JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

CHARACTERIZATION OF YEASTS ISOLATED FROM DIFFERENT SOURCES AS PROBIOTICS

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https://doi.org/10.15414/jmbfs.937

ARTICLE INFO

Received 3. 10. 2018
Revised 4. 3. 2021
Accepted 24. 3. 2021
Published 1. 8. 2021

Regular article

OPEN ACCESS

ABSTRACT

Probiotics are living microorganisms which are similar to beneficial microorganisms found in the human gut. These microorganisms can be taken as dietary supplements or as food products provides positive effect to the host. In the present study, soil, milk and curd samples were used as source for the isolation of probiotic yeast. The isolates were checked for the probiotic properties such as antimicrobical production, pH & temperature tolerance, NaCl tolerance, cell adhesion, bile tolerance and cholesterol removal. Initially, twelve yeasts were isolated from each of the three different sources and characterization tests were performed. Among the 12 isolates, M_1 showed maximum activity in all characterization tests and the conditions optimized are temperature (35^0C), pH (3 & 7) and salt tolerance (5% of NaCl) for the growth of the probiotics. It can be used as a major food supplements and also with high nutritive value for human.

Keywords: Probiotics, Yeast, Antibiotic Resistance, Cell Adhesion and Extracellular Polysaccharides

INTRODUCTION

Probiotics are given to human in sufficient amount to provide health benefits to the host system **(Basavaraju and Jamil, 2014)**. Our digestive tract consists of variety of microbial species that have a symbiotic relationship with the host **(Ragavan and Das, 2017; Sridevi *et al.*, 2015)**. Most commonly used probiotic microbes are bacteria and yeast **(Hamed and Elattar, 2013; Del Carmen *et al.*, 2011),** which performs a significant function in host system by improving immune system, food digestion, production of short-chain fatty acids and essential vitamins, and colonization resistance against infectious agents **(Mishra and Sharma, 2014; Guo *et al.*, 2010).**
In general, most of the probiotic organisms belong to lactic acid bacteria, they are Gram-positive, catalase- negative and rods shaped with rounded ends. It usually appears in combined form either as short or long chains. They are a non-flagellated, non-motile and non-spore forming organism **(Alander *et al.*, 1999)**. They are used to produce various beneficial compounds such as antimicrobial, lactic acid, hydrogen peroxide and many bacteriocins. Also, have an ability to interact with host microbial flora and need to possess the antagonistic property against infectious agents such as bacterial, viral and fungal agents **(Gorbach, 2002).**
They help in influencing several aspects of innate and acquired immunity by inducing phagocytosis and IgA secretion, modifying T-cell responses, enhancing TH_1 (T Helper) responses and attenuating TH_2 responses **(Kechagia *et al.*, 2012).** Major mechanism of probiotics includes enhancement of epithelial barrier, increased adhesion to the intestinal mucosa, inhibition of pathogenic adhesion, production of antimicrobial substances and immune system modulation **(Bermudez-Brito *et al.*, 2012).**
Many food products containing probiotics organisms are wide and still growing in markets. Examples of food products with probiotics are dairy based including fermented milk, cheese, yogurts, buttermilk, ice creams and milk powder **(Ankita and Jayati, 2015; Arena *et al.*, 2014).** Non-dairy food products include soy based products, nutrition bars, cereals and variety of juices **(Kechagia *et al.*, 2012; Král *et al.*, 2012; Pundir *et al.*, 2013).** The most important properties for the selection of probiotics are non- pathogenic, ability to adhere to gut, acid & bile tolerance, thermotolerance, Cholesterol removal, GIT tolerance, EPS production, pH resistance, production of antimicrobial substance and resistance to antibiotics **(Basavaraju and Jamil, 2014; Mishra and Sharma, 2014; Qvirist *et al.*, 2016).** Also, it has the ability to survive in the digestive tract and colonize in lumen and colon for long period of time. Additionally, it should be safe for human consumption and should not cause any harmful toxic compounds that affect the human health **(Collado *et al.*, 2007).**
The advantage of using yeast as probiotic food supplements because of its eukaryotic nature and has the ability to change post-translational modification. This makes them to express various therapeutic proteins with proper conformation. Also helps in maintaining microbial flora in gut, prevent proliferation of infectious agents, improves food digestion, production of short-chain fatty acids and essential vitamins **(Mishra and Sharma, 2014; Klaenhammer and Kullen, 1999).** The main aim of the present study is to isolate and characterize yeast as probiotics from different sources.

MATERIALS AND METHODS

Isolation of yeast

Sample such as soil (food waste dumped site), milk (milk vendor) and curd sample (local market) was collected from Eachanari, Coimbatore, Tamil Nadu, India. The collected samples were serially diluted and directetly plated on YEPD (Yeast peptone dextrose) agar plates and incubated at 37^0C for 24-48 hours. An antimicrobial agent chloramphenicol (100µg/ml) was added to inhibit the growth pf bacteria. After 48 hours of incubation, totally 12 isolates, 4 from each of the three sample were isolated includes S_1-S_4, M_1-M_4 & C_1-C_4 and used for further analysis **(Basavaraju and Jamil, 2014; Mishra and Sharma, 2014)**.

Characterisation tests for probiotics

Antimicrobial production test

The microbial resistance of yeast isolates were tested against pathogenic cultures; *E.coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas sp* and *Klebsiella pneumonia*. The crude extracts from all the isolates were collected by centrifuging the culture at 10,000 rpm for 10 minutes. Then agar well diffusion method was carried out by swabbing the pathogenic culture on each plate and using gel puncture well was created and the extracts were added. After incubation, these plates were analysed for the zone of inhibition. The well containing distilled water was used as the control **(Basavaraju and Jamil, 2014; Pundir *et al.*, 2013).**

pH tolerance test

This test checks the ability of the isolates to grow in the acidic and basic environment by finding the growth of the organism at various pH. The YEPD broth containing selected isolates was adjusted to pH 1, 2, 3, 4, 5, 7, 9 and 11 using 1N HCl and 1N NaOH. Then, the samples were incubated at 37^{0}C for 24-48 hours. The growth of all the isolates was measured at 600nm **(Basavaraju and Jamil, 2014; Pundir *et al.*, 2013).**

Thermotolerance test

In this test, the selected yeast isolates were checked for its ability to withstand at various temperatures. The selected 12 yeast isolates were incubated at various temperatures, i.e., 15, 25, 35 and 45^{0}C for 24-48 hours. The growth of the organisms was calculated by measuring the absorbance at 600nm **(Mishra and Sharma, 2014).**

Salt tolerance test

The salt tolerance ability of the 12 isolates was tested by growing the isolates with NaCl at different concentrations (0-10%) at 37^{0}C for 24-48 hours. The NaCl tolerance ability of these isolates was analyzed by measuring the absorbance at 600nm **(Escamilla-Montes *et al.*, 2015; Hoque *et al.*, 2010; Sieladie *et al.*, 2011).**

Cell adhesion test

The ability of the probiotic isolates to adhere onto the cells were measured by microbial adhesion to solvents (MATS). The cultures were centrifuged and the pellets were suspended in potassium phosphate buffer. Chloroform was used as a solvent and mixed in the ratio of 1:3 to all the cell suspensions and kept for 10 minutes incubation and mixed vigorously. Then, these samples were incubated for 20 minutes at room temperature and OD was taken at 600nm **(Escamilla-Montes *et al.*, 2015)**. The percentage (%) of cell adhesion to solvent was calculated by the formula:

$$\%\ \textbf{of cell adhesion} = \frac{A_0 - A_1}{A_0} * 100$$

where A_1 is absorbance after incubation and A_0 is absorbance reading before incubation with solvent.

Bile tolerance test

The bile tolerance of the probiotic yeast isolates were analysed in the presence of various concentrations of bile salts (0-100 ppm). After incubation, absorbance was measured at 600nm **(Pundir *et al.*, 2013).**

Cholestrol removal test

The cholesterol removal potential of the probiotic isolates were analysed with 1% bile salt & 100µg of water-soluble cholestrol and incubated at 37^{0}C at different time intervals (4, 8, 12, 24 and 48 hours). 5ml of the sample was taken at each interval and centrifuged at 5000rpm. After centrifugation, the supernatant was collected and absorbance was measured at 600nm **(Ragavan and Das, 2017).** Assimilation rate of cholesterol was calculated by the formula:

$$\textbf{Rate of cholesterol removal} = \frac{\textbf{Cholesterol conc. in control} - \textbf{Cholesterol conc. in sample}}{\textbf{Cholesterol conc. in control}} * 100$$

RESULTS AND DISCUSSION

Characterization test

Antimicrobial production test

The production of antimicrobial compound against specific pathogenic microorganisms by the yeast isolates were given in Table 2. The results showed that only strain C_1 produced antimicrobial substance against *E. coli* and isolates M_1, S_1 and C_4 exhibit antimicrobial activity against *Bacillus subtilis.* The isolates M_1, M_4 and C_4 developed resistance for *Staphylococcus aureus* and M_1 strain showed exceptionally good results against *Salmonella typhi* compared to all the isolates. Similarly, S_2, M_1 and M_4 were able to grow in presence of *Pseudomonas sp.* and all other isolates were insensitive to grow. Among the 12 isolates, M_1 and M_4 strain showed good results for *Klebsiella pneumoniae.*

Table 2 Antimicrobial production test

Name of the pathogens	Name of the isolates											
	S_1	S_2	S_3	S_4	C_1	C_2	C_3	C_4	M_1	M_2	M_3	M_4
E.coli	-	-	-	-	++	-	-	-	-	-	-	-
Bacillus subtilis	+	-	-	-	-	-	-	++	++	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-	+	+++	-	-	+
Salmonanella typhii	-	-	-	-	-	-	-	-	+	-	-	-
Pseudomonoas sp	-	+	-	-	-	-	-	-	+	-	-	+
Klebsiella pneumoniae	-	-	-	-	-	-	-	-	++	-	-	+

Legend: + indicates moderate activity of antimicrobical compounds from isolates, ++ indicates good activity of antimicrobical compounds from isolates, +++ indicates excellent activity of antimicrobical compounds from isolates, – indicates absence of antimicrobical compounds from isolates.

pH tolerance test

The effect of different pH on the growth of 12 isolates was shown in figure 1. From the graph, it was found that most of the selected isolates exhibited maximum tolerant growth at pH 3, 5 and 7, whereas S_3 exhibited at pH 11 and C_3 at pH 9.

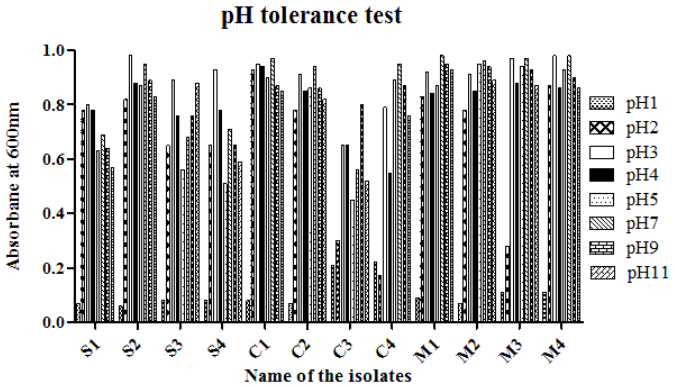

Figure 1 pH Tolerance test for the growth of the probiotic yeast isolates from different sources.

Also, there was an slightly decreased growth at pH 1, 2 and 4. From the above results, it was clearly revealed that all the isolates had maximum tolerance at pH 3 & 7 and also growth was observed among the other pH ranges. During the primary screening, 12 among the 20 yeast isolates showed good growth under acidic condition (pH- 2) **(Ragavan and Das, 2017)**.

Thermotolerance test

The isolated yeast isolates S_1 to S_4, C_1 to C_4, M_1 to M_4 was exposed to a different temperature (15^{0}C, 25^{0}C, 35^{0}C, 45^{0}C) and the results were shown in figure 2. The results confirmed that all stains had maximum growth at 35^{0}C except C_3, M_3 showed maximum growth at 25^{0}C. All the isolates were sensitive to grow at 15^{0}C and strain S_1 exhibited minimum growth at 45^{0}C.

According to **Ragavan and Das, (2017),** thermotolerance for 20 yeast isolates conducted from which 12 isolates showed best resistance at 35^{0}C. All the selected isolates from the study of **Sornplang and Piyadeatsoontorn (2016),** had an ablity to withstand the temperature in the range of 25, 30, 37 and 40^{0}C.

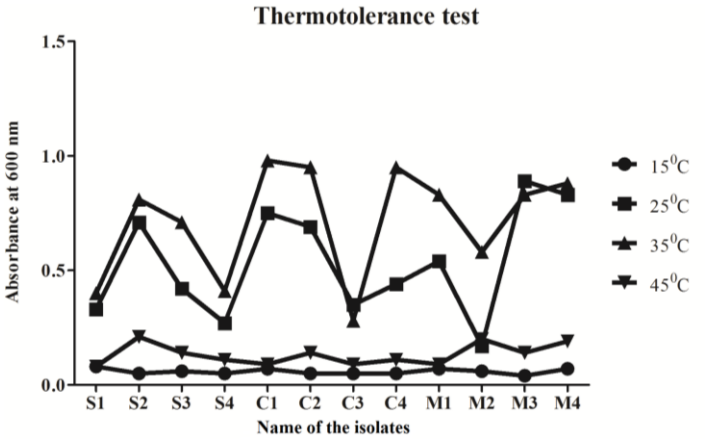

Figure 2 Effect of various temperatures for the growth of the probiotic yeast isolates from different sources.

Salt tolerance test

For salt tolerance test, the selected isolates (S_1 to S_4, C_1 to C_4, M_1 to M_4) were incubated in a different concentrations of NaCl (2-10%) in YEPD broth. The growth of the isolates was represented in figure 3. Only isolates S_2 & C_1- C_4 has the ability to grow in NaCl concentration of 2%. Also observed that all the isolates isolated from milk sample (M_1-M_4) showed increased growth in 2-4% of NaCl. Except strain M_3, all the other isolates of milk sample showed growth in 6% of NaCl. But the growth was reduced by increasing NaCl concentration of about 8- 10 % for all the isolates.

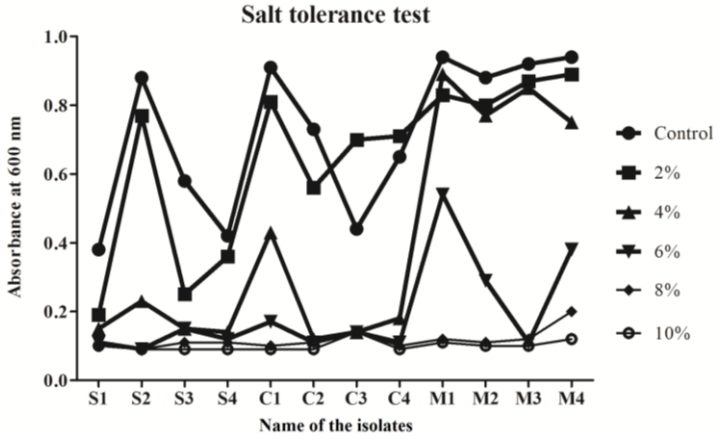

Figure 3 Salt tolerance test for the growth of the probiotic yeast isolates from different sources.

All the isolates that involved in saline tolaerance test had the competence to grow in 0.5 to 9% NaCl concentrations **(Escamilla-Montes *et al.*, 2015)**. Similar test was performed by **Ankita and Jayati (2015)** and the results from the test showed that most of the selected isolates were able to show resistance in 4% NaCl.

Cell adhesion test

The cell adhesion for the selected isolates was measured by cell adhesion hydrophobicity, (i.e) microbial adhesion to solvents (MATS). The percentage of cell adhesion was presented in figure 4. It was observed that the isolates S_1, M_1, M_2 and M_4 showed very good adhesion ability of above 50% i.e., it possess ultrahydrophobic capacity towards the solvent. The hyperhyphobicity level was observed in isolates S_3, S_4, $C_1$and M_3 showed the adhesion ability between 30-50% whereas all other isolates were able to adhere below 30% (possess hybrophobicity). The adhesion ability was tested with n-Hexadecane and found that most of the selected isolates showed good adhesion ability of about 60% **(Ragavan and Das, 2017).**

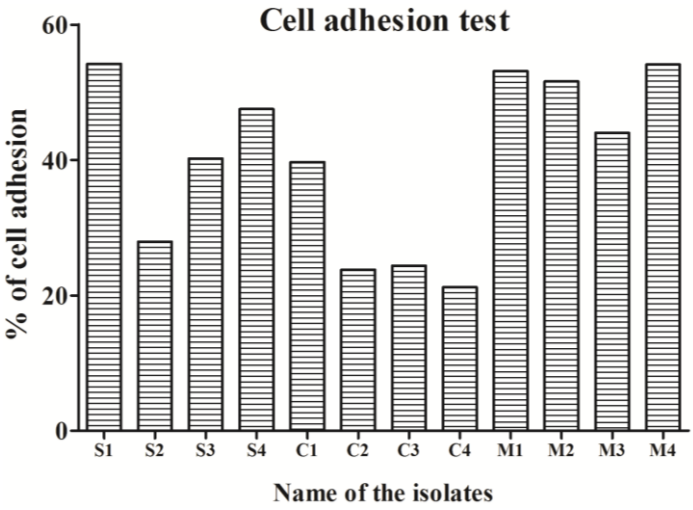

Figure 4 Cell adhesion test for the growth of the probiotic yeast isolates from different sources.

Bile tolerance test

The bile tolerance ability of 12 isolates was revealed in figure 5. From the graph, the bile tolerance ability of the isolates were found to be higher in 25 ppm for the isolates S_2 to S_4, C_1, C_3, C_4 and M_4, whereas S_1 showed maximum tolerance for 100 ppm. The isolates C_2 and M_2 were able to grow at 75 ppm concentration of bile salt. It was found that other two isolates of milk sample M_1 and M_3 showed higher resistance to 50 ppm than other bile concentrations.

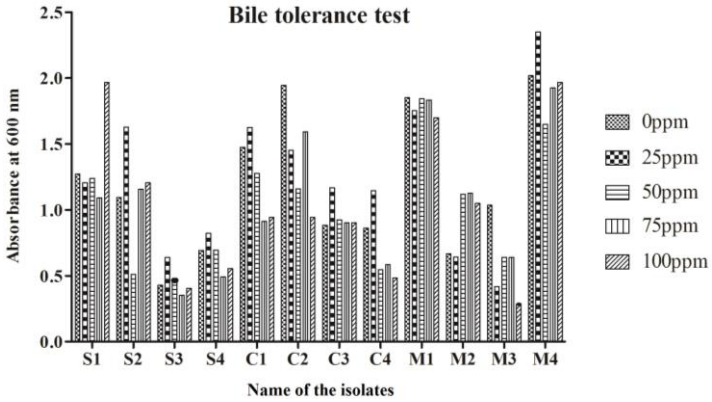

Figure 5 Bile Tolerance test for the growth of the probiotic yeast isolates from different sources.

Bile tolerance ability carried out by **Ragavan and Das (2017**), the bile salt concentration of 0.6% showed maximum growth of the selected isolates. Another work done by **Pundir *et al.*, 2013,** depicted that the selected isolates were able to tolerate different concentrations of bile salt (0.5 – 2%). The concentrations of bile salt used in the test were 0.05, 0.1, 0.15 and 0.3% and noticed that all isolates were able to multiply in the all concentration **(Hoque *et al.*, 2010)**.

Cholesterol removal test

The rate of cholesterol removal ability was found at different time intervals (4, 8, 24, 48th hours) and resulted in figure 7. The result was exhibited that all strains S_1 to S_4, C_1 to C_3, M_1 and M_4 indicated the increased reduction of cholesterol level from 4th to 48th hour of incubation. Similar results were found in the cholesterol removal test during 24th hour interval **(Ragavan and Das, 2017)**.

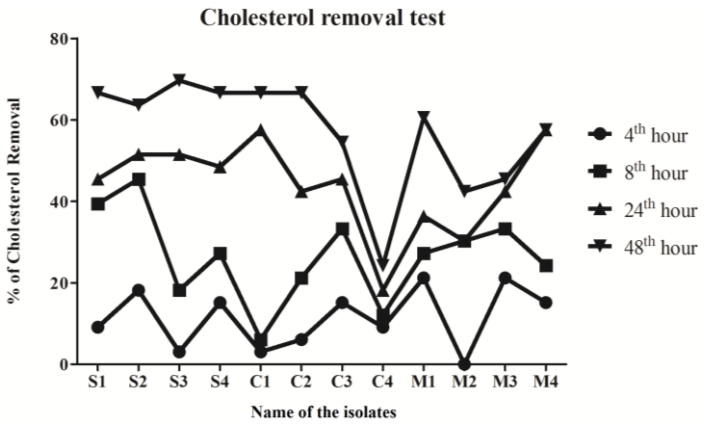

Figure 6 Cholesterol removal test for the growth of the probiotic yeast isolates from different sources.

CONCLUSION

Thus, the results from the present study it was found that the M_1 isolate from the milk sample showed good results against most of the characterization tests and it has an ability to withstand high temperature, pH and also able to show resistance against most of the microorganisms used by producing antimicrobial compounds compared to other isolates from soil and curd samples. It could be the potential source for the replacement of dietary food intake in the near future.

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