03)**	7.85 (0.04)**	7.76 (0.03)**
11	Sc11	10.88 (0.02)	10.45 (0.03)**	9.91 (0.02)**	9.49 (0.04)**	8.76 (0.02)**	8.06 (0.02)**	7.55 (0.03)**
12	Sc12	10.67 (0.03)	10.59 (0.03)	10.16 (0.04)**	9.46 (0.01)**	8.94 (0.02)**	8.45 (0.02)**	7.76 (0.03)**
13	Sc13	10.89 (0.01)	10.84 (0.01)	9.49 (0.02)**	8.88 (0.01)**	8.26 (0.03)**	7.96 (0.01)**	7.9 (0.01)**
14	Sc14	10.64 (0.04)	10.61±(0.04)	9.46 (0.030)**	9.05 (0.01)**	8.87 (0.01)**	8.15 (0.04)**	7.75 (0.02)**
15	Sc15	10.85 (0.03)	10.36 (0.02)**	9.44 (0.04)**	9.01 (0.01)**	8.27 (0.03)**	8.2 (0.03)**	7.65 (0.03)**
16	Sc16	10.8 (0.02)	10.77 (0.02)	10.7 (0.02)**	9.56 (0.02)**	9.02 (0.02)**	7.9 (0.02)**	7.84 (0.02)**
17	Sc17	10.75 (0.04)	10.53 (0.03)**	9.74 (0.03)**	9.48 (0.02)**	8.89 (0.04)**	7.77 (0.04)**	7.69 (0.04)**
18	Sc18	10.74 (0.03)	10.25 (0.02)**	9.62 (0.03)**	9.55 (0.02)**	9.06 (0.04)**	8.36 (0.02)**	7.96 (0.01)**
19	Sc19	10.59 (0.04)	10.54 (0.04)	9.57 (0.04)**	9.16 (0.02) **	8.75 (0.03)**	8.16 (0.02)**	7.86 (0.03)**
20	Sc20	10.95 (0.04)	10.86 (0.04)	10.04 (0.05)**	9.75 (0.04)**	8.83 (0.05)**	8.05 (0.04)**	7.81 (0.01)**
21	Sc21	10.77 (0.02)	10.64 (0.02)**	9.56 (0.03)**	8.94 (0.03)**	8.45 (0.03)**	7.85 (0.04)**	7.31 (0.01)**
22	Sc22	10.8 (0.01)	10.71(0.01)**	10.55 (0.03)**	8.93 (0.01)**	7.81 (0.01)**	7.72 (0.01)**	6.95 (0.03)**
23	Sc23	10.8 (0.02)	10.7 (0.02)	9.56 (0.02)**	9.02 (0.02)**	8.65 (0.02)**	8.07 (0.03)**	7.76 (0.01)**

Results are shown as mean (± standard deviation), Number of replications=3; Independent sample, testing of significance of variances by F -test, significance testing of two means by using Fishers t-test and Cochran and Cox t-test ,*P < 0.05 but > 0.01; ** P< 0.01.

and reported variable tolerance to low pH (2.0 to 3.0) and high bile concentrations (0.2 to 2.0%). van der Aa Kqhle et al. (2005) investigated 18 *S. cerevisiae* strains isolated from various foods or beverages, and found that all yeast strains were able to withstand pH 2.5 and 0.3% oxgall which are important probiotic traits. One isolate that is Sc03 showed augmented growth in the presence of ox bile with a lag time of just -0.13 h. It is difficult to assign any particular reason for this behaviour of isolate Sc03 as authors could not find appropriate kinetic studies on yeast isolates in presence of bile salts.

Bile salt deconjugation activity

Deconjugation has been included by the World Health Organization (WHO) experts as one of the main activities of intestinal microorganisms (FAO/WHO, 2002). For qualitative assaying of the deconjugation activity, the formation of opaque or whitish halo zone around the colony due to the release of free bile acids on deconjugation of added bile salts was taken as an indication of deconjugation ability of an organism as reported by Dashkevicz and Feighner (1989). None of the 23 yeast isolates was able to show precipitation/deconjugation activity on PDA plates supplemented with 0.5% (w/v) of various glycine and taurine conjugated bile salts; however, all isolates could grow in the presence of these bile salts, a property which needs to be ascertained further.

Microbial adherence to hydrocarbon

After tolerating upper gastrointestinal transit, the next challenge for an effective probiotic is to adhere to small intestinal cells. Adhesion is a complex trait that could be a multistep process in which both non-specific and specific mechanisms play a role. Cell surface hydrophobicity is considered to be an important factor in the adhesion and proliferation of microorganisms on the intestinal epithelial cells (Del Re et al., 1998). The adsorption of microbes to hydrocarbons and their partitioning in a hydrocarbon-aqueous biphasic system,

Table 2. Survival of indigenous yeast isolates in simulated intestinal juice (pH 8).

S/N	Isolate	Viable count (log CFU/ml)			
		0 h	1 h	4 h	8 h
1	Sc01	6.91 (0.01)	6.89 (0.03)	6.81 (0.02)**	6.72 (0.01)**
2	Sc02	7.01 (0.02)	6.99 (0.02)	6.97 (0.02)*	6.93 (0.02)**
3	Sc03	7.28 (0.03)	7.15 (0.03)**	7.02 (0.02)**	6.94 (0.01)**
4	Sc04	5.81 (0.02)	5.39 (0.05)**	5.15 (0.03)**	5.05 (0.02)**
5	Sc05	6.87 (0.01)	6.83 (0.02)	6.81 (0.01)**	6.72 (0.01)**
6	Sc06	7.06 (0.02)	7.05 (0.02)	7.01 (0.02)*	6.98 (0.01)*
7	Sc07	6.65 (0.07)	6.60 (0.04)	6.54 (0.02)	6.48 (0.04)*
8	Sc08	6.52 (0.02)	6.50 (0.01)	6.37 (0.02)**	6.21 (0.02)**
9	Sc09	6.50 (0.02)	5.45 (0.01)*	6.37 (0.02)	6.26 (0.02)*
10	Sc10	6.65 (0.07)	6.65 (0.07)	6.57 (0.02)	6.17 (0.04)**
11	Sc11	6.86 (0.07)	6.78 (0.07)*	6.69 (0.02)**	6.66 (0.04)**
12	Sc12	7.60 (0.02)	7.58 (0.02)*	7.44 (0.02)**	7.39 (0.01)**
13	Sc13	7.82 (0.01)	7.41 (0.01)**	7.40 (0.02)**	7.35 (0.01)**
14	Sc14	5.81 (0.02)	5.66 (0.02)**	5.48 (0.02)**	5.11 (0.04)**
15	Sc15	7.01 (0.02)	7.01 (0.02)	6.97 (0.01)*	6.89 (0.02)**
16	Sc16	7.22 (0.02)	7.06 (0.02)**	6.87 (0.02)**	6.78 (0.02)**
17	Sc17	5.48 (0.01)	5.44 (0.02)	5.11 (0.01)**	5.04 (0.01)**
18	Sc18	6.76 (0.01)	6.55 (0.02)**	6.34 (0.02)**	6.15 (0.03)
19	Sc19	5.89 (0.02)	5.79 (0.02)**	5.75 (0.03)**	5.62 (0.03)**
20	Sc20	5.75 (0.02)	5.66 (0.03)	5.62 (0.01)	5.46 (0.01)**
21	Sc21	5.98 (0.02)	5.92 (0.08)*	5.82 (0.04)**	5.68 (0.01)**
22	Sc22	5.82 (0.01)	5.73 (0.02)*	5.65 (0.02)**	5.53 (0.03)**
23	Sc23	5.84 (0.02)	5.78 (0.03)	5.66 (0.03)**	5.51 (0.03)**

Results are shown as mean (± standard deviation), Number of replications=3; Independent sample, testing of significance of variances by F -test, significance testing of two means by using Fishers t-test and Cochran and Cox t-test ,*P < 0.05 but > 0.01; ** P< 0.01.

has been suggested to be a good method for measuring cell surface hydrophobicity (Rosenberg et al., 1980). Strains possessing high hydrophobicity exhibit good adhesion property to intestinal cell lines (Pan et al., 2006; Marin et al., 1997; Wadstrom et al., 1987). Therefore, hydrophobicity was used to ascertain the adhesive potential of the indigenous isolates. Hydrophobicity was determined with n-hexadecane because it has been reported to give more reliable results without any cell lysis as compared to other hydrocarbons for evaluation of adhesion ability of probiotics (Vanhaecke and Pijck, 1988; Richard et al., 1999). A great variability in hydrophobicity values was observed in 23 indigenous yeast isolates (Table 4).

For only nine isolates, the hydrophobicity values were above 50%. Highest (59.65%) hydrophobicity was recorded for isolate Sc01 and lowest (13.46%) for isolate Sc18. In general, the hydrophobicity values for indigenous yeast isolates were overall lower than the values as high as 79.69% reported for bacteria (Sourabh et al., 2010; Del Re et al., 2000; Collado et al., 2008). Isolates possessing low hydrophobicity values may have low adhesion ability, which needs to be confirmed using *in vivo* experiments/cell line studies. It was reported that usually, yeast strains show variable adherence property in comparison to bacteria, that is why these microorganisms are to be administered repeatedly to achieve steady-state concentrations in the colon (Kumura et al., 2004). Since these yeast indigenous isolates are an integral part of traditional fermented foods being consumed regularly by people of Western Himalayas, therefore role of these isolates as probiotics seems to be important in this population.

Autoaggregation ability

Autoaggregation ability of probiotics is another trait which is associated with the adhesion ability of microorganisms (Del Re et al., 1998; Perez et al., 1998). Autoaggregation (%) ability values were found to be in the range of 18.47 to 67.65% (Table 5) for all the tested indigenous yeast

Table 3. Effect of ox bile (0.3 % w/v) on the growth rate of indigenous yeast isolates.

S/N	Isolate	Time required to increase A_{620} nm by 0.3 units [Lag time (in hours)]
1	Sc01	0.13^{j}
2	Sc02	1.26^{bc}
3	Sc03	-0.13^{k}
4	Sc04	0.72^{e}
5	Sc05	1.37^{b}
6	Sc06	$0.17^{l\ j}$
7	Sc07	0.13^{j}
8	Sc08	$0.67^{e\ f\ i}$
9	Sc09	$0.64^{e\ f}$
10	Sc10	0.99^{d}
11	Sc11	0.73^{e}
12	Sc12	$0.51^{f\ g}$
13	Sc13	$1.10^{c\ d}$
14	Sc14	0.96^{d}
15	Sc15	$1.08^{c\ d}$
16	Sc16	$0.70^{e\ f}$
17	Sc17	$0.61^{e\ f}$
18	Sc18	$0.31^{h\ j}$
19	Sc19	1.83^{a}
20	Sc20	$0.29^{h\ l\ j}$
21	Sc21	$0.25^{h\ l\ j}$
22	Sc22	$0.35^{g\ h\ i}$
23	Sc23	$0.38^{g\ h}$

Number of replications=3. Mean values within the same column followed by different superscript letters differ significantly when compared by Duncan's Multiple Range Test.

Table 4. Microbial adhesion (expressed in terms of % hydrophobicity) to n-hexadecane exhibited by indigenous yeast isolates.

S/N	Isolate	% Hydrophobicity
1	Sc01	59.65 (0.58)^{abcde}
2	Sc02	41.55 (2.78)^{mn}
3	Sc03	54.52 (1.26)^{gh}
4	Sc04	34.55 (1.92)^{opq}
5	Sc05	21.53 (0.94)^{stu}
6	Sc06	51.92 (1.55)^{i}
7	Sc07	20.91(1.14)^{tu}
8	Sc08	57.47 (1.25)^{abcdefg}
9	Sc09	30.80 (2.51)^{qr}
10	Sc10	42.38 (1.59)^{lm}
11	Sc11	49.22 (0.39)^{k}
12	Sc12	57.39 (1.67)^{abcdefg}
13	Sc13	49.55 (1.51)^{jk}
14	Sc14	28.40 (3.43)^{r}
15	Sc15	58.28 (0.95)^{abcdef}
16	Sc16	58.79 (0.46)^{abcde}
17	Sc17	55.21 (0.77)^{efgh}
18	Sc18	13.46 (2.63)^{wx}
19	Sc19	21.37 (0.52)^{tuv}
20	Sc20	56.12 (1.40)^{cdefgh}
21	Sc21	35.63 (1.83)^{no}
22	Sc22	32.66 (1.39)^{pqr}
23	Sc23	13.81(0.70)^{v}

Results are shown as mean (± standard deviation); Number of replications=3. Mean values within the same column followed by different superscript letters differ significantly when compared by Duncan's Multiple Range Test.

isolates. Although hydrophobicity values for most of the isolates were low, isolates having good autoaggregation ability in conjunction with the good hydrophobicity values can strongly be related to the adhesion ability of these microorganisms. Though these traits are independent of each other; they are still related to adhesion property of a particular microbe (Rahman et al., 2008). Autoaggregation ability has been more strongly associated to adhesion as compared to hydrophobicity (Del Re et al., 2000) therefore, good amount of autoaggregation ability in spite of low hydrophobicity values may account for adherence property of these indigenous isolates.

Production of exopolysaccharide

For microbial cells, EPS are thought to play a role in protection against desiccation, toxic compounds, bacteriophages, osmotic stress, and to permit adhesion to solid surfaces and biofilm formation (De Vuyst and Degeest, 1999). Therefore, the native yeast isolates were screened for the production of EPS by using ruthenium red dye in the medium where the positive isolates gave white colonies (ropy colonies) whereas, the negative isolates gave red or pinkish colonies (non-ropy colonies). Out of 23 yeast isolates, nine yeast isolates namely, Sc03, Sc11, Sc12, Sc13, Sc15, Sc19, Sc20, Sc21 and Sc22 were positive for production of EPS (Figure 1A). Isolates Sc09 and Sc17 showed no exopolysaccharide production but they exhibited proteolysis on the medium used for detection of exopolysaccharide (Figure 1B). EPS has immunostimulatory (Hosono et al., 1997) and anti-tumoral (Ebina et al., 1995) activities, and phosphate groups in EPS play an important role in the activation of macrophages and lymphocytes (Kitazawa et al., 2000; Uemura et al., 2003). Therefore, with this background, the indigenous yeast isolates can be used as immunostimulatory adjuvants and further studies in our laboratory are going on.

Antioxidant activity

Out of 23 yeast isolates, only four isolates that is Sc01,

Table 5. Autoaggregation (%) ability of indigenous yeast isolates.

S/N	Isolate	% Autoaggregation ability
1	Sc01	64.59 (0.19)^{cd}
2	Sc02	67.59 (0.27)^{ab}
3	Sc03	63.90 (0.42)^{efg}
4	Sc04	42.43 (0.27)^{r}
5	Sc05	57.37 (0.16)^{jkl}
6	Sc06	32.84 (0.22)^{t}
7	Sc07	60.52 (0.25)^{g}
8	Sc08	31.15 (0.10)^{u}
9	Sc09	49.50 (0.32)^{n}
10	Sc10	18.47 (0.43)^{w}
11	Sc11	63.52 (0.26)^{fg}
12	Sc12	58.28 (0.22)^{h}
13	Sc13	64.38 (0.24)^{def}
14	Sc14	47.90 (0.25)^{pq}
15	Sc15	67.65 (0.33)^{ab}
16	Sc16	54.64 (0.11)^{m}
17	Sc17	57.16 (0.41)^{jkl}
18	Sc18	47.87 (0.27)^{q}
19	Sc19	35.18 (0.21)^{s}
20	Sc20	55.63 (0.34)^{l}
21	Sc21	48.57 (0.26)^{o}
22	Sc22	57.63 (0.37)^{ijk}
23	Sc23	27.74 (0.53)^{v}

Results are shown as mean (± standard deviation), Number of replications=3. Mean values within the same column followed by different superscript letters differ significantly when compared by Duncan's Multiple Range Test.

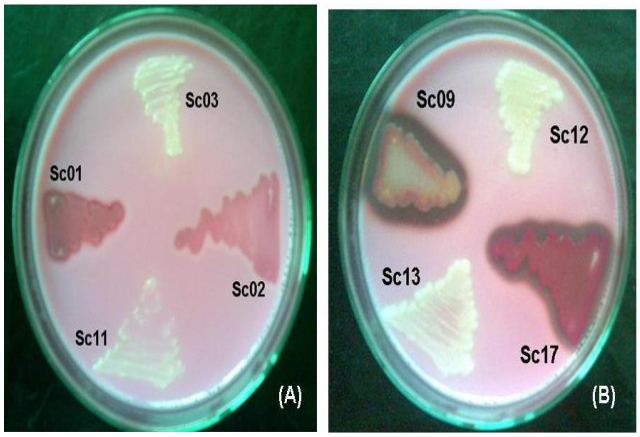

Figure 1. Exopolysaccharide production by yeast isolates.

Sc02, Sc03 and Sc16 showed scavenging of DPPH free radical in both methanol and buffered methanol reaction systems (Table 6). The intracellular cell free extract of isolate Sc16 showed maximum antioxidant activity (22.07%) while isolate Sc01 showed minimum activity (11.43%) with DPPH assay (methanol). With whole cell extract, the highest scavenging activity was shown by isolate Sc02 (25.47%). In case of DPPH assay employing buffered methanol, isolate Sc01 and Sc02 showed maximum antioxidant activity with intracellular cell free (33.30%) and whole cell extract (42.51%) respectively.

The results of the present study indicate that the radical scavenging ability of both intracellular cell-free extracts and whole cell extract of native isolates contribute towards the antioxidative effect. Thus, making indigenous yeast isolates as potential candidate for the production of functional food supplements. Various workers have reported the exhibition of antioxidative activity by bacteria like *Lactobacillus* species (Kullisaar et al., 2002; Saide and Gilliland, 2005; Jarvenpaa et al., 2007) whereas, in yeasts it is less reported. Therefore, presence of this activity in yeast isolates is an additional protective trait which may be useful as natural antioxidant.

Galactosidase enzyme production

An important way in which probiotics beneficially affects the health of the host is by providing enzymatic activities that improve the utilization of nutrients within the intestine (Rowland, 1992; Mustapha et al., 1997). The primary mechanism which is involved in this case is through the intraintestinal hydrolysis of the lactose by β - galactosidase (EC 3.2.1.23) enzyme therefore, the indigenous yeast isolates were assayed for β - galactosidase enzyme. None of the yeast isolates was found positive for this enzyme.

Production of siderophore

These indigenous yeast isolates were also subjected to screening for siderophore production, a iron chelating metabolite known to exert antagonistic effect (Kline et al., 2000). Only one yeast isolate that is Sc15, was found positive for siderophore production as judged by the formation of orange halo zone on CAS agar medium (Figure 2). Most bacteria and fungi synthesize, secrete, and take up at least one type of siderophore and yet also have the capacity to take up siderophores secreted by other organisms (Byers and Arceneaux, 1998). Although *S. cerevisiae* do not secrete siderophores, it is capable of taking up siderophore-bound iron (Neilands, 1995; Lesuisse et al., 1987), and a specific transport system for the hydroxamate-type siderophore ferrioxamine B(FOB) has been described (Yun et al., 2000). In this study, the production of siderophore by indigenous yeast isolate (*S. cerevisiae*) is an interesting observation.

The results of the present study confirm that indigenous yeast isolates showed better survivability in simulated gastrointestinal juices and in the presence of ox bile. Some of these isolates also exhibited good hydrophobicity

Table 6. Antioxidant activity of indigenous yeast isolates using scavenging of DPPH free radical.

S/N	Isolate	Protein concentration (µg/ml) of sample	Dry weight (mg) per 500 µl of sample		DPPH (methanol) in 100 µl extract		DPPH (buffered methanol) in 100 µl extract	
			Intracellular cell free extract	Whole cell extract	Intracellular cell free extract	Whole cell extract	Intracellular cell free extract	Whole cell extract
1	Sc01	179.08	0.8	1.8	11.43 (±0.40)^{c}	19.47 (±0.06)^{b}	33.30 (±0.30)^{a}	36.27 (±0.15)^{b}
2	Sc02	205.32	0.8	0.9	17.27 (±0.31)^{b}	25.47 (±0.21)^{a}	25.27 (±0.31)^{b}	40.40 (±0.20)^{a}
3	Sc03	327.45	0.9	1.4	17.10 (±0.10)^{b}	16.40 (±0.10)^{c}	18.43 (±0.21)^{c}	32.77 (±0.25)^{c}
4	Sc16	323.52	1.7	6.6	22.07 (±0.93)^{a}	15.40 (±0.36)^{d}	25.67 (±0.25)^{b}	27.67 (±0.95)^{d}

Results are shown as mean, mean (± standard deviation); Number of replications=3. Mean values within the same column followed by different superscript letters differ significantly when compared by Duncan's Multiple Range Test.

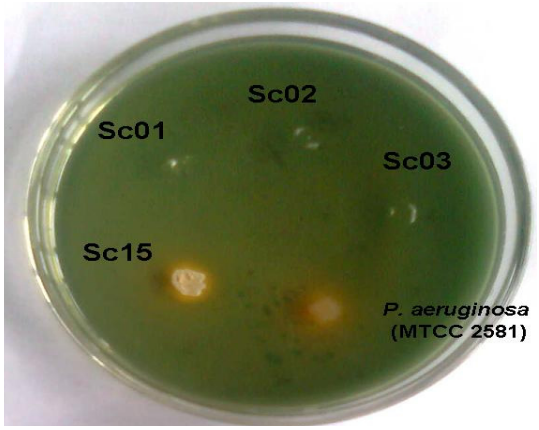

Figure 2. Siderophore production by yeast isolate Sc15 and *Pseudomonas aeruginosa*-MTCC 2581 (positive control).

and autoaggregation abilities which are one of the essential markers for the selection of adherence potential of probiotics. In addition, the exhibition of some other traits such as antioxidant activity, exopolysaccharide and siderophore production makes them appropriate candidate for the development of functional foods.

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