



Isolation and enumeration of probiotic microorganisms from fermented idli batter

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Abstract

Probiotics are defined as nonpathogenic living microorganisms, that have beneficial effects on host health. The most important and frequently used functional food compounds are probiotics and fermented foods. Functional foods have numerous beneficial nutritional effects. Probiotics dairy foods, idli, dhokla, pickles etc are traditional Indian foods that beneficially affect the host. They improve survival and implantation of live microbial supplements. In our study, probiotics were isolated from Fermented Idli batter. There is a difference observed in the pH of fermented idli batter. It decreases from 6 to 4.3-5.3 during fermentation. Yeasts were isolated from the fermented batter on Sabouraud's agar. Serial dilutions were performed, furthermore spread plate of these dilutions upto 10^{-5} was done on Rogosa agar plates and incubated for 72 h in a candle jar under microaerophilic condition. Colonies of lactic acid bacteria were observed on Rogosa agar plate. The count of bacteria in the fermented batter was 10^8 cells/ml. This experiment shows the presence of probiotic microorganisms, both bacteria and yeasts. Lactic acid bacteria and yeasts facilitate idli batter fermentation thereby enhancing their nutritional value and development of desirable texture and flavor.

Keywords: Fermentation, Probiotics, Lactic acid bacteria, Yeast

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1. Introduction

Probiotics are defined as live microorganisms which confer health benefits to the host. The most important and frequently used functional food compounds are probiotics and fermented foods. Functional foods have numerous beneficial nutritional effects (De Vuyst and Leroy, 2007). Probiotic dairy foods, idli, dhokla, pickles, etc. are traditional Indian foods that beneficially affect the host. Live microbial supplements that beneficially affect the host animal by improving its intestinal microbial balance, probiotics are generally used to improve the health of both animals and humans through the modulation of the intestinal microbiota (Fijan, 2014).

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There are both beneficial and harmful bacteria in the human body. When a person gets an infection, there are more bad bacteria in their system out of balance. Good bacteria help to eliminate the extra bad bacteria, returning the balance. Probiotic-supplements or probiotic foods are a way to add good bacteria to the body (Hoffmann et al., 2013). Good bacteria can also be found naturally in many Indian foods, especially fermented foods. There is an abundant opportunity available for food microbiologists to explore the Indian fermented foods for the isolation of new strains of lactic acid bacteria for their potential role in probiotic research (Satish Kumar et al., 2013). Majority of microorganisms used as probiotics belong to lactic acid bacteria and bifidobacteria. Within the group of lactic acid bacteria, *Lactobacillus* species are most commonly utilized group of microorganisms for their potential beneficial properties as probiotics (Pundir et al., 2013). Probiotics have a wide range of beneficial effects on human health, which include immunomodulation, lowering of cholesterol, treatment of rheumatoid arthritis, prevention of cancer, improvement in lactose intolerance, and prevention or reduction of the effects of atopic dermatitis, Crohn's disease, diarrhoea, and constipation as well as candidiasis and urinary tract infections (Reid, 1999).

Fermentation is a metabolic process that converts sugars to acids, alcohol or gases. Most of the dishes in South Indian cuisine that include butter milk, idli, dosa, dhokla, uttapam are made by fermentation, which makes them rich in live cultures of good bacteria. Like most other traditional fermented foods, idli is rich in probiotics. Idli is a traditional fermented rice and black gram-based breakfast food and can be a potential source of probiotic microorganisms. It is consumed as a healthy breakfast. Among several Indian traditional foods, idli, a fermented steamed product with a soft and spongy texture is highly popular and very widely consumed throughout India and is also becoming popular in other countries (Steinkraus et al., 1967; and Venkatasubbaiah et al., 1984). Manufacture of ready-to-cook idli mix and fermented idli batter in India is a growing business, both on small scale and industrial scale. The flavor and texture of idli depends upon the ratio of raw materials, fermentation conditions and the properties of the batter. Probiotics possess important functional aspects for basic nutritional requirement. Idli, dosa, dhokla, uttapam all are made by fermenting rice and lentils, which makes them rich in live cultures of good bacteria. The culture used in making curd, buttermilk, the dosa or idli batter, fermented beverages etc are all rich in probiotics. Idli is a source of vitamins, mainly B-complex vitamins, proteins, and calories due to the fermentation process. Lactic acid bacteria isolated from fermented food can be a good source of probiotics because of their health promoting and non-pathogenic characteristics (Vishwanathan and Kadirvelu, 2017). Fermented foods consumed in India are categorized on the basis of their base material (Satish Kumar et al., 2013).

Table 1 lists the lactic acid bacteria and yeasts commonly present in idli batter. The lactic acid and carbon dioxide produced due to the metabolic activity of lactic acid bacteria reduce the pH of the batter and make a favorable environment for the growth of yeasts.

Table 1: Organisms present in idli batter (Agrawal et al., 2000)	
<i>Lactobacillus delbrueckii</i> , <i>Lactobacillus fermentum</i> , <i>Lactobacillus brevis</i> , <i>Lactococcus lactis</i> , <i>Leuconostoc mesenteroides</i> , <i>Pediococcus cerevisiae</i> , and <i>Streptococcus faecalis</i>	<i>Torulopsis holmii</i> , <i>Torulopsis candida</i> , <i>Trichosporon pullulans</i> , <i>Geotrichum candidum</i> , <i>Candida fragilola</i> , <i>Candida tropicalis</i> , <i>Candida kefir</i> , <i>Rhodotorula graminis</i> , and <i>Hansenula anomala</i> (Mukherjee et al., 1965)

2. Materials and methods

Idli batter was prepared from rice and black gram dal taken in the ratio of 1:2. Black gram dal and rice taken were separately soaked in water for 6 h, after which they were ground in a blender. The two were mixed thoroughly and required amount of salt was added. The bowl was incubated at ambient temperature for 6 h for fermentation.

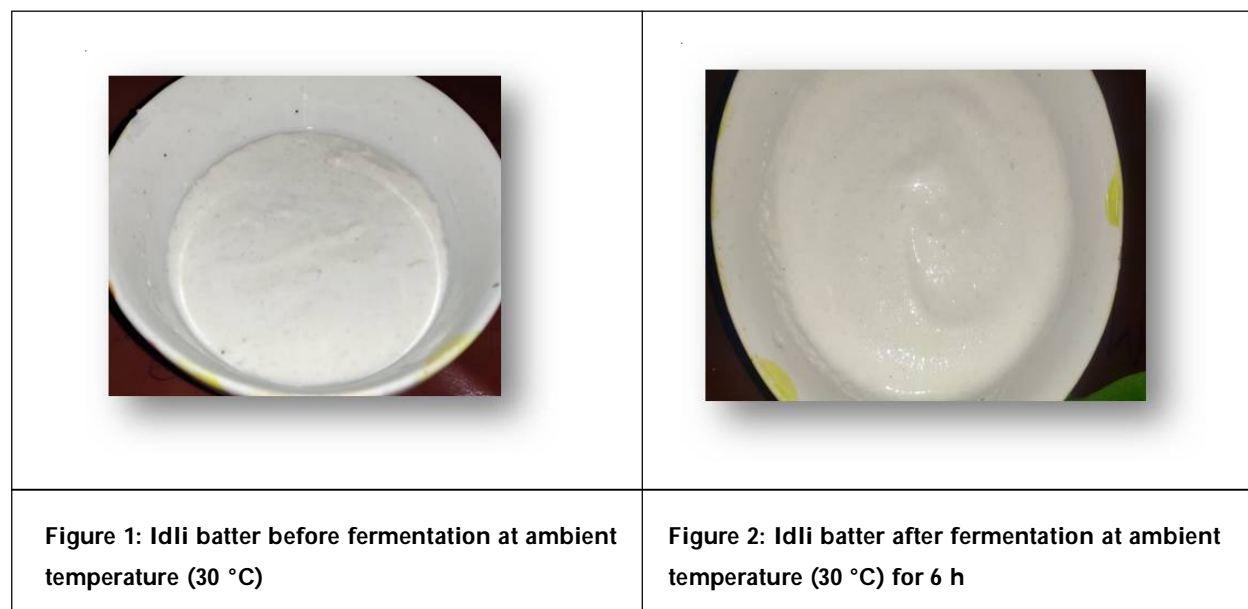
Sterile Sabouraud's agar and Sterile Rogosa agar were used for the cultivation of yeasts and lactic acid bacteria respectively.

2.1. Composition of Rogosa agar

Tryptone 10.000 g/L, Yeast extract 5.000 g/L, Dextrose (Glucose) 20.000 g/L, Potassium dihydrogen phosphate 6.000 g/L, Polysorbate 80 (Tween 80) 1.000 g/L, Triammonium citrate 2.000 g/L, Sodium acetate 15.000 g/L, Magnesium sulphate heptahydrate 0.575 g/L, Manganese (II) sulphate monohydrate 0.110 g/L, Iron (II) sulphate heptahydrate 0.034 g/L, Agar 15.000 g/L. Final pH after addition of glacial acetic acid at 25 °C) 6.2 ± 0.1 (M1899, HiMedia Technical data).

Principle: Rogosa Agar is primarily a selective medium for the cultivation of *Lactobacillus*. High acetate concentration and low pH effectively suppress other bacteria, but also many strains of other lactic acid bacteria. The modification of the pH to 6.2 instead of 5.5 alters the selectivity of the medium for the whole group of lactic acid bacteria. Tryptone, yeast extract provides nitrogenous compounds, sulphur, trace elements and vitamin B complex, essential for growth of Lactobacilli (Rogosa et al., 1951).

Determination of pH: pH of batter before and after fermentation was determined with the help of a pH meter. Yeasts were isolated on Sabouraud's agar by T-Streak method using a loopful of diluted idli batter. Plates were incubated at room temperature for 48 h. Colony characteristics of the isolates were observed. Further, gram staining of the isolated colony was carried out. Enumeration of lactic acid bacteria was done on Rogosa agar. The fermented idli batter was homogenized and tenfold serial dilutions upto 10⁻⁵ were done using sterile saline. Furthermore, 0.1 mL of these dilutions were surface spread on Rogosa agar plate. These plates were then incubated for 72 h in a candle jar for microaerophilic conditions.



3. Results and discussion

There was a change in the characteristics of idli batter. After fermentation there was a decrease in pH of batter, the volume of the batter increases, change in texture is also observed. The batter became soft, fluffy and porous. The rise in the dough and increase in volume can be clearly seen in Figures 1 and 2. The pH of unfermented batter was found to be 6 while fermented batter was 4.5. Isolation on Sabouraud's agar: After incubation at room temperature for 48 h well isolated colonies were observed on Sabouraud's agar plate. Colony characteristics of an isolated colony of the most abundant organism were studied. These are described in Table 2.

Table 2: Colony characteristics of an isolated colony from Sabouraud's agar plate							
Size	Shape	Color	Margin	Elevation	Opacity	Consistency	Gram staining
2 mm	Circular	White	Entire	Convex	Opaque	Butyrous	Gram positive oval cells

3.1. Enumeration of LAB on Rogosa agar plate

As seen in Figure 3, bacterial colonies were obtained on Rogosa agar plate after incubation for 72 h in a candle jar. As per the principle of Rogosa agar, the medium is selective for lactic acid bacteria (Rogosa et al., 1951). Catalase test was performed to confirm that the colonies were of lactic acid bacteria. The colonies were catalase negative. Thus, these colonies were of lactic acid bacteria. Count of bacteria before and after fermentation was 2.0×10^3 CFU/ml and 1.15×10^8 CFU/ml respectively clearly indicating an increase in the number of lactic acid bacteria during fermentation.

A lot of further studies can be done for identification of the various organisms isolated from the batter. Identification of isolated organism from idli batter can be done up to species level. Study of succession of microorganisms as fermentation proceeds can be undertaken. Study of change in pH at different time intervals and the growth of microorganisms at different pH during fermentation process can be studied. The nutritional profile, microbiological profile, carbohydrate factor, presence of vitamins, amino acid and proteins before and after fermentation process of idli batter can also be analyzed.



Figure 3. Spread plate of dilutions on Rogosa agar plate

4. Conclusion

Microbial analysis of the homemade idli batter was done to study the role of yeasts and lactic acid bacteria in its fermentation. No pure starter cultures were added to the batter indicating that the fermentation was exclusively due to the indigenous organisms present on the grains. Yeasts were isolated from fermented Idli batter by T streak isolation of diluted batter on Sabouraud's agar plate. A number of colonies were observed on Rogosa agar plate which is a selective medium for lactic acid bacteria. Therefore, it can be concluded that Lactic acid bacteria are present in large numbers. This experiment shows the presence of probiotic microorganisms, both bacteria and yeasts, in fermented idli batter. Lactic acid bacteria and yeasts work synergistically to facilitate idli and dosa batter fermentation thereby enhancing their nutritional value and development of desirable texture and flavor. This study shows that in natural idli fermentation lactic acid bacteria and yeasts play a major role and affect the physical, chemical and textural properties of the batter, thereby enhancing the organoleptic properties of the steamed idli.

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