CHARACTERIZATION OF YEASTS ISOLATED FROM DIFFERENT SOURCES AS PROBIOTICS

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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; Sridivi 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).

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 directly plated on YEPD (Yeast peptone dextrose) agar plates and incubated at 37°C for 24-48 hours. An antimicrobial agent chloramphenicol (100µg/ml) was added to inhibit the growth of pathogenic bacteria. After 48 hours of incubation, totally 12 isolates, 4 from each of the three sample were isolated includes S. aureus, 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; Pandir et al., 2013).

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; Pandir et al., 2013).

Keywords: Probiotics, Yeast, Antibiotic Resistance, Cell Adhesion and Extracellular Polysaccharides
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 YEFPD 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°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°C for 24-48 hours. The growth of the organisms was calculated by measuring the absorbance at 600nm (Misra 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°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:

\[
\% \text{ of cell adhesion} = \frac{A_0 - A_1}{A_0} \times 100
\]

Table 2 Antimicrobial production test

| Name of the isolates | Name of the isolates |
|----------------------|----------------------|
| E. coli | C. jejuni | C. perfringens | P. aeruginosa | K. pneumoniae |
| ++ | ++ | ++ | ++ | ++ |

Legend: + indicates moderate activity of antimicrobial compounds from isolates, ++ indicates good activity of antimicrobial compounds from isolates, +++ indicates excellent activity of antimicrobial compounds from isolates, – indicates absence of antimicrobial 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 S1 exhibited at pH 11 and C1 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 S1 to S4, C1 to C4, M1 to M4 was exposed to a different temperature (15°C, 25°C, 35°C, 45°C) and the results were shown in figure 2. The results confirmed that all strains had maximum growth at 35°C except C4, M1 showed maximum growth at 25°C. All the isolates were sensitive to grow at 15°C and strain S1 exhibited minimum growth at 45°C. According to Ragavan and Das, (2017), thermotolerance for 20 yeast isolates conducted from which 12 isolates showed best resistance at 35°C. All the selected isolates from the study of Sornplang and Piyaedasootorn (2016), had an ability to withstand the temperature in the range of 25, 30, 37 and 40°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 (S₁ to S₄, C₁ to C₄, M₁ to M₄) 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 S₂ & C₁–C₄ has the ability to grow in NaCl concentration of 2%. Also observed that all the isolates isolated from milk sample (M₁–M₄) showed increased growth in 2–4% of NaCl. But the growth was reduced by increasing NaCl concentration of about 8–10% for all the isolates.

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 S₁, M₁, M₂ and M₄ showed very good adhesion ability of above 50% i.e., it possess ultrahydrophobic capacity towards the solvent. The hyperhydrophobic level was observed in isolates S₃, S₄, C₁ and M₄ showed the adhesion ability between 30–50% whereas all other isolates were able to adhere below 30% (possess hyrophobicity). 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).

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 S₂ to S₄, C₁, C₃, C₄ and M₄ whereas S₁ showed maximum tolerance for 100 ppm. The isolates C₂ and M₂ were able to grow at 75 ppm concentration of bile salt. It was found that other two isolates of milk sample M₁ and M₃ showed higher resistance to 50 ppm than other bile concentrations.

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 S₁ to S₄, C₁ to C₄, M₁ and M₄ 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).
CONCLUSION

Thus, the results from the present study it was found that the M1 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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