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PROBIOTIC CHARACTERIZATION OF *SACCHAROMYCES CEREVISIAE* Y196 AND Y197 ISOLATED FROM RICE *CHHANG*- A FERMENTED BEVERAGE OF LAHAUL SPITI

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ABSTRACT

Fermented foods and beverages are rich source of probiotic microorganisms and yeasts are the most common microorganisms found in these fermented products. Fermented beverages are less explored for probiotics, therefore; the present study was focused on isolation, screening and characterization of probiotic yeast from rice *Chhang*, a fermented beverage of Lahaul Spiti. Ten yeast isolates were initially isolated from rice *Chhang* and out of which two isolates *Saccharomyces cerevisiae* Y196 and *Saccharomyces cerevisiae* Y197 were selected based on their antimicrobial activity. These two yeast isolates were examined for various probiotic properties such as survival at low pH, bile tolerance, microbial adhesion to hydrocarbons, cholesterol assimilation, exopolysaccharide production and haemolytic activity. Both isolates showed antimicrobial activity against all the tested pathogens used in this study, also showed high acid tolerance at pH 2 and pH 3 and can withstand high bile concentration as compared to control. Percentage of hydrophobicity was found to be 57 % and 41 % in *Saccharomyces cerevisiae* Y196 and *Saccharomyces cerevisiae* Y197, respectively. Both isolates were able to show high cholesterol assimilation with a maximum of 90 % in case of *Saccharomyces cerevisiae* Y197 with taurocholate. They were also found to be positive for exopolysaccharide production and showed no haemolytic activity. Hence, keeping in view the results of present study, these isolates can be proposed for preparation of various probiotic food products.

Keywords: Yeast, rice *Chhang*, probiotics, cholesterol assimilation, Himachal Pradesh

INTRODUCTION

Probiotics are live bacteria, when administered in adequate amounts they boost the host's health (**FAO/WHO, 2002**). Probiotics are utilized as food or pharmaceutical supplements to improve human or animal health by changing one of the three primary roles of normal intestinal microflora, namely colonization resistance, immunomodulation, and nutritional contribution (**Martins *et al*., 2005**). Various *in vitro* studies validate that probiotics have been shown to provide anticancer, antioxidant, antiobesity, inflammatory bowel disease alleviation, improved lactose tolerance, resistance to pathogens, cholesterol reduction, allergy relief and increase the immunity (**Grau *et al*., 2017; Lenoir *et al*., 2016; Markowiak and Slizewska, 2017; Valdes-Varela *et al*., 2018**).

The majority of probiotics now in use are prokaryotic in origin. Prokaryotic probiotics include lactic acid bacteria, *bifidobacteria*, *enterococci*, and a variety of other bacteria (**Savitri and Lata, 2021**). *Lactobacilli* and *bifidobacteria* are increasingly being added to foods as dietary supplements to improve human health. During food fermentation, microorganisms change the chemical contents of raw substrates of plant or animal origin, enhancing the nutritional value of the products. They also enhance the flavor and texture, maintain perishable foods, increase food shelf life, and supplement products with health-improving bioactive substances. Microbes breakdown noxious chemicals and anti-nutritive factors present in foods, generate antioxidants and antibacterial substances, and boost probiotic effects **(Tamang, 1998; Farhad *et al*., 2010**).

Traditional fermented foods and beverages are very common and popular in Himachal Pradesh. A variety of fermented products are prepared and consumed in Himachal and these products are unique and distinct from those found in other regions of the country (**Thakur *et al*., 2004**). The fermented beverages prepared in this region are less explored and are rich source of probiotics with the potential to benefit a variety of health conditions. It is therefore very important that microorganisms involved in the fermentation of these products must be assessed for their probiotic attributes. *Chhang* is a main cultural alcoholic beverage of tribal and rural areas of Lahaul Spiti of Himachal Pradesh **(Thakur *et al*., 2015)**. It is prepared by solid-state fermentation of cooked rice/barley using inoculum traditionally called '*phab*' which contains a consortium of lactic acid bacteria and yeast (**Angmo and Bhalla, 2014; Handa and Sharma, 2016**).

The most prevalent and essential microorganisms found in fermented foods and beverages are yeasts. Yeasts have long been used in fermented rice beverages for their health benefits (**Mishra *et al*., 2018**). The ability of yeasts to survive the passage through the human gastrointestinal (GI) tract, tolerating exposures to low pH and bile salts make them potential candidates as probiotics. Different yeast species such as *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Debaryomyces hansenii*, *Torulaspora delbrueckii* (**Psani and Kotzekidou, 2006**), *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Kluyveromyces lodderae* (**Kumara *et al*., 2004**) have shown tolerance to passage through the gastrointestinal tract and found to inhibit enteropathogens.

The main issue linked with the use of *Lactobacillus* and bacterial probiotics is the genetic exchange of drug resistance genes from these bacteria to infections (**Czerucka *et al*., 2007**). However, no eukaryotic probiotics have been observed to transfer antibiotic resistance genes, which is a serious problem for bacterial probiotics. *Lactobacillus* spp. utilized as probiotics, such as *Lactobacillus lactis* and others, have been extensively researched for the genes that confer resistance to common antibiotics such as tetracycline, vancomycin, and erythromycin (**Egervarn *et al*., 2009**). Yeasts are not affected by antimicrobial substances, and they contain a variety of immunostimulant molecules (e.g., β-glucans, nucleic acids, and mannan-oligosaccharides), which aid in conferring protection against a variety of infections (**Li and Gatlin, 2006; Lokesh *et al*., 2012).** The aim of present study is to isolate the yeasts present in rice *Chhang* and to characterize the yeast isolates for probiotic potential.

MATERIAL AND METHODS

Isolation of yeasts

Yeast isolates were isolated from rice *Chhang* using serial dilution and spread plate method. One gram of sample was homogenized with 9 ml of saline (0.9 % NaCl) and serial dilutions were prepared. An aliquot of 0.1 ml of each dilution was plated on Yeast Malt Agar (peptone 5.0 g/L, yeast extract 3.0 g/L, malt extract 3.0 g/L, dextrose 10.0 g/L, agar 20.0 g/L (HiMedia, Mumbai, India) 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. To obtain a pure culture, the isolated yeasts were streaked on yeast malt agar plates.

Morphological and biochemical characterization

The yeast isolates were checked for morphological characters like color, surface and margin, etc. For biochemical characterization catalase test, urease test and sugar fermentation test were carried out.

Catalase test

Catalase activity of yeasts was evaluated by adding 3 % (v/v) of hydrogen peroxide onto the fresh culture broth, according to the **Whittenbury (1964)**.

Urease test

This test was performed in the Christensen's urea agar plate (Oxoid), containing phenol red as pH indicator. After adding the yeast inoculum, plates were incubated at 30 ºC for 4 days; color turning to purple showed the presence of urea hydrolysis due to increase in pH.

Sugar fermentation

Sugar fermentation was tested by using API 20C AUX identification kit (Biomereux India Pvt. Ltd.).

Growth at different temperature

Yeast isolates were examined for their growth and stability at different temperature (20, 25, 30, 37, and 45 ºC). Yeast isolates were inoculated in YPD (yeast peptone dextrose) broth and incubated at different temperature for 24 h.

Antimicrobial activity

Antimicrobial activity of yeast isolates were 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.* Agar well diffusion method was used to test the antimicrobial activity as described by **Mishra and Prasad (2005)**. The supernatants of 20 h grown yeast cells were used against indicator pathogens.

Identification and Sequencing of finally selected yeast isolates

The yeast isolates were sequenced and identified at Biologia Research India Pvt. Ltd., New Delhi, India. ITS DNA region of isolated DNA was amplified by using universal primers ITS1: TCCGTAGGTGAACCTGCGG and ITS4: TCCTCCGCTTATTGATATGC. 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 (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi). The assembled sequences were deposited in Genbank database under accession number OL454190 and OL444816. A phylogenetic tree was constructed to determine the closest yeast species by the neighbor-joining approach (**Saitou and Nei, 1987**), using MEGA 11 (**Tamura *et al*., 2011**).

Probiotic Characterization

Survival to low pH

The yeast isolates were studied for their tolerance to acidic conditions by the method of **Maragkoudakis *et al*. (2006)**. The ability of isolates to grow in the acidic conditions was checked at pH 2, pH 3 and pH 7 (control) for 3 h. The survival of yeast isolates was calculated in terms of Log cfu/mL.

Bile salt tolerance

The method used for testing bile tolerance was performed by **Gilliland *et al*. (1984)**. Yeast isolates were inoculated into sterilized 9 ml of YPD broth containing 0.5 %, 1 % and 2 % Ox-bile and incubated at 30 ºC for 3 h. The survival of yeast isolates was calculated in terms of Log cfu/mL.

Microbial adhesion to hydrocarbons

The adhesion of yeast isolates to various hydrocarbons was analyzed by the modified method of **Rosenberg *et al*. (1980**). Different hydrocarbons viz. n-hexadecane, xylene and toluene were used to assess the hydrophobicity of yeast isolates. Experiments were performed in triplicate and hydrophobicity was calculated in percentage by using formula:

Hydrophobicity (%) = [(A0-A)/A0] ×100

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

Cholesterol assimilation

Cholesterol assimilation by yeast isolates was done using o-phthalaldehyde method according to **Liong and Shah (2005)**. Three different bile salts viz. cholic acid, sodium taurocholate and ox bile were used in the study.

Exopolysaccharide production

The production of exopolysaccharide was assessed using the approach used by **Mora *et al*. (2002)**.

Haemolytic activity

Haemolytic activity of yeast isolates were evaluated by the method of **Lombardi *et al*. (2002)**. The Columbia 5 % Sheep Blood agar plates were streaked with 24 h grown culture and incubated at 30 °C for 24 h to observe the haemolysis.

Statistical analysis

The data was obtained from three separate experiments and provided as mean values. The statistical analysis was carried out with SPSS Inc. software (version 21.0). ANOVA and Tukey's multiple comparison test ($p < 0.05$) were used to compare the results.

RESULTS AND DISCUSSION

In total, 10 yeast isolates were isolated from rice C*hhang.* Earlier studies showed that yeasts are predominately present in fermented beverages like *Chhang*, rice wine, etc (**Thakur *et al*., 2015**). **Bhardwaj *et al*. (2016)** isolated the yeast mainly *Saccharomyces* spp. from the traditional alcoholic beverage and *balma* prepared by Bhotiya tribe of Uttarakhand, India. Similarly, number of lactic acid bacteria, acetic acid bacteria and yeast grew marginally during rice '*calugi*' fermentation (**Miguel *et al*., 2012**).

Morphological and biochemical characterization

Morphological, biochemical characteristics and sugar fermentation of yeast isolates are shown in Table 1 & 2. The colonies of yeast isolates on YM agar plates were of large sized, white/cream colored and having smooth surface. All the screened isolates were found to be negative for urease activity and three (Y1, Y4 and Y9) out of ten isolates were found positive for catalase activity (Table 1).

Sugar fermentation

All the yeast isolates ferment glucose, galactose and sucrose, while some isolates also ferment maltose, raffinose, methyl-αD-glucopyranoside and sorbitol (Table 2).

Table 1 Morphological and biochemical characteristics of yeast isolates

Sr. no.	Yeast isolates	Color	Surface	Margin	Elevation	Catalase	Urease test
1.	Y1	White	Smooth	Entire	Convex	+	-
2.	Y2	Cream	Smooth	Entire	Convex	-	-
3.	Y3	White	Smooth	Entire	Convex	-	-
4.	Y4	White	Smooth	Entire	Convex	+	-
5.	Y5	White	Smooth	Entire	Convex	-	-
6.	Y6	White	Smooth	Entire	Convex	-	-
7.	Y7	Cream	Smooth	Entire	Convex	-	-
8.	Y8	Cream	Smooth	Entire	Convex	-	-
9.	Y9	Cream	Smooth	Entire	Convex	+	-
10	Y10	Cream	Smooth	Entire	Convex	-	-

+ Presence, - absence of enzyme

Table 2 Sugar fermentation by yeast isolates

Sugars	**Yeast isolates**									
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10
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	-	+	-	+	-	+	+	+	-	+

+ Fermentation, - no fermentation

Growth at different temperature

Growth of all the yeast isolates at different temperature after 24 h of incubation is depicted in Table 3. All the isolates were viable upto 30 ºC and showed no viability at 45 ºC.

Table 3 Growth of yeast isolates at different temperature

Sr. no.	**Yeast isolates**	**20** ºC	**25** ºC	**30** ºC	**37** ºC	**45** ºC
1.	Y1	+	+	+	+	-
2.	Y2	+	+	+	+	-
3.	Y3	+	+	+	-	-
4.	Y4	+	+	+	+	-
5.	Y5	+	+	+	+	-
6.	Y6	+	+	+	-	-
7.	Y7	+	+	+	-	-
8.	Y8	+	+	+	+	-
9.	Y9	+	+	+	-	-
10	Y10	+	+	+	-	-

+ Presence, - absence of growth

Antimicrobial activity

An essential requirement of probiotics is their inhibitory effect against pathogenic organisms. The inhibitory effect of probiotic organisms is either by decreasing the pH, or generation of certain organic acids, competition for substrates and space, and the production of metabolites such as bacteriocins, hydrogen peroxide and antibiotics (**Dey *et al*., 2017**). The yeast isolates were checked for antimicrobial activity against eight pathogenic strains (Table 4). Out of 10 isolates, two Y2 and Y8 exhibited the antimicrobial activity against all pathogenic strains used.

Identification and phylogenetic analysis of finally selected yeast isolates

The best two yeast isolates were chosen based on their broadest antimicrobial activity against pathogenic strains. After partial sequencing, sequences of *Saccharomyces cerevisiae* Y196 (Y2) with accession number OL454190 and *Saccharomyces cerevisiae* Y197 (Y8) with accession number OL444816 were deposited in GenBank database. The phylogenetic analysis of yeast isolates was also performed by neighbor-joining approach by using MEGA 11 software (figure 1).

Table 4 Antimicrobial activity of yeast isolates against pathogens by well agar diffusion method

Isolates	***S. typhi*** **(mm)**	***E. coli*** **(mm)**	***Shigella*** **(mm)**	***P. aeruginosa*** **(mm)**	***B. cereus*** **(mm)**	***S. aureus*** **(mm)**	***A. hydrophilla*** **(mm)**	***L. monoctyogenes*** **(mm)**
Y1	-	-	10.00±0.50	-	-	11.00±0.00	15.00±0.50	-
Y2	11.00±0.00	12.00±1.00	9.50±0.50	10.00±0.00	12.50±0.50	11.00±0.00	20.00±2.00	11.00±1.00
Y3	8.00±0.00	10.00±1.00	-	11.00±0.50	9.00±0.50	8.00±0.50	14.50±0.00	-
Y4	11.00±0.50	-	-	-	-	9.00±0.50	17.00±0.50	-
Y5	-	10.00±1.00	-	-	9.00±0.00	-	15.00±1.50	8.50±0.50
Y6	-	-	-	11.00±0.00	-	-	14.00±1.00	-
Y7	10.00±0.50	-	10.00±0.00	9.50±0.00	10.00±0.00	-	18.00±0.00	-
Y8	11.00±1.00	9.50±0.50	11.50±0.50	10.00±0.00	12.00±0.00	11.50±0.50	16.50±1.50	10.00±0.00
Y9	-	11.00±0.00	12.00±0.00	-	10.00±1.50	-	10.00±0.00	8.00±1.00
Y10	10.00±0.00	10.50±1.00	-	9.00±1.00	-	-	-	11.00±0.00

Values represented as mean ± standard deviation (SD) of triplicate analysis, - no zone of inhibition

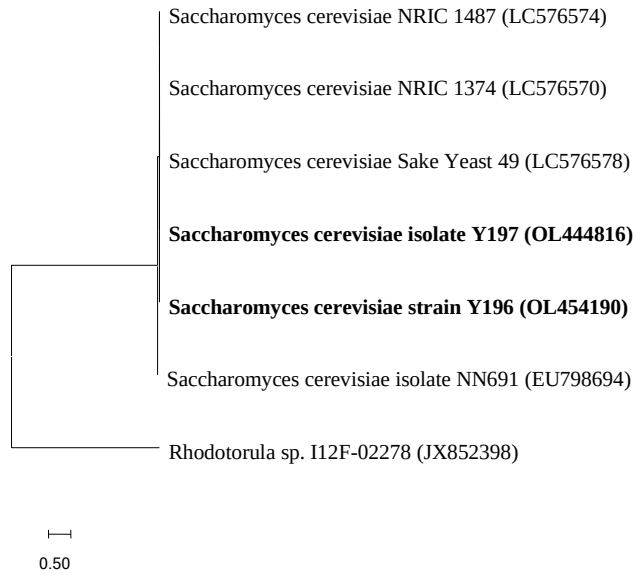

Figure 1 Phylogenetic tree of yeast isolates based on homology of ITS gene sequences

Probiotic characterization

Survival at low pH

Probiotic organisms have to survive in the severe circumstances of gastrointestinal tract therefore acid and bile tolerance is widely considered a key assessment factor for probiotic evaluation. The result of acid tolerance of both isolates is given in Table 5. The results showed that the isolates were quite tolerant to pH 2 and pH 3 after 3 h. There was no significant difference in the survival of isolates at pH 2, pH 3 when compared to pH 7 (control). These findings are similar to those published by **Adesokan *et al*. (2021)**, in which after 3 h of incubation, two *Saccharomyces cerevisiae* strains, BKT07 and OBB17, had the maximum growth of 7.2 log cfu/mL, while *Saccharomyces cerevisiae* SC01 had the lowest growth of 6.1 log cfu/mL at pH 3.

Bile salt tolerance

Concentration of bile varies in the gastrointestinal tract and depends on the type of food that we eat. During the first hour of digestion, the concentration of bile in different area of the gut varies from 0.5 % to 2 %. Both the isolates showed the significant resistance to the bile salt concentration, sustaining their viability with minimal decline viable count. Studies showed that yeast strains were resistant to bile salts at different concentrations i.e., 0.3, 0.5, 1.0, 1.5 and 2.0 % (**Bajwa and Sharma, 2018**). In another study, three yeast strains demonstrated significant bile salt tolerance in another investigation, with bile concentrations ranging from 0.2 to 1.0 percent. The bile tolerance ability of *Yarrowia lipolytica* VIT-ASN04 was found to be very good in a study reported by **Dey *et al*. (2017)**.

Table 5 Acid and Bile tolerance of yeast isolates

Isolates	Acid tolerance (Log cfu/mL)			Bile tolerance (Log cfu/mL)			
	pH 7	pH 3	pH 2	Control	0.5 %	1 %	2 %
Saccharomyces cerevisiae Y196	6.35^{aA}±0.09	6.32^{aA}±0.16	6.20^{aA}±0.07	7.50^{aB}±0.10	7.46^{aB}±0.02	7.45^{aB}±0.02	7.40^{aB}±0.04
Saccharomyces cerevisiae Y197	6.48^{aA}±0.18	6.39^{aA}±0.18	6.28^{aA}±0.11	6.59^{aA}±0.31	6.50^{aA}±0.32	6.46^{aA}±0.15	6.41^{aA}±0.05

Values represented as mean ± standard deviation (SD) of triplicate analysis
[a] = average in the rows with same superscript letter are not significantly different as measured by 2 sided Tukey's post hoc range test between replications.
[A-B] = average in the column with same superscript letter are not significantly different as measured by 2 sided Tukey's post hoc range test between replications.

Microbial adhesion to hydrocarbons

A nonspecific connection between microbial cells and their hosts is cell surface hydrophobicity. This connection/interaction is considered as an essential factor in the adhesion and proliferation of microorganisms on the intestinal cells (**Del Re *et al*., 1998**). Cell adhesion is usually determined by the properties of their cell surface. Three hydrocarbons such as n-hexadecane, xylene and toluene were used to assess the adhesion property of the isolates. Both isolates showed greater cell surface hydrophobicity (%) towards toluene and least with the n-hexadecane (figure 2). It was observed that *Saccharomyces cerevisiae* Y196 illustrated the highest hydrophobicity i.e. 57 % against toluene and lowest (16 %) against n-hexadecane. Similar study also revealed that seven yeast isolates from *idli* and *jalebi* batter demonstrated 32 to 67 percent and 45 to 86 percent microbial adhesion with xylene and n-hexadecane, respectively (**Syal and Vohra, 2013**). **Hossain *et al*. (2020)** reported that a novel strain of *Saccharomyces boulardii* isolated from soya paste showed a higher affinity for chloroform (40.31%) than n-hexadecane (23.11%).

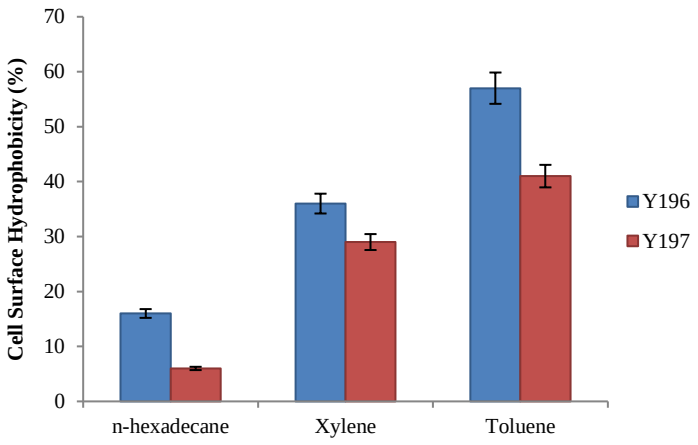

Figure 2 Microbial adhesion to different hydrocarbons by *Saccharomyces cerevisiae* isolates

Cholesterol assimilation

High blood cholesterol levels have been linked to an increased risk of heart diseases, so the use of probiotic bacteria to lower cholesterol levels has attained a lot of attention. The assimilation of cholesterol during 24 h of growth of yeast isolates is depicted in Table 6. With taurocholate, cholic acid and oxbile, cholesterol uptake was highest, intermediate, and least in the medium, respectively. The range of cholesterol assimilation in *Saccharomyces cerevisiae* Y196 and *Saccharomyces cerevisiae* Y197 was 46-87 %, and 28-90 %, respectively. *Saccharomyces boulardii*, *Saccharomyces cerevisiae* and *Pichia kudriavzevii*, have been studied as prospective probiotics for cholesterol absorption (**Psomas *et al*., 2003**). In another study**, Dey *et al*. (2017)** found that three yeast isolates isolated from fruits, *Wickerhamomyces anomalus* VIT-ASN01, *Saccharomyces cerevisiae* VIT-ASN03, and *Yarrowia lipolytica* VIT-ASN04, absorb cholesterol by 51, 46, and 56 percent after 6 h, 12 h, and 24 h of incubation, respectively.

Table 6 Cholesterol assimilation of yeast isolates

Isolates	Oxbile (%)	Cholic acid (%)	Taurocholate (%)
Saccharomyces cerevisiae Y196	46.10^{aB}±1.35	64.61^{bA}±0.72	87.27^{cA}±1.73
Saccharomyces cerevisiae Y197	28.36^{aA}±1.02	75.00^{bB}±0.78	90.14^{cB}±0.23

Values represented as mean ± standard deviation (SD) of triplicate analysis
[a-c] = average in the rows with same superscript letter are not significantly different as measured by 2 sided Tukey's post hoc range test between replications.
[A-B] = average in the column with same superscript letter are not significantly different as measured by 2 sided Tukey's post hoc range test between replications.

Exopolysaccharide production

Exopoysaccharides are polymeric compounds that help probiotics colonize the gastrointestinal tract by enhancing cell-cell interactions (**Kanmani *et al*., 2013**). The considerable changes in pH act as a regulatory element in the production of exopolysaccharide by yeasts (**Adami and Cavazzoni, 1990; Elinov *et al*., 1992**). Both yeast isolates produced exopolysaccharide on skimmed milk-ruthenium red plates (figure 3 a). During a study on yeasts isolated from traditional fermented foods **Syal and Vohra (2013)** found similar results for exoplysaccharide synthesis.

Haemolytic activity

The absence of haemolysis is regarded as a safety requirement for probiotic strain selection (**FAO/WHO, 2002**). Several studies have reported that the lactic acid bacteria isolated from fermented foods are non haemolytic (**Santini *et al.*, 2010**). In the present study, no haemolysis was observed when both isolates were streaked on blood agar plates (figure 3 b).

(a) (b)

Figure 3 a) Exopolysaccharide production by *Saccharomyces cerevisiae* on ruthenium red plate, **b)** Yeast isolates streaked on blood agar plates showing no haemolysis

CONCLUSION

The yeast isolates namely *Saccharomyces cerevisiae* Y196 and *Saccharomyces cerevisiae* Y197 isolated from rice *Chhang*, a fermented beverage of Lahaul Spiti has been characterized for probiotic properties. These isolates were found to be resistant to acidic conditions (pH 2) and bile salts (2 %). Yeast isolates showed antimicrobial activity against eight foods spoilage causing bacteria and were also non-haemolytic which suggested their use in probiotic products. The percentage of assimilation of cholesterol was very high in both the isolates especially in *Saccharomyces cerevisiae* Y197 (90 %) in medium with toluene which revealed their hypocholesterolemic properties. Isolates showed adhesion to hydrocarbons and were determined to be positive for exopolysachharide production. However, more research on the influence of these isolates on health of the consumers will further expand their significance as probiotics and their use in food industry.

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