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COMPARISON OF THE PROBIOTIC EFFECT OF *LACTOBACILLUS ACIDOPHILUS, STREPTOCOCCUS THERMOPHILUS* AND *SACCHAROMYCES CEREVISIAE* ON STAPLE SOUTH INDIAN FERMENTED FOODS

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ABSTRACT

India is a well diversified country with various food cultures. South Indian food is gaining popularity, throughout the world, which holds rice, pulses (dhal) and milk as its staple food. Rice and Dhal in the form of batter is used after fermentation and used in making various delicacies in South India. Milk is used predominantly in the form of curd and buttermilk. The probiotic effect of these items is the major factor favoring its world wide acceptance. In an attempt to evaluate and standardize the fermentation of these items, the present study compared the production of pre-biotic lactic acid production as a marker of probiotic effect. It has been found that approximately 50 hr of fermentation at standard condition favors maximum probiotic effect evident by maximum production of lactic acid. Among the three probiotic agents evaluated, *L.acidophilus* has maximum capability followed by *S. cerevisiae* and *S. thermophilus.*

Key words: Probiotics, Lactic acid, fermented food, batter, *L.acidophilus, S.thermophilus, S.cerevisiae.*

INTRODUCTION

India is a subcontinent having variety of food culture and local practices. Recent surveys show that south Indian food is gaining popularization in the global food market. Rice, pulses in the form of dhal and milk are the main ingredients in South Indian food. This is preferred

worldwide due to less oil content and high nutritional value. The added nutritional value is attributed to the probiotic effect of this fermented food. Probiotics are dietary supplements of live microorganisms thought to be healthy for the host organism. According to the currently adopted definition by FAO/WHO, probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host. [1] Lactic acid bacteria (LAB) and Bifidobacteria are the most common types of microbes used as probiotics but also certain yeasts and bacilli are available. At first, probiotics were thought to be beneficial to the host by improving their intestinal microbial balance and inhibiting pathogens and toxin producing bacteria.[2] The basic concept behind probiotics is that our body is host to microorganisms or bacteria known as gut flora that are essential to our health. Substances containing these microbes can be taken as a way to improve our beneficial microbial population. Probiotics have become an important part of nutrition because our microbial populations have been altered by the use of antibiotics and other substances that are designed to kill germs; they are also effective in killing the beneficial bacteria. Probiotic products introduce friendly microbes to our gut flora which strengthen the resident micro flora. Although, probiotics have not proven to be effective at taking up residence themselves, they can strengthen our resident friendly microbes over a period of time**.**

Baker's yeast *(Saccharomyces cerevisiae)* is the traditional probiotic agent used for fermentation in South Indian foods. Recently, there are a large number of organisms reported to be probiotic and capable of fermenting traditional food items. Large scale industrial production use standardized protocol and more efficient microorganism such as Lactic acid bacteria (LAB) and Bifidobacteria. Other suggested organisms are *Streptococcus thermophilus* and *Lactobacillus acidophilus*.

L. acidophilus is a homo-fermentative species, fermenting sugars into lactic acid, which grows readily at rather low pH values (below pH 5.0) and has an optimum growth temperature of 37 °C (98.6 °F). *L. acidophilus* occurs naturally in the human and animal gastrointestinal tract, mouth, and vagina. Some strains of *L. acidophilus* may be considered to have probiotic characteristics. These strains are commercially used in many dairy products, sometimes together with *S. salivarius ssp. thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* in the production of acidophilus-type yogurt.

Streptococcus thermophilus is another commercially important of lactic acid bacteria. It has an important role as a probiotic, alleviating symptoms of lactose intolerance and other

gastrointestinal disorders. *S.thermophilus* is a facultative anaerobe, which is an organism that is capable of generating ATP through both aerobic respiration and fermentation. Enzymes in *S. thermophilus*, such as aminopeptidase, are used as starter cultures for food fermentation. Exopolysaccharides created by these peptidases are essential to generate the texture of fermented milk products and organoleptic properties of dairy products. Food industries have high demands for an improved *S. thermophilus* strain, which can provide stable fermentation qualities, resilience to bacteriophage, and consistent flavor and texture of dairy products.

S. cerevisiae has medical applications. However, research has shown that use of *S. cerevisiae* probiotics may actually be harmful. Munoz et al. documented an incident of *S. cervisiae* fungemia and pointed out the possibility of emerging infections due to that though the substantial evidence is lacking. Their research indicated that the only apparent risk factor for this disease is having been treated with a probiotic made from *Saccharomyces boulardii*. This probiotic is used in Europe to treat *Clostridium difficile*-associated diarrhea. Based on their findings, Munoz et al. concluded that the use of these probiotics should be reconsidered. [3]

The probiotic effect and nutritional value varies greatly according to the local conditions. These include the agent of fermentation, duration and temperature of fermentation, purity of the microbial agent etc. The process of fermentation of South Indian food is traditional house hold method rather than standardized protocol. The agents used for fermentation are typically uncharacterized microbial culture (a portion of the previous batter) serially maintained for generations. There is little effort to improve the probiotic efficiency by introducing better microbial agent, optimizing the fermentation conditions without affecting the quality, taste and texture which can be easily followed in the traditional way. In an effort to improve the probiotic efficiency, in the present study the lactic acid production by traditional agent *S. cerevisiae* is compared with *Lactobacillus acidophilus* and *Streptococcus thermophilus.* A known quantity of substrates was subjected to fermentation by these agents and production of lactic acid was monitored at fixed time intervals using conventional and HPLC method.

MATERIALS AND METHODS

Culturing and Quantitations: Microbial agents (*S. cerevisiae, L. acidophilus, S. thermophilus*) were isolated from batter and curd samples. All organisms were identified by standard biochemical methods. Cultures were maintained at 37 to 45 ^{0} C. Quantitations were done using spectrophotometer method and diluted down to get a microbial count of 10^{8} organism per ml.

Preparation of substrate: Three substrates were used in this study namely rice flour, urad dhal flour, and milk powder. Triplicates of each substrate were used to prepare the batter by adding 100 ml of autoclaved distilled water under sterile conditions. The batters were inoculated with one ml of organism (10^{8}) so that each batter is tested with all three organisms. The inoculated samples were incubated at 37^{0} C in an incubator shaker at 150 rpm for 3 days.

Sampling*:* Samples were drawn at 2hr, 4hr, 24hr, 26hr, 48hr, 50hr, 72hr and 74hr. Approximately 2ml of batter was transferred to autoclaved micro-centrifuge tube. The samples were then centrifuged for 15min at 5000 rpm. The supernatant was collected in fresh autoclaved tubes and labeled appropriately**.** This supernatant was further utilized for HPLC analysis.

HPLC Characterisation: Aliquots of the sample taken in the given intervals was used to determine lactic acid concentration. Briefly, samples were thermal treated at 95^{0} C for 20 minutes and stored at 18^{0} C. The mobile phase used was acetonitrile (75vol %) + Water (25vol %) with a flow rate of 1ml/min. The detection of lactic acid was set at λ= 210nm. The calculation of lactic acid was made from the peak height registered at specific retention time for lactic acid.

Standardization of equipment: Standard solution of lactic acid (pure lactic acid) with varying concentration (by diluting with mobile phase) was injected and was allowed to elute in 15 min. The peak was obtained at a retention time of 2.59 min and height of each peak was noted. A standard graph was plotted for concentration against peak height. Thus a linear dynamic range was established for the standard lactic acid solution.

Sample analysis: The flow rate of the equipment was adjusted to 1ml/min. Approximately 1ml of sample was injected, the loop capacity was 20µl. The sample was allowed to run and eluted out; the run was terminated at 25min (to resolve the peaks). The peak obtained at retention time 2.59 min was matched with the peak of standard solution and the peak height was noted. The concentration of the lactic acid present in the sample was obtained by tracing back using the peak height in the standard graph. Thus the concentration of lactic acid was calculated using peak height.

RESULTS

All three organisms evaluated in this study were capable of producing pre-biotic lactic acid in sufficient quantities. The production of lactic acid varied according to organism used, substrate as well as the time of incubation. The amount of lactic acid produced by each organism on each substrate is shown in the table (Table.1, 2, 3). Lactobacillus was the most successful lactic acid producer at all occasions closely followed by yeast and Streptococcus. Among the substrates, as expected milk was the most productive substrate followed by rice and dhal. Amount of lactic acid steadily increased as time increases till 50 hr. Highest peaks in HPLC observed at 50 hr samples as shown in the fig 1-3. The samples at 52 hr showed slight decrease in the lactic acid concentration and it steadily decreased thereafter. At 72 hr testing, it was found that the lactic acid production marginally decreased. The decrease was parallel and comparative in all samples.

Tables and Graphs

Table 1. Lactic acid concentration in µg/ml observed and calculated using HPLC in the given time interval for the substrate Milk.

	Milk							
Time(hr)	2	4	24	26	48	**50**	72	74
L.acidophilus	7.4	8	18.6	27.2	37.4	**46.1**	36.4	35.2
S.thermophilus	7.3	8.1	13.2	22.8	30	**40.7**	35.5	33.3
S.cerevisiae	7.9	8.2	17.8	24.5	34.9	**43.5**	36.8	35.6

Table 2. Lactic acid concentration in µg/ml observed and calculated using HPLC in the given time interval for the substrate Rice.

	Rice							
Time(hr)	2	4	24	26	48	**50**	72	74
L.acidophilus	5.8	7.3	18.4	24.3	34.6	**42.1**	36.2	35.6
S.thermophilus	5.5	7	12.5	20.9	28.7	**37.8**	34.6	33.9
S.cerevisiae	6.1	7.5	16.3	22.2	33.7	**39.1**	36.5	34.6

Table 3. Lactic acid concentration in µg/ml observed and calculated using HPLC in the given time interval for the substrate Dhal.

	Dhal							
Time(hr)	2	4	24	26	48	**50**	72	74
L.acidophilus	5.9	6.1	13.8	20.5	28.4	**40.8**	33.5	30.5
S.thermophilus	5.2	5.8	10.8	15.5	24	**35.2**	30	26.5
S.cerevisiae	5.3	6.3	11.7	18	27.8	**37.4**	32.3	28.1

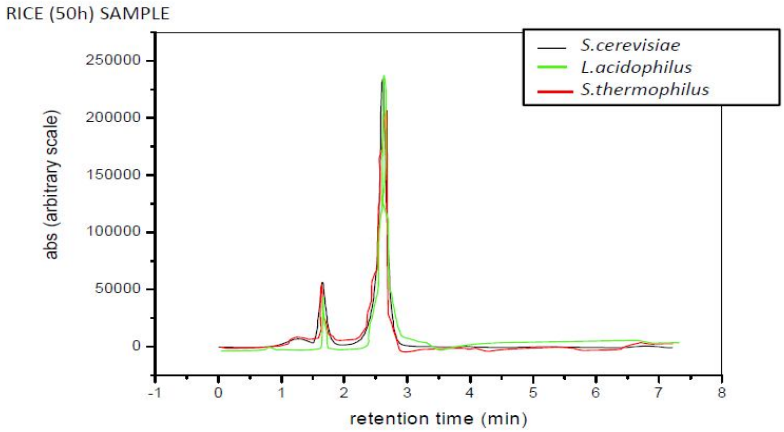

Fig. 1: 50th hour sample of rice (maximum lactic acid concentration)

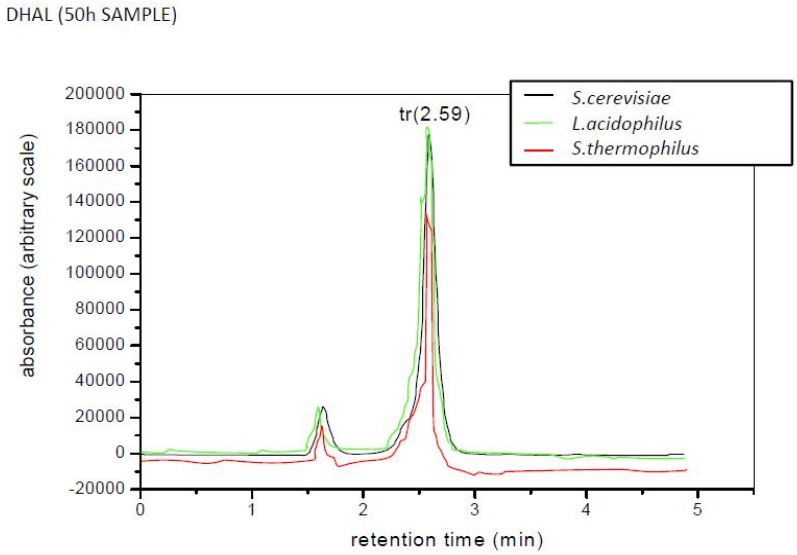

Fig. 2: 50th hour urad dhal sample (maximum lactic acid concentration)

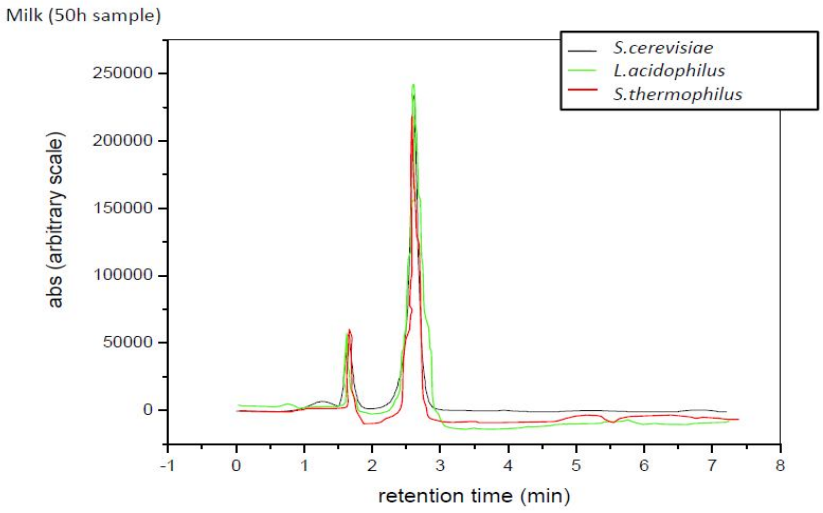

Fig. 3: 50th hour milk sample (maximum lactic acid concentration)

DISCUSSION

India is a land of early civilization and the most diversified eating habits in the world with different delicacies including fermented foods. Traditionally fermented products are rich source of Lactic acid bacteria (LAB), which is the major probiotic agent. South Indian fermented foods like *Idly*, *Dosa*, *Curd*, *Kallappam*, *koozh* and *Mor Kuzhambu* were reported as a source of potential probiotics and which is also useful as biopreservative. These foods are traditionally fermented using yeast. The Yeast fermentation of South Indian food is more of a traditional method. It is very effective and easily done. The common yeast (baker's yeast) is capable of fermenting a wide variety of food stuffs including rice, dhal, milk etc. It can be safely consumed by people who have specific intolerance to certain food stuffs, for example: people suffering from lactose intolerance as they may consume fermented rice and pulses without any discomfort. The ability of *S.cerevisiae* to ferment specific sugars is a major factor that differentiates it from other yeasts.

A probiotic in terms of its beneficial effects, *S.cerevisiae* has many properties from the most basic to highly advanced. When ingested in a quantity of two table spoons daily provided the Recommended Dietary Allowance (RDA) recommended amount of protein, fiber, vitamin B and folic acid.

Nutritional yeast also has the presence of β-1, 3 glucans, which have been shown to stimulate the body's immune system. Vetvicka et al. established a receptor found on the surface of immune cells is known for binding itself to β - glucans and recognize it as dissimilar. [4] β -

Glucans appear to assist the immune system without causing over activity while some pharmaceutical drugs over stimulate the immune system.[5] They also prevent formation of tumors and development of cancers.[6,7,8]

But many researches have shown that consumption of yeast in large quantities can cause considerable harmful effects in humans. People have contact with *S.cerevisiae* daily either by inhalation or ingestion. Wolochow et al. in 1961 showed that extremely high consumption of yeast would result in passage and colonization to draining of the lymph nodes. However it was also noticed that the daily levels of exposure are below the risk dosage.[9] Studies conducted by Eng et al. indicated that *S.cerevisiae* as being partially capable in causing infections in patients who were also exposed to strong antibiotics.[10] According to Posteraro et al. in very rare cases *S.cerevisiae* was found to cause vaginitis in women in the child bearing age.[11] For these reasons the consumption of yeast as probiotics on a daily basis in large quantities and also when administered to patients has to be reconsidered. It is a logical idea to use probiotic bacteria which are similar to our microflora and thus benefit from it. Thus the exploration of potential probiotic bacteria such as *L.acidophilus* and *S.thermophilus* as a substitute for fermenting South Indian foods.

Lactic acid bacteria have been widely used as starter culture for the manufacturing of various fermented foods such as dairies, beverages, meat and vegetables. LAB and their food products are thought to confer a variety of important nutritional and therapeutic benefits and have many documented health promoting benefits or probiotic effects in human such as inhibition of pathogenic organisms, antimutagenic and reduction of blood cholesterol. *Lactobacillus acidophilus* is found naturally in humans in the mouth, the intestines and the vagina. It assists in the breakdown of foods and thereby produces hydrogen peroxide, lactic acid and other substances that create an acidic, unfriendly environment for harmful organisms. Russian scientist and Nobel laureate Elie Metchnikoff, while investigating the diets of people living in the Balkans and the Near East speculated that the lactic acid produced during yogurt fermentation can conquer decaying gut microbes and probiotics can prevent the formation of the harmful microbes.[12]

Reid et al. stated that daily oral intake of probiotic strains of Lactobacillus resulted in some asymptomatic bacterial vaginosis patients reverting to a normal lactobacilli dominated vaginal microflora. [13,14] Burton et al. showed that when lactobacilli are introduced vaginally for patients suffering from yeast vaginitis, the outcome might significantly benefit the

patient.[15] *L. acidophilus* also confers other benefits like absorption of nutrients like vitamins K and B, calcium, lactase and fatty acids.

Few negative effects have been reported with appropriate use of this probiotic. During initial use, there may be a period of excessive gassiness and flatulence. These conditions will decrease in frequency as the body becomes accustomed to the presence of *Lactobacillus acidophilus.*

S. thermophilus is used along with LAB as a starter culture for the manufacture of several important fermented dairy foods. It has an important role as probiotic, alleviating symptoms of lactose intolerance and other gastro intestinal disorders. The cell structure of *Streptococcus thermophilus* allows the bacteria to endure elevated temperatures, such as the many industrial dairy fermentation processes that require high temperatures. *Streptococcus thermophilus* also lacks genes which contain surface proteins. This is important because harmful bacteria use these surface proteins to attach to mucosal tissues and hide from the body's defensive actions.

Ongoing research and experimentation have improved the *Streptococcus thermophilus* strain even beyond its natural beneficial state. This improved strain is responsible for the consistent taste and texture of many dairy products. It also provides stable fermentation and a resilience to bacteriophage. *Streptococcus thermophilus* also produces exopolysaccharides essential to the texture of fermented milk products and also to the production of reduced-fat dairy products that maintain similar characteristics to their full-fat counterparts.[16] One of *Streptococcus thermophilus*' unique abilities is that it can break down casein, the protein in dairy products like cheese. In addition, *Streptococcus thermophilus* also prevent transformation of nitrates into cancer-causing nitrites. One strain which is undergoing exciting research is *Streptococcus thermophilus* TH-4. [17] Chemotheraphy often causes intestinal mucositis which can be treated using this strain.

Considering the comparative advantages of these bacterial probiotics over the traditional yeast fermentation of South Indian foods, the present study evaluated the probiotic efficiency using lactic acid production as a marker. HPLC being the most accurate method of measurement was utilized in this study. The time bound comparative evaluation shows that all three agents are good fermenters of South Indian food stuffs. But yeast has distinct advantage in the early stages of fermentation, this is probably due to the better enzyme system present in the eukaryote. As time advances *L.acidophilus* has more efficiency because

of the short replication time. The bacterial growth outnumbers yeast and produces large number of metabolically active bacteria available for speedy fermentation. The acidophilic nature of this bacterium clearly shows its advantage compared to *S.thermophilus*. The accumulation of acid may competitively inhibit lactic acid production shown by lesser quantity throughout the period of fermentation.

This is the first comparative study exploiting the fermentative properties of the three organisms in the given time scale for the production of lactic acid, a probiotic. Rice (carbohydrate) was better fermented by all three organisms when compared to dhal (protein). LAB is a better alternative to *S.cerevisiae* for South Indian food in terms of efficiency, safety and its versatility towards various substrates.

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