Evaluation of probiotic potential of *Saccharomyces* spp. isolated from traditional fermented beverages of Himachal Pradesh by *in vitro* studies and principal component analysis

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Abstract

In the present study, 60 yeast isolates were obtained from traditional fermented beverages of Himachal Pradesh. After primary screening, only 10 yeast isolates were selected for further studies. Molecular identification of the isolates revealed that S. cerevisiae was main microorganism associated with fermented beverages. All the yeast isolates were found to be tolerant to the stressed conditions of gastrointestinal tract. All yeast isolates demonstrated excellent autoaggregation ability. Autoaggregation of the isolates enhanced with time. The variation in the percentage of hydrophobicity was observed among the isolates. Regarding the beneficial health effects, all the selected yeast isolates showed antimicrobial activity against the tested pathogens. S. cerevisiae Y97, S. cerevisiae Y137 and S. cerevisiae Y154 showed excellent cholesterol assimilation percentage which was more than 85 %.

All the isolates were observed to produce exopolysaccharide. None of the isolates showed haemolysis and thus were considered as safe. The present study revealed that Saccharomyces spp. was mainly found in fermented beverages and these microorganisms have better probiotic potential.

Keywords: Fermented beverages, Himachal Pradesh, Probiotics, *S. cerevisiae*, Survival rate.

Introduction

Himachal Pradesh is located between 30° 22′ 44″ and 33° 12′ 40″ N latitude and 75° 45′ 55″ to 79° 04′ 20″ E longitude. It is a hilly State and the climate, geography and culture variety of State are quite diverse. Locals in Himachal Pradesh prepare traditional fermented foods and beverages during festivals to symbolize the celebrations. These products are made by native people using locally available raw materials and their inherited traditional knowledge. The preparation of a fermented product involves either natural fermentation or the addition of starter culture or a local inoculum to the substrate to convert it into an edible item that is customary among the local population's ethnic and socio-economic groups[12]. Some of the well-known traditional beverages in Himachal Pradesh's tribal districts are *chhang*, *lugari*, *aara*, *chakti*, *daru*, *sura*, *kinnauri*, *angoori* etc.[15] The traditional inocula *phab* and *dhaeli* were used for the preparation of these beverages. The different raw materials include rice, barley, or finger millet is used to prepare these beverages. In undistilled beverages, the ethanol concentration ranged from 5 % to 12 % (v/v), whereas in distilled beverages, it ranged from 13 % to 19 %. Distilled beverages have also been shown to contain very small amount of acetaldehyde, methanol, ester, n-propanol and other chemical components. Various types of bacteria, yeasts, filamentous moulds, viruses and archaea arise and persist during fermentation[19]. The microflora of these products revealed yeast's dominance.

Antibiotics were believed to have negative consequences on the body including super infection and drug resistance, by killing both good and bad bacteria, upsetting the body's microbiota and disrupting the immune system. Therefore, consuming probiotics aids in restoring the digestive tract's natural microbial flora. The ability of yeasts to survive transit through the human gastrointestinal tract (GIT), which is required to confer health benefits on the host, has been suggested as a probiotic[14]. Probiotics have been proven to have anticancer, antioxidant, inflammatory bowel disease alleviation and enhanced lactose tolerance, resistance to infections, cholesterol reduction and increased immunity[9,21].

Yeast has several benefits over probiotic bacteria including the resistance to antibiotics, capacity to minimize pathogen adhesion to mucosal surfaces and retain viability in the intestinal biota while receiving antibiotic therapy. In contrast to bacteria, the transfer of yeast genes has never been documented[16]. The benefits of yeast probiotics are well documented, yet only a few strains are used for humans. Only *Saccharomyces cerevisiae* var. *boulardii* are now commercially available for human use. It would be important for research and the food sector to choose novel yeasts that can help to increase the variety of strains that have enhanced probiotic potential. Keeping in view the above considerations, the present work was designed to examine the various yeast isolates obtained from traditional indigenous fermented beverages of Himachal Pradesh for probiotic potential through *in vitro* screening and principal component analysis.

Material and Methods

Materials and Microorganisms: The chemicals and media used in this study were purchased from Hi-Media (Mumbai,

India) and Sigma-Aldrich (St. Louis, Missouri, USA). Probiotic strain *Saccharomyces cerevisiae* ATCC-MYA-796 procured from American Type Culture Collection (ATCC®) Manassas, VA 20110 USA was used as reference strain for evaluating the probiotic potential of isolated yeasts.

Sample collection, Isolation and Characterization of yeast isolates: The samples of fermented beverages (*chhang* and *sura*) were collected from different regions of Himachal Pradesh. One gram of sample was homogenized with 9 ml of saline and serial dilutions ranging from 10^{-1}-10^{-10} were prepared. An aliquot of 0.1 ml of each dilution was spread on YM Agar (Yeast Malt Agar) supplemented with ampicillin (0.05 g/L) and acidified with 1N HCl to pH 5.0. The inoculated plates were incubated for 48 h at 30 ºC. Isolates were affirmatively identified as yeast by culturing on YM agar and examined for colony and cell appearance, catalase activity and Gram staining.

Biochemical characterization: Sugar fermentation of yeast isolates was tested by using API 20C AUX identification kit (Biomereux India Pvt. Ltd.). Growth of yeast isolates at different pH values (2.5, 3.5, 8.5 and 9.5) and temperature (15, 37 and 45 °C) was measured.

DNA sequencing, Identification and Phylogenetic analysis of yeast isolates: For molecular identification, ITS DNA sequencing was conducted at Biologia Research India Pvt. Ltd., New Delhi, India. The fragments of sequences were assembled and the consensus sequences were compared to those deposited in the GenBank DNA database using the Basic Local Alignment Search Tool. Sequence was submitted in National Center for Biological Information (NCBI) using BankIt sequence submission tool. A phylogenetic tree was constructed to determine the closest yeast species by the neighbor-joining approach, using MEGA 11.

Probiotic characterization

Acidic pH and bile salts tolerance: Acid and bile salts tolerance of the isolates was studied according to the method of Maragkoudakis et al.[8] The survival of yeast isolates at different pH (2, 3 and 7) and bile salt (0.5 %, 1 % and 2 %) was determined. Survival of yeast isolates was calculated in terms of log cfu/ml.

Cell surface hydrophobicity: The ability of isolate to adhere to different hydrocarbons (n-hexadecane, xylene and toluene) was studied as per the method described by Rosenberg et al.[13] Hydrophobicity was calculated by using formula:

$$\text{Hydrophobicity (\%)} = [(A0\text{-}A)/A0] \times 100$$

whereas A0 and A are absorbance before and after mixing with solvents at 600 nm.

Autoaggregation: Autoaggregation ability of yeast isolate was performed by the modified method of Collado et al.[2] The autoaggregation ability was checked at different time intervals (0 h, 3 h and 24 h) and autoaggregation ability was expressed in percentage by using formula as:

$$\text{Autoaggregation (\%)} = [1 - At/A0] \times 100$$

where At represents the absorbance at time t and A0 the absorbance at t = 0.

Antimicrobial activity: The yeast isolates were screened for antimicrobial activity by using agar well diffusion method[10]. The eight food spoilage pathogenic strains were used for antimicrobial activity. The diameter of zone of inhibition around the wells was measured and clear zone of 1 mm or more was considered as positive inhibition.

***In vitro* cholesterol assimilation:** According to Liong and Shah[6], o-phthalaldehyde was used to assimilate cholesterol by yeast isolates. The study used three distinct bile salts: cholic acid, sodium taurocholate and ox bile. The filter sterilize cholesterol solution (Sigma-Aldrich), 10 mg/ml in 96 percent ethyl alcohol was prepared. 70 µl of cholesterol solution was added to 10 ml of YPD broth (final cholesterol concentration 70 µg/ml) with 0.2 % (w/v) bile salts (oxbile/cholic acid/sodium taurocholate). The yeast isolates (1%) were added to YPD broth, which was then incubated for 20 h at 30 °C. An uninoculated sample was used as control.

Exopolysaccharide production: Overnight grown yeast isolate was streaked on the ruthenium red milk agar plate (10 % w/v, skim milk powder, 1 % w/v, sucrose, 0.08 g/l ruthenium red and 1.5 % w/v agar) and incubated at 30 ºC for 24 h[11]. The non ropy strains gave red colonies due to the staining of the yeast cell wall and ropy strains appeared as white colonies.

Safety Assessment

Haemolytic activity: Overnight cultures were streaked on the surface of Columbia blood agar plates (Oxoid) supplemented with 5 % sheep blood and incubated at 30 °C for 48 h. After 48 h, the haemolytic reaction was recorded by observation of zone of clearance[7].

Statistical analysis: Statistical analysis was carried out with SPSS Inc. software (version 21.0). One-way analysis of variance (ANNOVA) was used to study significant difference between means, with significance level at $p<0.05$. 2-sided Tukey's HSD test was used to perform multiple comparisons between means. All data presented are mean values of two determinations and three replicates.

Principal Component Analysis (PCA) for the selection of promising probiotic isolates: Statistical differences among the isolates were pointed out through the Principal Component Analysis. The relationship among the isolates

was determined by principal component analysis (PCA) using XLSTAT™ 2021 software. The cases introduced in the analysis were the 12 identified yeast isolates along with the one standard probiotic strain while the discriminating variables were acid and bile tolerance, hydrophobicity, cholesterol assimilation, autoaggregation and antimicrobial assay. Results of the quantitative probiotic characterization were converted into three coded values (0, 1 and 2) and used as input data for PCA. PCA was done by using varimax rotation.

Results

Isolation of Yeast: On YM agar plates, a total of 60 yeast isolates were obtained from various traditional fermented beverages of Himachal Pradesh. Only 25 of the 60 isolates were chosen based on their morphological and biochemical characteristics and of those 25, only 10 matched the requirements for probiotic properties.

Morphological, Biochemical characterization and Molecular Identification of Yeast Isolates: All the yeast isolates showed large sized, white/cream colored colonies having smooth surface on YM agar plates. All the ten isolates were found to be negative for catalase test and showed significant growth at 15 °C and 37 °C. Most of the isolates demonstrated growth at 45 °C and at low pH value (pH 2.5 and 3.5). All the tested yeast isolates fermented D-glucose, D-galactose, D-maltose, D-saccharose and D-raffinose except isolate Y126 (Table 1).

The ITS DNA sequences of all the 10 yeast isolates were determined and phylogenetic tree was constructed based on ITS DNA sequences (Fig. 1). Taxonomic identification of yeast isolates was performed by comparing the resulting sequences of each isolate using online tool BLAST. The isolates were identified based on the highest >99 % sequence identity and the sequences of all the yeast isolates were deposited in the National Center for Biotechnology Information with accession numbers (Fig. 1). The evolutionary history was constructed using the Neighbor-Joining method by using MEGA 11 software.

Probiotic characterization

Acidic pH and bile salts tolerance: In this study, all isolates showed tolerance to pH 2 and 3 for 3 h despite fewer variations in the degree of viability. In comparison of the reference strain, most of the isolates showed the higher viability index. There was no significant difference in the survival rate of all the selected isolates at pH 2 and pH 7 that was control (Table 2).

Table 1
Carbohydrates fermentation profile of yeast isolate

Carbohydrates	Yeast isolates									
	Y50	**Y81**	**Y97**	**Y98**	**Y126**	**Y134**	**Y137**	**Y141**	**Y143**	**Y154**
Control	-	-	-	-	-	-	-	-	-	-
D-glucose	+	+	+	+	+	+	+	+	+	+
Glycerol	-	-	-	-	-	-	-	-	-	-
calcium 2-Keto-Gluconate	-	-	-	-	-	-	-	-	-	-
L-arabinose	-	-	-	-	-	-	-	-	-	-
D-xylose	-	-	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-	-	-
Xylitol	-	-	-	-	-	-	-	-	-	-
D-galactose	+	+	+	+	+	+	+	+	+	+
Inositol	-	-	-	-	-	-	-	-	-	-
D-sorbitol	-	-	-	-	-	-	-	-	-	-
Methyl-αD-Glucopyranoside	+	+	-	-	+	-	-	-	-	-
N-Acetyl-Glucosamine	-	-	-	-	-	-	-	-	-	-
D-cellobiose	-	-	-	-	-	-	-	-	-	-
D-lactose	-	-	-	-	-	-	-	-	-	-
D-maltose	+	+	+	+	+	+	+	+	+	+
D-saccharose	+	+	+	+	+	+	+	+	+	+
D-trehalose	-	-	-	-	+	-	+	-	-	+
D-melezitose	-	-	-	-	-	-	-	-	-	-
D-raffinose	+	+	+	+	-	+	+	+	+	+

+ Presence, - absence of growth

The effects of different concentration (0.5 %, 1 % and 2 %) of bile salt on the growth of yeast are presented in table 2. *S. cerevisiae* Y126 and *S. cerevisiae* Y141 showed full tolerance to bile at 0.5 %, but their survival rate declined as bile concentration increased from 0.5 % to 2 %.

Cell surface hydrophobicity: In the present study, the results showed a wide variation in hydrophobicity among the isolates as well as same isolate for different hydrocarbons. *S. cerevisiae* Y126 and *S. cerevisiae* Y134 exhibited higher percent hydrophobicity towards toluene than reference strain (Fig. 2a).

Autoaggregation: The autoaggregation percentage was measured for yeast isolates for 3 h and 24 h and it was found that autoaggregation ability increased with time and was higher at 24 h than 3 h. Majority of the yeast isolates showed significantly ($p<0.05$) higher autoaggregation as compared to R after 3 h of incubation. All the studied isolates exhibited excellent autoaggregation ability (Fig. 2b).

Antimicrobial activity: Antimicrobial activity of selected yeast isolates was screened against eight food spoilage causing bacteria such as *Listeria monocytogenes* MTCC 657, *Bacillus cereus* MTCC 1272, *Staphylococcus aureus* subsp. *aureus* MTCC 96, *Pseudomonas aeruginosa* MTCC 424, *Escherichia coli* MTCC 118, *Shigella, Salmonella typhi and Aeromonas hydrophilla*. The results showed that there was no significant difference among the many isolates for same pathogenic strain. All the studied yeast isolates showed good antimicrobial activity against the food spoilage bacteria (Table 3).

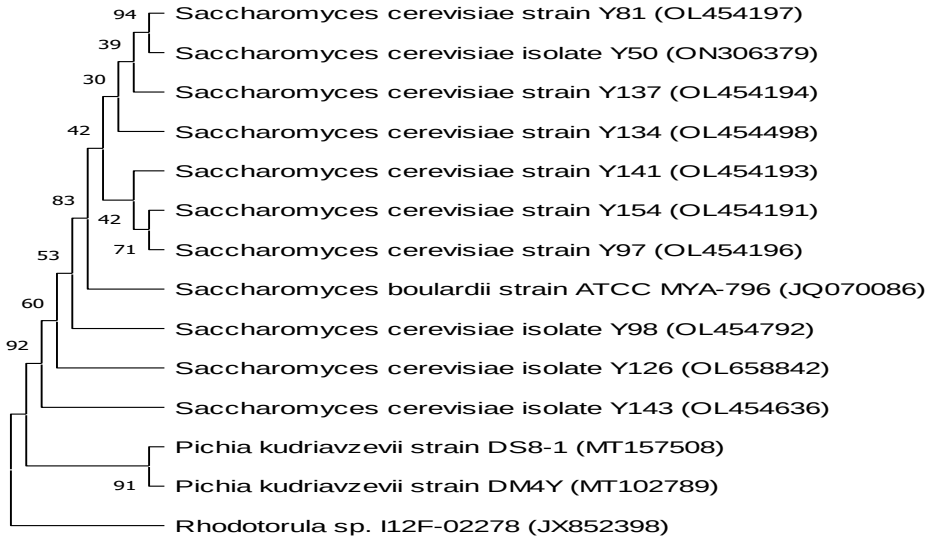
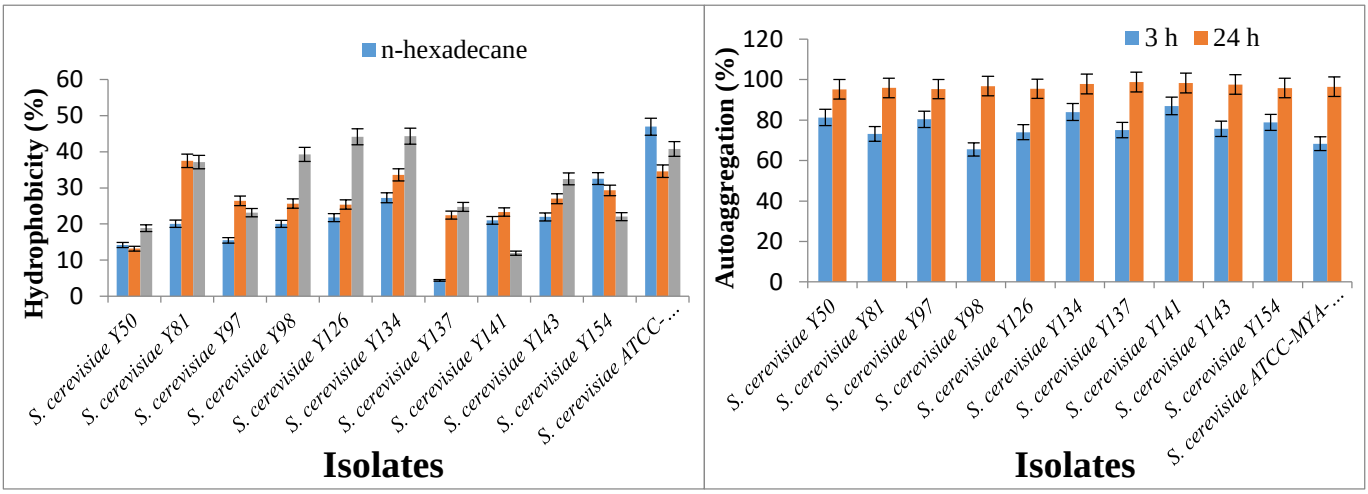


Figure 1: A neighbor joining phylogenetic tree based on ITS DNA sequences belonging to 10 yeast isolates and reference strain (*Saccharomyces cerevisiae* ATCC-MYA-796).

Table 2
Survival of yeast isolates in acidic and bile conditions

Isolates	Survival rate (%)						
	Acid tolerance			Bile tolerance			
	pH 7 (control)**	pH 3**	pH 2**	Control**	0.5 %**	1 %**	2 %**
S. cerevisiae Y50	100±0.10	99±0.01	99±0.01	100±0.07	99±0.13	99±0.07	97±0.15
S. cerevisiae Y81	100±0.13	100±0.04	99±0.04	100±0.12	99±0.07	98±0.08	95±0.19
S. cerevisiae Y97	100±0.06	100±0.04	97±0.08	100±0.26	99±0.17	97±0.25	96±0.14
S. cerevisiae Y98	100±0.06	98±0.06	97±0.06	100±0.07	99±0.02	99±0.07	98±0.03
S. cerevisiae Y126	100±0.19	100±0.08	98±0.06	100±0.07	100±0.08	99±0.13	98±0.14
S. cerevisiae Y134	100±0.30	98±0.08	95±0.09	100±0.06	99±0.16	98±0.06	97±0.18
S. cerevisiae Y137	100±0.27	99±0.12	95±0.14	100±0.25	97±0.28	97±0.30	96±0.24
S. cerevisiae Y141	100±0.16	97±0.04	96±0.09	100±0.12	100±0.10	99±0.14	98±0.03
S. cerevisiae Y143	100±0.17	99±0.08	99±0.17	100±0.07	99±0.16	98±0.04	97±0.19
S. cerevisiae Y154	100±0.20	99±0.05	97±0.08	100±0.04	99±0.05	98±0.44	97±0.22
S. cerevisiae ATCC-MYA-796	100±0.09	97±0.08	96±0.01	100±0.15	99±0.02	99±0.02	98±0.04

Values represented as mean ± standard deviation (SD) of triplicate analysis
** Significant at $p<0.05$ measured by 2 sided Tukey's post hoc range test between replications



(a) **(b)**

Figure 2: (a) Hydrophobicity (%) of yeast isolates towards n-hexadecane, xylene and toluene (b) autoaggregation percentage of yeast isolate

Table 3
Antimicrobial activity (mm) of yeast isolates against pathogens

Isolates	*S. typhi*	*E. coli*	*Shigella*	*P. aeruginosa*	*B. cereus*	*S. aureus*	*A. hydrophilla*	*L. monocytogenes*
S. cerevisiae Y50	12.00^{b} ±1.00	12.50^{bc} ±0.50	10.50^{abc} ±1.50	14.00^{cd} ±1.00	12.50^{e} ±0.50	11.50^{cde} ±0.50	24.00^{f} ±1.00	12.50^{d} ±0.50
S. cerevisiae Y81	12.00^{ab} ±1.00	11.50^{bc} ±0.50	10.00^{ab} ±0.00	12.00^{bc} ±1.00	12.00^{de} ±0.00	12.50^{ef} ±0.50	24.00^{f} ±1.00	11.00^{c} ±0.00
S. cerevisiae Y97	9.50^{ab} ±0.50	12.00^{a} ±0.00	10.00^{ab} ±0.00	10.00^{ab} ±1.00	12.50^{e} ±0.50	10.50^{bc} ±0.50	14.50^{ab} ^{c}±0.50	10.00^{ab} ^{c}±0.00
S. cerevisiae Y98	10.00^{ab} ±0.00	11.00^{ab} ±0.00	11.50^{bc} ±0.50	10.00^{ab} ±0.00	11.00^{cde} ±0.00	12.00^{def} ±0.00	12.50^{a} ±0.50	10.50^{bc} ±0.50
S. cerevisiae Y126	9.50^{ab} ±0.50	11.50^{a} ±0.50	11.00^{bc} ±1.00	14.50^{d} ±0.50	9.50^{abc} ±0.50	11.00^{bcd} ±0.00	12.50^{a} ±0.50	10.50^{bc} ±0.50
S. cerevisiae Y134	11.00^{ab} ±1.00	11.00^{abc} ±0.00	10.00^{ab} ±0.00	11.00^{ab} ±0.00	8.50^{a} ±0.50	10.00^{b} ±0.00	14.00^{ab} ±0.00	9.50^{ab} ±0.50
S. cerevisiae Y137	11.00^{a} ±1.00	10.50^{ab} ^{c}±0.50	9.00^{a} ±0.00	10.50^{ab} ±0.50	11.00^{cde} ±0.00	8.50^{a} ±0.50	18.50^{e} ±1.50	9.50^{ab} ±0.50
S. cerevisiae Y141	13.00^{b} ±0.00	12.50^{c} ±0.50	12.00^{c} ±0.00	13.50^{cd} ±0.50	12.50^{e} ±0.50	13.00^{f} ±0.00	17.50^{de} ±0.50	12.50^{d} ±0.50
S. cerevisiae Y143	11.00^{ab} ±0.00	11.50^{abc} ±0.50	10.00^{ab} ±0.00	9.00^{a} ±1.00	9.00^{ab} ±0.00	11.00^{bcd} ±0.00	15.50^{bcd} ±0.50	10.50^{bc} ±0.50
S. cerevisiae Y154	11.00^{ab} ±0.00	11.00^{abc} ±1.00	10.00^{ab} ±1.00	10.00^{ab} ±0.00	10.50^{bcd} ±0.50	11.00^{bcd} ±0.00	17.00^{cde} ±1.00	9.50^{ab} ±0.50
S. cerevisiae ATCC-MYA-796	17.00^{c} ±1.00	19.00^{d} ±1.00	17.50^{d} ±0.50	18.00^{e} ±0.00	18.50^{f} ±0.50	20.00^{g} ±1.00	24.50^{f} ±0.50	18.00^{e} ±0.00

Values represented as mean ± standard deviation (SD) of triplicate analysis
$^{a-g}$ = average in the columns with same superscript letter, not significantly ($p<0.05$) different as measured by 2 sided Tukey's post hoc range test between replications

***In vitro* cholesterol assimilation:** The majority of isolates had the highest, intermediate and lowest cholesterol assimilation in the medium with taurocholate, cholic acid and oxbile respectively. In the medium containing taurocholate, the overall cholesterol assimilation was observed to be significantly ($p<0.05$) highest for isolates *S. cerevisiae* Y97, *S. cerevisiae* Y137 and *S. cerevisiae* Y154 i.e. cholesterol assimilation more than 85 % (Table 4). Overall cholesterol assimilation for most of the yeast isolates was significantly higher than reference strain for oxbile and taurocholate.

Exopolysaccharide production: All the yeast isolates including reference produced exopolysaccharide on ruthenium red agar plates (Fig. 3a).

Safety Assessment

Haemolytic activity: All of the yeast isolates were γ-haemolytic i.e. no haemolysis was observed when these isolates were streaked on Columbia sheep blood agar plates (Fig. 3b).

Principal Component Analysis (PCA): The first four main components (PCs) of the PCA explained 61.39 % of the overall variation whereas PC1 and PC2 explained 19.22% and 16.63% respectively in fig. 4a and b. Fig. 4a displays the homogenous distribution of variables on the plane of principal components. Based on the arrangement of the variables in the factorial space of PCA, the yeast isolates were distributed (Fig. 4b). The probiotic qualities of the yeast isolates within the circle were at their maximum levels, displaying the same properties as the reference strain and some were superior to reference strain in terms of probiotic attributes. This gives a clear idea that yeast isolates (*S. cerevisiae* Y98, *S. cerevisiae* Y81, *S. cerevisiae* Y134, *S. cerevisiae* Y126, *S. cerevisiae* Y143, *S. cerevisiae* Y154, *S. cerevisiae* Y141 and *S. cerevisiae* Y50) exhibited probiotic activity that is more comparable to or effective than that of the reference strain.

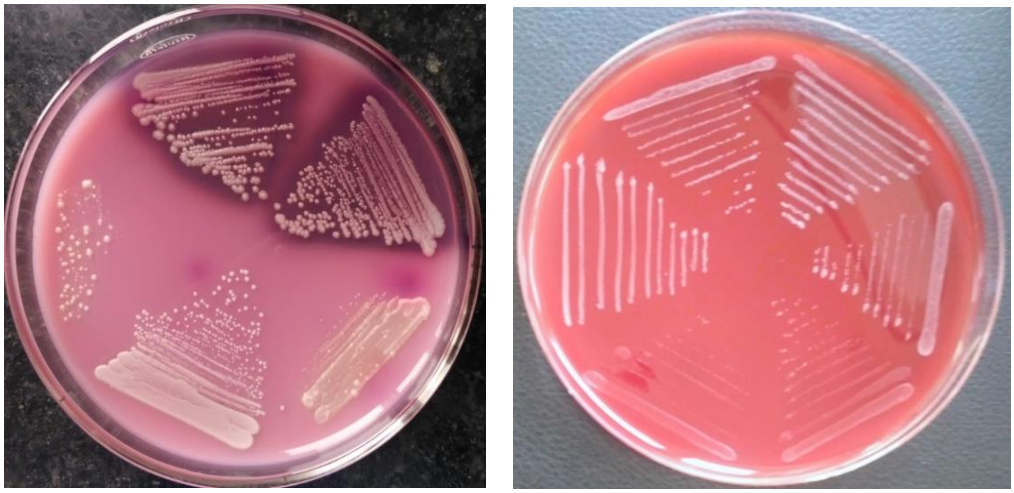
Discussion

In Himachal Pradesh, several fermented beverages are prepared and consumed in rural and tribal areas. People in these parts of Himachal Pradesh enjoy a variety of cereal-based, fruit-based and millet-based indigenously prepared fermented beverages. Yeasts play a crucial role in food safety and the imparting of physiochemical properties, making them an essential component of the microflora of many fermented beverages.

Table 4
Cholesterol assimilation (%) of yeast isolates in different bile salts

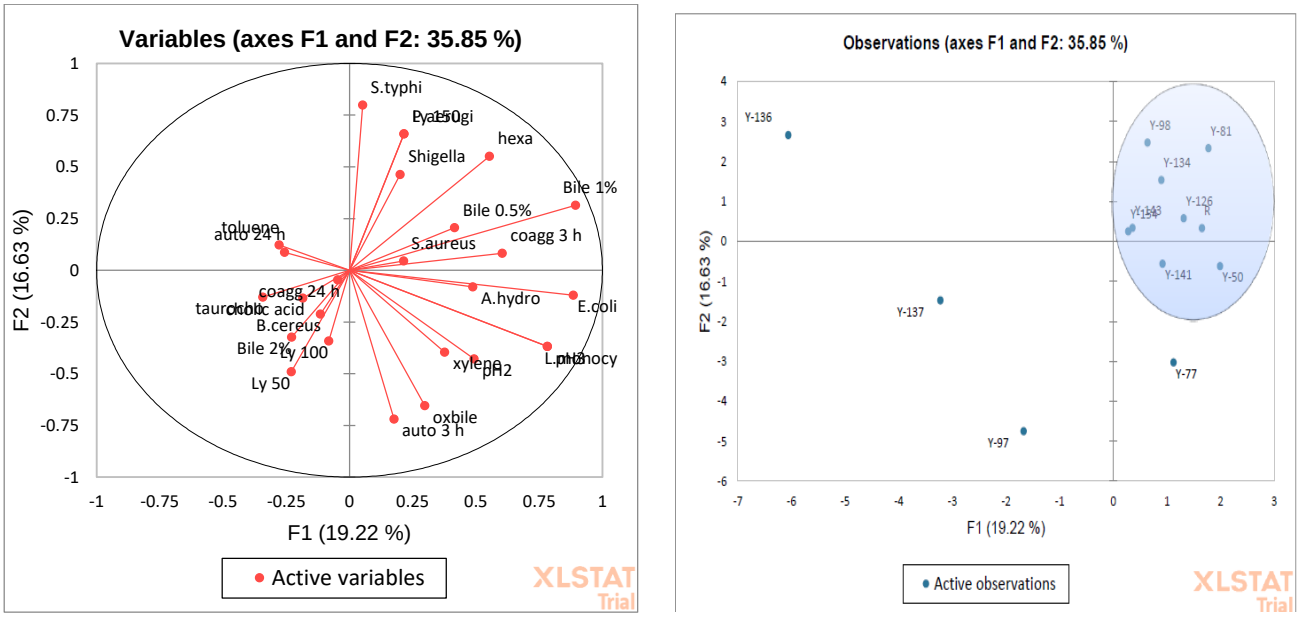
Isolates	Oxbile	Cholic acid	Taurocholate
S. cerevisiae Y50	49.83^{fgC}±0.18	19.93^{aA}±0.50	48.49^{bB}±0.49
S. cerevisiae Y81	26.97^{aA}±0.93	33.61^{cB}±0.79	79.37^{eC}±1.06
S. cerevisiae Y97	38.48^{cB}±1.52	34.79^{cA}±1.06	86.95^{hiC}±1.00
S. cerevisiae Y98	25.12^{aA}±0.82	28.19^{bB}±0.12	83.75^{fgC}±0.23
S. cerevisiae Y126	40.80^{cdA}±0.28	52.15^{eB}±1.15	53.19^{cB}±1.10
S. cerevisiae Y134	46.29^{efB}±1.43	35.90^{cA}±1.61	43.08^{aC}±1.22
S. cerevisiae Y137	52.80^{ghA}±0.24	75.44^{gB}±1.03	88.05^{iC}±0.29
S. cerevisiae Y141	45.43^{eA}±1.23	54.98^{eB}±1.26	84.26^{ghC}±1.42
S. cerevisiae Y143	34.73^{bA}±1.58	63.53^{fB}±1.99	62.28^{dB}±1.06
S. cerevisiae Y154	55.36^{hA}±2.28	81.97^{hB}±2.66	86.59^{ghiB}±1.75
S. cerevisiae ATCC-MYA-796	43.60^{deA}±0.72	78.61^{ghB}±0.83	80.26^{eB}±0.74

Values represented as mean ± standard deviation (SD) of triplicate analysis
$^{a-i}$ = average in the columns with same superscript letter, not significantly ($p<0.05$) different as measured by 2 sided Tukey's post hoc range test between replications
$^{A-C}$ = average in the rows with same superscript letter, not significantly ($p<0.05$) different as measured by 2 sided Tukey's post hoc range test between replications



a) **b)**

Figure 3: a) Exopolysaccharide production by yeast isolates b) Non haemolytic activity of yeast isolates



(a) **(b)**

Figure 4: a) Projection of the variables in PC1 and PC2 b) Projection of the yeast isolates in PC1 and PC2

Thakur et al[20] reported that microorganisms present in the starter culture (*phab* and *dheli*) of fermented beverages were *Pediococcus pentosaceus, Enterococcus faecium, Lactobacillus plantarum, Saccharomyces fibuligera* and *Saccharomyces cerevisiae.* In the present study, all the ten yeast isolates were identified as *S. cerevisiae* by DNA sequences from different traditional fermented beverages of Himachal Pradesh. Since probiotics are frequently administered orally, they must possess the ability to make it through the GIT and into the colon.

Probiotic properties include the capacity to withstand the physiological temperature, bile salts and the acidic gastric juice[4]. During the present study, all the isolates were found to be resistant to the acidic conditions (pH 2). Most of the isolates showed high degree of viability as compared to the reference strain. These results were similar with those of the previous studies where yeast isolates exhibited tolerance to acidic conditions[5,17]. All the studied isolates demonstrated excellent growth at 0.5 %. Slight decrease in the tolerance was observed when concentration was increased to the 2 % of bile salt. The results of present study coincide with an evaluation of probiotic properties of yeasts isolated from fermented fish product of North East India[1].

Cells with high hydrophobic characteristics adhere to hydrophobic surfaces more strongly. All the yeast isolates demonstrated excellent autoaggregation ability and their autoaggregation ability increased with increase in time. After 24 h of incubation, autoaggregation ability of all the yeast isolates was higher than 95 %.

Syal and Vohra[18] during their study revealed that seven yeast isolates from *idli* and *jalebi* better demonstrated 32 to 67 % and 45 to 86 % microbial adhesion with xylene and n-hexadecane respectively and the percentage autoaggregation of these yeast isolates ranging from 47-100 %. Yeasts isolated from Brazilian fermented table olives showed intermediate to high autoaggregation rate (41-91 %) after 4 h of incubation[17].

The inhibiting effect against the growth of harmful bacteria is another pre-requisite for yeast to exhibit probiotic properties. All the studied yeast isolates exhibited strong and broad antimicrobial activity against food spoilage bacteria. No significant difference was observed among the isolates for antimicrobial activity. Yeast isolates obtained from traditional Indian fermented foods exhibited antimicrobial activity against pathogens such as *E. coli*, *Staphylococcus aureus*, *Pseudomonas* sp., *Vibrio* sp. and *Salmonella* sp.[18].

In the present study, yeast isolates showed good percentage of cholesterol assimilation and this was observed highest with taurocholate. Cholesterol assimilation was found to be higher in most of the yeast isolates than reference strain. Dey et al[3] found that three yeast isolates isolated from fruits, *Wickerhamomyces anomalus* VIT-ASN01, *Saccharomyces cerevisiae* VIT-ASN03 and *Yarrowia lipolytica* VIT-ASN04, absorbed cholesterol by 51, 46 and 56 percent after 6 h, 12 h and 24 h of incubation respectively.

EPS are exocellular polymeric substances that remain in the gastrointestinal tract for a longer period of time which facilitates probiotic microbe colonization. In the present study, all the yeast isolates were found to produce exopolysaccharide. Similar results were observed during a study on yeasts isolated from traditional fermented foods where all the yeast isolates were found to produce slimy white ropy colonies on skimmed-milk ruthenium agar plates[18]. Haemolysis activity was evaluated to assess the pathogenicity of yeast isolates. No haemolysis was observed during the study. Simões et al[17] have observed that none of

the yeasts isolated from Brazilian fermented table olives possess haemolytic activity.

Conclusion

Fermented products are home for various beneficial microorganisms and are gaining attention nowadays. In the past, people were efficient at cultivating healthy microorganisms, primarily lactic acid bacteria, yeasts and filamentous moulds, to produce food for consumption. The yeasts isolated from traditional fermented beverages of Himachal Pradesh, were shown to have probiotic properties. All the yeast isolates were tolerant to acidic and bile conditions. The studied isolates showed good cell surface characteristics which help in adhesion of the probiotics to the gastrointestinal tract. In addition to the probiotic properties, these isolates also exhibited various beneficial health effects such as cholesterol assimilation, antimicrobial activity and exopolysaccharide production.

The present study suggests that the selected probiotic *Saccharomyces* isolates can be exploited further for potential benefits to human health. Although these yeast isolates met the essential requirements for a probiotic, additional *in vivo* or animal studies on the isolates safety are necessary to demonstrate its potential for therapeutic use and significant health benefits. The study indicates yeast as potential probiotic candidate for the preparation of various probiotic products with improved functional properties.

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References

1. Bajwa J. and Sharma N., Evaluation of probiotic properties of yeasts isolated from Sidra–An ethnic fermented fish product of North East India, *Int J Curr Microbiol Appl Sci*, **7**, 2632-43 **(2018)**

2. Collado M.C., Meriluoto J. and Salminen S., Adhesion and aggregation properties of probiotic and pathogen strains, *Eur Food Res Technol*, **226**, 1065-1073 **(2008)**

3. Dey A., Ragavan M.L., Mandal S.K. and Das N., Isolation, identification and in vitro characterisation of probiotic yeast strains, *Res J Pharm Technol*, **10**, 726-732 **(2017)**

4. Gut A.M., Vasiljevic T., Yeager T. and Donkor O.N., *Salmonella* infection–prevention and treatment by antibiotics and probiotic yeasts: a review, *Microbiology*, **164**, 1327-44 **(2018)**

5. Hsiung R.T., Fang W.T., Le Page B.A., Hsu S.A., Hsu C.H. and Chou J.Y., *In vitro* properties of potential probiotic indigenous yeasts originating from Fermented Food and Beverages in Taiwan, *Probiotics Antimicrob Proteins*, **13**, 1-12 **(2020)**

6. Liong M.T. and Shah N.P., Optimization of cholesterol removal by probiotics in the presence of prebiotics by using a response surface method, *Appl Environ Microbiol*, **71**, 1745-1753 **(2005)**

7. Lombardi A., Dal Maistro L., De Dea P., Gatti M., Giraffa G. and Neviani E., A polyphasic approach to highlight genotypic and phenotypic diversities of *Lactobacillus helveticus* strains isolated from dairy starter cultures and cheeses, *J Dairy Res*, **69**, 139-149 **(2002)**

8. Maragkoudakis P.A., Zoumpopoulou G., Miaris C., Kalantzopoulos G., Pot B. and Tsakalidou E., Probiotic potential of *Lactobacillus* strains isolated from dairy products, *Int Dairy J*, **16**, 189-99 **(2006)**

9. Markowiak P. and Śliżewska K., Effects of probiotics, prebiotics and synbiotics on human health, *Nutrients*, **9**, 1021 **(2017)**

10. Mishra V. and Prasad D.N., Application of in vitro methods for selection of *Lactobacillus casei* strains as potential probiotics, *Int J Food Microbiol*, **103**, 109-115 **(2005)**

11. Mora D., Fortina M.G., Parini C., Ricci G., Gatti M., Giraffa G. and Manachini P.L., Genetic diversity and technological properties of *Streptococcus thermophilus* strains isolated from dairy products, *J Appl Microbiol*, **93**, 278-287 **(2002)**

12. Rawat K., Kumari A., Kumar S., Kumar R. and Gehlot R., Traditional fermented products of India, *Int J Curr Microbiol App Sci*, **7**, 1873-1883 **(2018)**

13. Rosenberg M., Gutnick D. and Rosenberg E., Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity, *FEMS Microbiology Letters*, **9**, 29-33 **(1980)**

14. Roussel C., Sivignon A., De Vallee A., Garrait G., Denis S., Tsilia V., Ballet N., Vandekerckove P., Van de Wiele T., Barnich N. and Blanquet-Diot S., Anti-infectious properties of the probiotic *Saccharomyces cerevisiae* CNCM I-3856 on enterotoxigenic *E. coli* (ETEC) strain H10407, *Appl Microbiol Biotechnol*, **102**, 6175-89 **(2018)**

15. Savitri and Bhalla T.C., Traditional foods and beverages of Himachal Pradesh, *Indian J Tradit Knowl*, **6**, 17-24 **(2007)**

16. Sen S. and Mansell T.J., Yeasts as probiotics: Mechanisms, outcomes and future potential, *Fungal Genet Biol*, **137**, 103333 **(2020)**

17. Simões L.A., Cristina de Souza A., Ferreira I., Melo D.S., Lopes L.A., Magnani M., Schwan R.F. and Dias D.R., Probiotic properties of yeasts isolated from Brazilian fermented table olives, *J Appl Microbiol*, **131**, 1983-97 **(2021)**

18. Syal P. and Vohra A., Probiotic potential of yeasts isolated from traditional Indian fermented foods, *Int J Microbiol Res*, **5**, 390 **(2013)**

19. Tamang J.P., Ethno-microbiology of ethnic Indian fermented foods and alcoholic beverages, *J Appl Microbiol*, **133**, 145-61 **(2022)**

20. Thakur N., Savitri, Saris P.E. and Bhalla T.C., Microorganisms associated with amylolytic starters and traditional fermented alcoholic beverages of North Western Himalayas in India, *Food Biosci*, **11**, 92-96 **(2015)**

21. Valdés-Varela L., Gueimonde M. and Ruas-Madiedo P., Probiotics for prevention and treatment of *Clostridium difficile* infection, *Advances in Experimental Medicine and Biology*, **1050**, 161–176 **(2018)**.

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