September 2010 ISSN : 0974 - 7435 Volume 4 Issue 3

Trade Science Inc.

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 4(3), 2010 [130-135]

Screening and evaluation of novel probiotic yeast from food products

N.S.Meera, M.Charitha Devi*, P.Mounica

Department of Virology, College of Sciences, Sri Venkateswara University, Tirupati, A.P., (INDIA)

E-mail : charithamekala@yahoo.co.in; nsmeera378@gmail.com

Received: 27th March, 2010 ; Accepted: 6th April, 2010

ABSTRACT

Yeast is emerging as potential probiotic organism. Already there is a marked increase in the sale of various yeast based probiotic products. However there is a need to screen more potential probiotic strains of *Sacharomyces* and to develop new probiotic based food products. The main emphasis of the present study is screening and evaluation of probiotic properties of the yeast cultures isolated from various food sources. In this study ten isolates of food grade yeast were isolated from various food sources and identified using morphological, physiological and biochemical characteristics. The ten isolates were screened for their thermo, osmo, acid tolerance, antimicrobial properties and β- galactosidase enzyme activity. Among the ten isolates one potential isolate was selected for the fermentative production of yeast. The medium and physical conditions were optimized. Baker's yeast is used as reference yeast culture throughout the study. The optimized medium resulted in increased production of yeast compared to the initial production. The *Sacharomyces sp.* isolated from grapes G4 shows maximum growth at optimized conditions and is having high osmo, thermo and acid tolerance as compared to baker's yeast and has high antimicrobial activity as compared to the other yeast cultures isolated from curd sample. Based on the above results obtained *Sacharomyces sp.* isolated from grapes G4 was found to be the potential isolate and may be used for further human application that is either direct food or in the preparation of functional foods to enhance the health by improving the balance of micro flora in the intestine.

KEYWORDS

Probiotic properties;
Yeast;
Optimization;
Production.

INTRODUCTION

"Probiotics are live dietary supplements or food ingredients that have a beneficial effect on the host by influencing the composition and or metabolic activity of the flora of the gastrointestinal tract.[11]". Certain strains are used as probiotics in pharmaceutical preparations, feed additives and so called functional foods, yeasts also possess some medicinal efficiency. In addition to their nutritive value, probiotic yeasts are generally resistant to gastro intestinal passage and are resistant to most antibiotics. Yeast preparations have also been successfully applied in combination with antibiotics to treat *Clostridium difficile* related diarrhea commonly known as antibiotic associated diarrhea[10].

Yeast is widely distributed in nature. They are

TABLE 1 : Morphological, physiological characteristics of isolated yeast strains

Morphological features	B1	B2	C1	C2	C3	C4	G1	G2	G3	G4
Color	White	White	White	White	Pale yellow	Pale yellow	Colorless	White	White	White
State	Leathery	Mucoid	Mucoid	Leathery	Powdery	Mucoid	Leathery	Filaments	Leathery	Mucoid
Shape of rising	Convex	Convex	Convex	Convex	Convex	Convex	Concave	Concave	Concave	Concave
Feature of surface	Rough	Smooth	Rough	Rough	Smooth	Smooth	Smooth	Rough	Rough	Smooth
Reproduction	-	-	Budding	Budding	Budding	-	-	-	-	Budding

Under the microscope the yeast cells observed were simple filaments to elaborate pseudohyphae, Budding cells were observed

present in orchids and wine yards, in air and soil and in the intestinal tract of animals. Varieties of the *Saccharomyces cerevisiae* genus are the most common yeast in fermented foods and beverages based on fruits and vegetables.

The selection of suitable strain of a microorganism can be regarded as the primary requirement for the use as a probiotic. The multi-strain probiotic can act in broad spectrum and expected to be active in different species of host animals and against microbial infections[13].

Yeast is carefully selected for high growth and fermentation rate, osmo tolerance, low pH fermentation optimum and high temperature fermentation optimum. The yeast culture '*S. cerevisiae'* used is mesophilic in nature and may not exert more beneficial action due to harsh environmental conditions (temperature, variation in pH and NaCl concentration under which they have to survive in the gastro intestinal tract). Hence identification of thermo, osmo and acid tolerant strains of yeast isolated from food products will have more pronounced beneficial effects. The main objective of the present study is to select a potential probiotic strain of yeast having thermo, osmo, acid tolerance and antimicrobial properties from various food products for human applications and optimization of cultural conditions for the fermentative production of yeast.

MATERIALS AND METHODS

Yeast strains were isolated from different food sources (Bakery waste, cow curd and grape samples) using the YEP agar medium (Yeast extract 2%, peptone 2%, glucose 1%,NaH_2Po_4 0.2%, $MgSo_4$ 0.05%, distilled water 100 ml.), and based on their morphological, biochemical parameters out of ten isolates two *Sacharomyces sp*. B1,B2 from bakery, four *Sacharomyces sp* C1,C2,C3 and C4 from cow curd and four *Sacharomyces sp* G1, G2, G3 and G4 from grapes were selected for *in vitro* screening of probiotic properties like acid, thermo, osmo tolerance and antimicrobial activity (B = bakery, C = curd, G = grapes).

Morphological, biochemical and physiological characteristics

The cell morphology was examined by microscopy by performing Gram staining and simple staining. The morphological features were presented in TABLE 1. Sugar fermentation was determined by growing the *Sacharomyces sp.* in minimal medium containing different sugars with inverted Durham's tube incubated at 37°C for 48 hrs. Phenol red indicator dye was added to the minimal medium, change in color and gas formation was checked after 48 hrs[9].

Invitro screening of thermo, acid and osmo tolerance

Thermo tolerance was determined by growing the isolated yeast culture at respective temperatures 35°C, 40°C, 45°C, 50°C for 24 hrs. The cell growth was measured at 570nm. To determine the optimum pH the yeast culture was grown in the pH range of 2-11 and incubated for 24 hrs at optimum temperature and the cell growth was measured at 570nm[8]. The isolated yeast spp. were grown in the culture broth consisting of NaCl ranging from 0.5-3% and the cell growth was determined at 570nm[5]. Bakers yeast culture was used as reference culture throughout the study for comparison.

Invitro screening of antimicrobial property

Antimicrobial property was determined by disc diffusion method. The actively growing *E.coli* suspension was used as the indicator strain. yeast culture filter paper disks and two antibiotic discs (streptomycin and ampicillin) as reference discs were placed on the sur-

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TABLE 2 : Sugar fermentation by isolated yeast strains

Sugars	Glucose		Fructose		Lactose		Galactose		Sucrose		Mannitol		Maltose		Xylose	
	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
B1	+	-	+	+	+	+	+	_	+	_	_	+	_	+	+	+
B2	-	+	-	+	-	+	+	+	+	+	+	+	+	-	+	+
C1	-	+	-	+	+	+	-	-	+	-	+	-	+	+	+	+
C2	-	+	-	+	-	+	-	-	-	-	+	+	+	+	+	+
C3	+	-	-	-	+	-	-	-	+	-	+	-	+	+	+	+
C4	-	+	-	+	-	+	+	-	+	-	+	-	+	+	+	+
G1	-	-	-	+	-	-	-	-	+	-	+	-	+	+	+	+
G2	-	-	-	+	-	-	-	-	-	-	+	-	+	+	+	+
G3	+	+	-	+	-	+	-	-	+	-	+	-	+	+	+	+
G4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

A-Acid production; G- Gas production; B- Bakery waste; C-Curd; G- Grape sample

TABLE 3 : Anti microbial activity

Strains	Zone diameter inhibition (mm)
B1	1.0
B2	1.1
C1	0.8
C2	-
G1	-
G2	1.2
G3	1.2
G4	1.7
C3	-
C4	-
Ampicillin	1.5
Streptomycin	1.2

'-'= indicates no zone formation

face of agar plate consisting indicator strain and incubated at 37°C for 24 hrs, and the diameter of the zone of inhibition was measured with the help of scale and the effective strain was determined by comparing with standard chart[12].

Antibiotic susceptibility test

Antibiotic susceptibility for ten yeast isolates was determined by disc diffusion method. The various antibiotic discs include Erythromycin (E), Gentamycin (G) Streptomycin (S), Tetracycline (T), Ampicillin (A), Methicillin (M) and Ceffazidine (C),were placed on culture plates and incubated at 37°C for 48 hrs. The isolates were classified as resistant (R), susceptible (S)[1].

β-Galactosidase assay (EC:3.2.1.22)

β-galactosidase enzyme activity was estimated by measuring o-nitrophenol liberated from o nitrophenol β-D-galactoside. The absorbance was measured at 420nm. One unit of enzyme activity was equal to the release of 1 μmol of o-nitro phenol/ minute[15].

$$\text{Galactosidase (units/ml)} = \frac{1000 \times A_{420}}{t \times V}$$

were t = reaction time in minutes; v = volume of sample used.

Optimization of cultural conditions

The factors like temperature (35°C, 40°C, 45°C and 50°C), pH (3.0, 5.0, 7.0 and 9.0) and various carbon (Glucose, fructose, lactose, galactose, sucrose, maltose and xylose) and nitrogen (peptone, tryptone, ammonium chloride, ammonium sulfate and ammonium nitrate) additives affecting the production of yeast were optimized by adapting search technique varying parameters one at a time approach.

RESULTS

Yeast strains were isolated from various food sources. Among these strains, ten isolates of, four from grapes, four from curd and two isolates from bakery waste samples were selected for *in vitro* screening of the probiotic properties. The morphological, biochemical and physiological examinations of the yeast strains were shown in TABLE 1. The yeast cells were creamy in color, circular in shape, regular margined, convex elevated, opaque density and rough textured on the culture agar plate. Cells were simple filaments to elaborate pseudo hyphae and budding cells also observed. Yeast grew within 48 hrs in the culture broth, partially settled to form sediment and formed a cream film on the bottom when the culture flask was kept undisturbed in static conditions. Acid and gas production were tested by sugar fermentation test for the following sugars glucose, fructose, lactose, galactose, sucrose, mannitol, maltose, and xylose are noted in TABLE 2.

Invitro screening of thermo, acid and osmo tolerance

The isolates C4 and *Sacharomyces sp.* isolated from grapes G4 have maximum growth at 40^0c as com-

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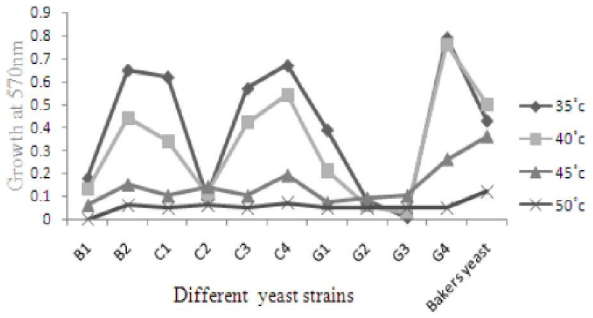

Figure 1 : Thermotolerence test

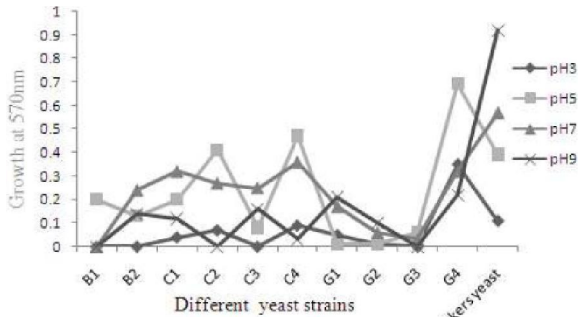

Figure 2 : Acid tolerance test

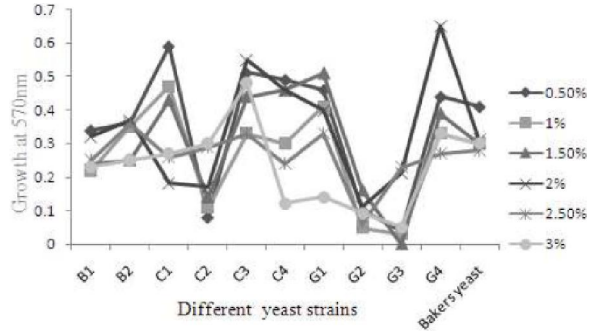

Figure 3 : Osmo tolerance test

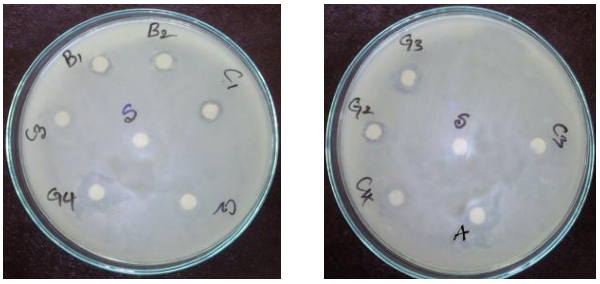

Figure 4 : Anti microbial activity: The isolates B_1, B_2, C_1, G_2, G_3 and G_4 shows resistance to *E.coli*, A-Ampicillin disc (10 mcg/disc), S-Streptomycin disc (10mcg/disc), B1, B2, C1, C2, C3, C4, G1, G2, G3, G4 are culture discs

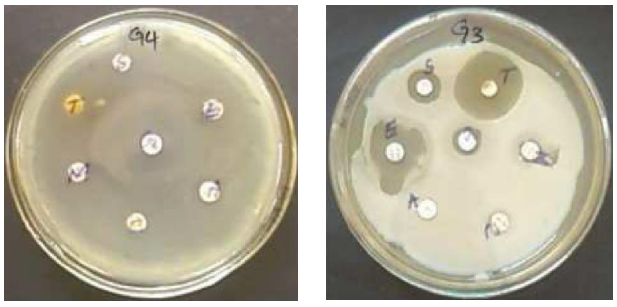

Figure 5 : Inhibition assay test: G_4 showing resistant to antibiotics; G_3 showing sensitivity to antibiotics

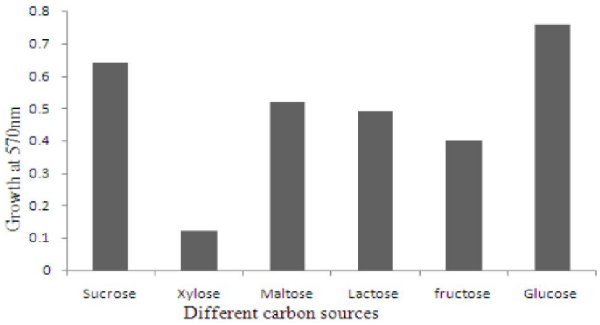

Figure 6 : Effect of carbon sources

pared to the bakers yeast culture (Figure 1). Among the ten isolates grown at various pH the maximum growth of the isolates C4 and *Sacharomyces sp.* isolated from grapes G4 was observed at pH5 as compared to other isolates (Figure 2). Osmotolerance was tested using various concentration of NaCl, maximum growth of *Sacharomyces sp*. isolated from grapes G4 and C3 isolate was noticed at 2% Nacl concentration (Figure 3).

Invitro screening of antimicrobial property and antibiotic susceptibility

A total of ten yeast strains isolated from bakery waste, grapes and curd was tested for antimicrobial activity. Only 5 strains of yeast were shown to produce antimicrobial activity against enteric pathogen *E.coli*. The anti microbial property of the strain *Sacharomyces sp* isolated from grapes G4 was more potent than the other isolated strains followed by B1, C1, G2, G3 strains. The antimicrobial property of yeast isolates against *E.coli* by disc diffusion method is shown in figure 4.

Antibiotic susceptibility test

The strians *Sacharomyces sp*. isolated from grapes G4, G2, and B2 are resistant to 7 antibiotics Erythromycin (E), Gentamycin (G) Streptomycin (S), Tetracycline (T), Ampicillin (A), Methicillin (M) and Ceffazidine (C) used.

The strains C3 and C1 resistant to only fewer antibiotics ampicillin and gentamycin. Inhibitory zones are shown in figure 5.

β-Galactosidase enzyme assay

The β-galactosidase enzyme produced by various

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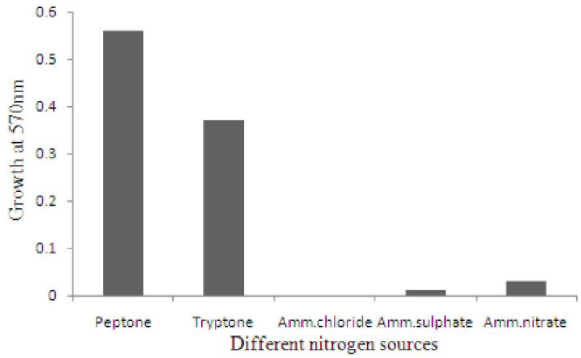

Figure 7 : Effect of nitrogen sources

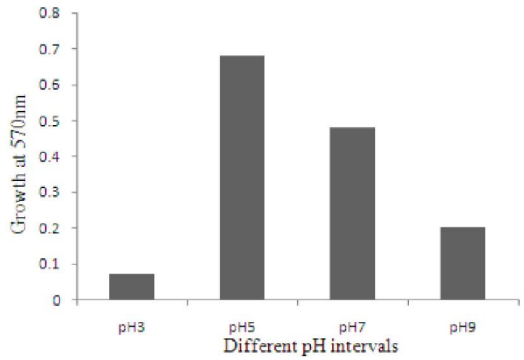

Figure 8 : Optimization of pH

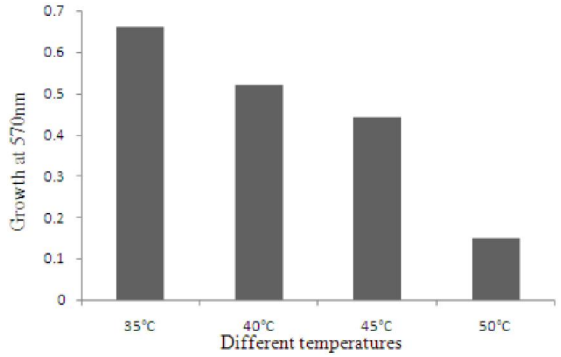

Figure 9 : Optimization of temperature

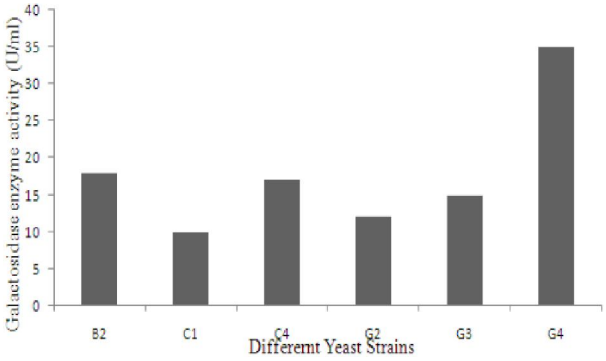

Figure 10 : Galactosidase enzyme activity

strains of yeast was assayed and presented in the figure 10. The *Sacharomyces sp.* isolated from grapes G4 has maximum enzyme activity of (35 IU/ml) followed by other strains B2 (18.5),C1(10), G2(12), G3(15.5), C4(16).

Optimization of cultural conditions

The *Sacharomyces sp.* isolated from grapes G4 was selected for the optimization of cultural conditions as this strain is having highly potent probiotic properties as compared to other strains isolated and is grown in various carbon and nitrogen sources and at different pH range and temperature range. Among different carbon sources tested glucose promoted maximum (yield) biomass of the yeast when compared to others and among the nitrogen sources tested rapid growth was observed in supplementation with peptone as compared to other nitrogen sources. The pH suitability for better production of yeast at different pH conditions was in the range of pH 3-9 with optimum pH at 5.5 and it was resistant to the temperature treatments like 35°C, 40°C, 45°C.

DISCUSSION

Yeast is emerging as potential probiotic organisms. Till now only mesophilic yeast has been used for human applications if we can replace the mesophilic yeast with thermo tolerant yeast, the results can be more beneficial. Apart from being thermo tolerant, if the yeast is bile, thermo and acid tolerant, yeast will have wide adaptability to tolerate the conditions in gastro intestinal tract. Already there is a marked increase in the sale of various yeast based probiotic products. However there is a need to isolate more potential probiotic strains of *Saccharomyces* and to develop new probiotic yeast based dairy products. Using probiotic yeast alone or in combination with lactic acid bacteria can enhance the nutritive value of fermented dairy products. Ten yeast isolates were screened from different sources using YEP agar plates[3]. The yeast isolates were identified using morphological, physiological and biochemical characteristics[2]. The above ten isolates were screened for acid, thermo, osmo tolerance and anti microbial properties. Baker's yeast is used as reference culture. The *Sacharomyces sp.* isolated from grapes G4 is having high thermo tolerance when compared to other isolates as well as with baker's yeast. Among the isolates screened for tolerance only one strain was selected for the optimization of fermentative condition. In the selected fermentation medium different components and

culture conditions were optimized for enhanced growth or production. The isolates B1, B2, C1, G2, G3, and *Sacharomyces sp.* isolated from grapes G4 have high antimicrobial activity against the pathogen *E.coli* strain. These isolates may be used as therapeutic agents against the pathogen[14], The maximum β-galactosidase was produced by *Sacharomyces sp*. isolated from grapes G4. β-galactosidase enzyme was used for lactose utilization are thus β-galactosidase and phospho β-galactosidase. The ability to ferment lactose is critical to success of dairy fermentation involving milk or whey where lactose is the dominant carbon and energy substrate for growth and acid production. There are two different biochemical pathways for lactose utilization[4]. In cheese manufacture, the homolactic lactose fermentation carried out by a phosphoenol pyruvate dependent lactose phospho transferase system, which results in the uptake of lactose into the cell as lactose-6-phosphate. This is hydrolyzed by 6- phospho β-D galactosidase. (Phospho β-galactosidase, E.C. 3.2.1.85) to give glucose and galactose 6 phosphate for sub is metabolized to lactic acid, where as the galactose is usually released into the medium by an energetically efficient galactose lactose antiport system[7]. The ability of strains to grow on galactosyl lactose, however invariably correlated with the presence of β-galactosidase. Since galactosyl lactose can be hydrolyzed by β-galactosidase[15]. These results indicate that β-galactosidase is involved in galactosyl lactose catabolism.

The *Sacharomyces sp*. isolated from grapes G4 could be able to assimilate a variety of carbon compounds like glucose, and sucrose; nitrogen compounds like peptone. The pH optimized was 5.0 and temperature was 45°C. The maximum growth was obtained when the fermentation media is optimized with the above fermentative parameters.

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