

**ASSESSING THE GROWTH OF PROBIOTIC BACTERIA IN SELECTED PREBIOTIC FOODS RICH IN OLIGOSACCHARIDES**

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**ABSTRACT:** The combination of both probiotic and prebiotic has the ability to heal and regulate the intestinal flora, particularly after the destruction of microorganisms following antibiotic, chemotherapy, or radiation therapies. Without the beneficial organism digestion, absorption, and manufacture of nutrients cannot take place. Probiotic bacteria were isolated from commercially available yoghurt and probiotic milk products. *Lactobacillus spp.* were isolated using MRS agar and incubated under anaerobic condition at 37°C for 24 hrs. MRS agar was used for the enumeration and isolation of probiotic bacteria. Both were incubated under anaerobic condition at 37°C for 24 hrs. Morphological, physiological and biochemical reactions were used to characterize the isolates. The isolated bacteria were grown in prebiotic foods such as wheat, oat and barley at varying temperature. The viability of *Lactobacillus spp.* in prebiotic foods under acidic condition was checked. The growth of *Lactobacillus spp.* were assessed in three different prebiotic foods and among the three different prebiotic foods barley served as best substrate for the growth of *Lactobacillus spp.*

**Keywords:** Probiotic, Prebiotic, *Lactobacillus*

**INTRODUCTION**

Gastrointestinal microfloras consist of different types of micro organisms and are biologically important. Among those different microorganisms, probiotics plays an important role in maintaining microbial balance in the intestine. Probiotics are defined as “Live micro organisms which when administered in adequate amounts confer health benefit to the host”. Lactic acid bacteria (LAB) and Bifidobacteria are the most common type of microbes used as probiotics. The probiotic should be non – pathogenic, non – toxic, should be resistant to gastric juice and produce antimicrobial substances (V. C. Suvarna and V. U. Bobby, 2005). The potential benefits of probiotics include lactose intolerance, prevention of colon cancer, lowering cholesterol, lowering blood pressure, improving immune functions and preventing infections. According to the international dairy federation, a minimum of  $10^7$  bacterial cells should be alive during consumption (Ouweland and Salminen, 1998).

On the other hand prebiotics are non-digestible food ingredients that stimulate growth and activity of bacteria in the digestive system (Gibson GR *et al*, 1995). Inulin, oligofructose, lactulose, galactooligosaccharides and synthetic fructose oligosaccharides are the examples of prebiotics. Some of the sources of prebiotics include soybeans, wheat, barley, and oats.

Combination of both probiotics and prebiotics is known as Synbiotics. This combination can improve the survival of probiotic organism. Consuming a probiotic supplement that also includes the appropriate Prebiotic has many beneficial effects. The combination of both probiotic and prebiotic has the ability to heal and regulate the intestinal flora, particularly after the destruction of microorganisms following antibiotic, chemotherapy, or radiation therapies. Without the beneficial organism digestion, absorption, and manufacture of nutrients cannot take place.

## MATERIALS AND METHODS

### Isolation of *Lactobacillus* spp.

De man rogosa sharp agar was prepared and autoclaved. Media were poured into sterile petriplates and allowed to solidify. One ml of curd was taken and it was serially diluted and spread plate was performed in MRS medium and incubated at 37°C for 24hr anaerobically. After incubation the isolated colonies were taken and sub cultured in MRS broth. Then for further purification the culture was streaked on MRS agar.

### Preparation of washed cell cultures

The *Lactobacillus* spp. cultures were grown overnight in MRS broth at 37°C. The cultures were then centrifuged (4500g, 10 min, 4°C), washed once in Ringers solution and re suspended in Ringer's solution to a volume equal to that of original culture.

### Preparation of acidified phosphate buffered media

A stock solution of phosphate buffered saline was prepared by dissolving NaCl (9 g/l), Na<sub>2</sub>HPO<sub>4</sub>.2H<sub>2</sub>O (9 g/l) and KH<sub>2</sub>PO<sub>4</sub> (1.5 g/l). 100 grams of powdered wheat, oat, and barley were mixed with 400 ml of tap water. The slurry was centrifuged (6000 x g) for 30 min at room temperature. Twenty ml of wheat, oat, and barley extracts supernatant was taken and mixed with twenty ml of stock solution. The pH was then adjusted to 2.40 and the acidified media was sterilized for 15 min at 121°C. Equal volumes of glucose and maltose was mixed with stock buffered solutions and subsequently acidified approximately to 2.35 and sterilized. The samples were serially diluted in sterile Ringer's solution and about 0.1 ml of the diluted sample was plated on MRS agar and incubated at 37°C for 48 hours.

### Growth of *Lactobacillus* spp. in prebiotic foods at varying temperature

The powdered wheat, oat and barely were mixed with water and made into slurry and sterilized. *Lactobacillus* spp. culture was added to the slurry and incubated at 37°C for 24 hours. Growth was checked by plating on plate count agar. The samples were serially diluted in sterile Ringer's solution and about 0.1 ml of the diluted sample was plated on PCA and incubated at 37°C for 48 hours.

### Growth of *Lactobacillus* spp. in Prebiotic foods under varying pH

The powdered wheat, oat and barley were mixed with water and made into slurry and sterilized. *Lactobacillus* spp. culture was added to the slurry. Growth of *Lactobacillus* spp. in Prebiotic foods have been carried out in different pH such as 4, 5, 6, 7 and incubated at 37°C for 24 hours. The samples were serially diluted in sterile Ringer's solution and about 0.1 ml of the diluted sample was plated on PCA and incubated at 37°C for 48 hours.

### Improved viability of *Lactobacillus* spp. in Prebiotic foods under refrigerated condition

Wheat, oat and barley were powdered and made into slurry using water. Then equal volume of glucose, maltose was added. The washed bacterial culture was added to the prebiotic foods. About 250mg of cysteine and ascorbic acid was added and sterilized. *Lactobacillus* spp. culture was centrifuged and the pellet was washed twice and re-suspended in sterile Ringer's solution and added to the slurry and incubated. The samples were serially diluted in sterile Ringer's solution and about 0.1 ml of the diluted sample were plated on MRS agar and incubated at 37°C for 48 hours.

## Determination of ethanol

Ethanol was analyzed by gas chromatography (GC). A volume of 10 ml of the fermented sample was centrifuged (8,000 rpm, 15 min) in a 15-ml tube. The supernatant (1 ml) was centrifuged further (12,000 rpm, 10 min) in a 1.5-ml micro centrifuge tube. An aliquot of 200 $\mu$ l of the final supernatant was added to a 15-ml test tube containing 5 ml of high-pressure liquid chromatography-grade water and thoroughly mixed. For standards, the 200 $\mu$ l supernatant was replaced by either 10% ethanol. Then, 1-ml portions of the final sample, standard solutions were transferred into GC vials for analysis.

## RESULTS AND DISCUSSION

*Lactobacillus spp* were isolated from curd sample

### Growth of *Lactobacillus spp* in Prebiotic foods under acidic condition

The three samples were serially diluted and about 1 ml was plated on MRS agar. Viable colonies were obtained when plated on MRS agar. Viable colonies indicate that *Lactobacillus* (figure-1) can able to tolerate the acidic environment. From this it is well understood that lactobacillus can able to survive the gastric pH in the stomach.

### Growth of *Lactobacillus* in Prebiotic foods under different temperature

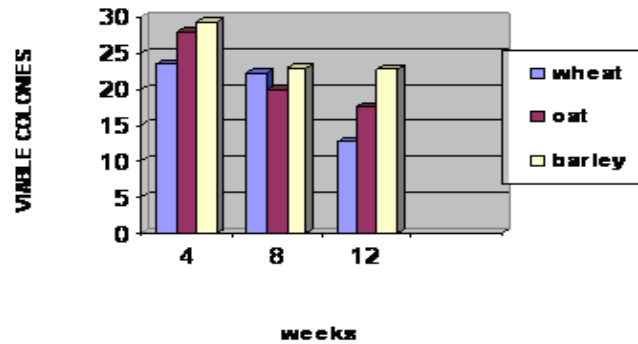
Maximum growths were obtained at 37°C when compared to 25°C. While considering temperatures, probiotic bacteria used prebiotic foods for their growth irrespective of the temperature. There was maximum growth at 37°C (Table: 1, Graph-1). This is due to the enhanced enzyme activity of probiotic bacteria at 37°C which is similar to internal body temperature.

**Table 1: Growth under different temperature**

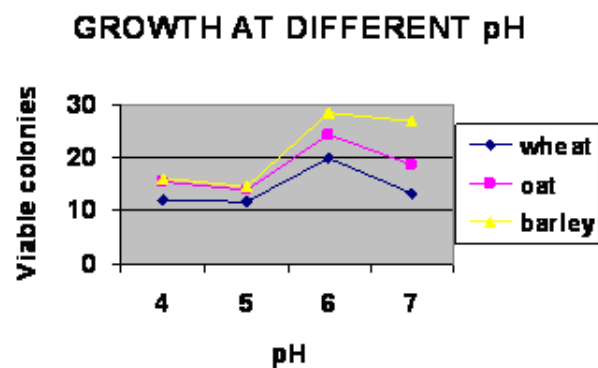
S.NO	PREBIOTIC FOODS	25°C	37°C
1.	WHEAT	++	+++
2.	OAT	++	+++
3.	BARLEY	++	+++

### Growth of *Lactobacillus* in Prebiotic foods under varying pH

Maximum growths were obtained under pH 6. Growth of *Lactobacillus* decreased at lower pH. Results indicate that growth of probiotic bacteria were maximum at pH 6 (Graph: 2). This indicates that the growth of probiotic bacteria on prebiotic foods is maximum at slightly acidic conditions.



Graph 1: Growth of *Lactobacillus* at varying temperature



Graph 2: Growth of *Lactobacillus* at varying pH

### Improving the viability of *Lactobacillus* spp in Prebiotic foods under refrigerated condition

From the graph it is well understood that the barley medium supported well the growth of *Lactobacillus* spp. Barley being a resistant starch cannot be altered by enzymatic hydrolysis. Next to barley, oats can be considered as good medium as oats is a good source of beta glucan and acts as a good carrier of probiotic bacteria during storage especially in cold conditions. Wheat fibre contains inulin which functions as support material for probiotic bacteria.

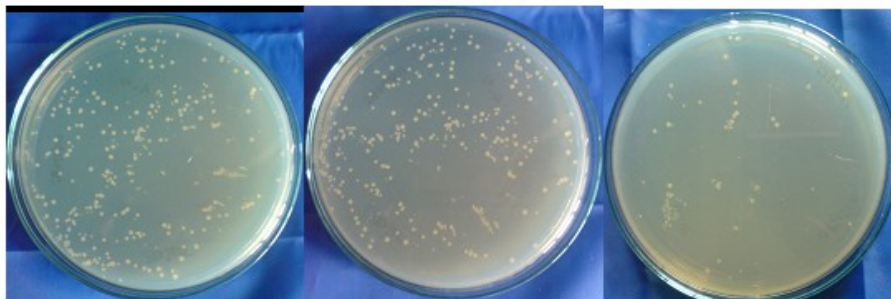


Figure 1: Viable *Lactobacillus* colonies

## Conclusion

The isolated *Lactobacillus spp* were grown in prebiotic foods such as wheat, oat and barley. Among these three foods barley served as best substrate for growth of *Lactobacillus spp*. when *Lactobacillus spp*. was grown in 25°C and 37°C, maximum growth was obtained in 37°C. While considering pH maximum growth was obtained at pH 6. Viable colonies of *Lactobacillus spp*. were obtained when grown under acidic condition. Shelf life of *Lactobacillus spp*. was extended when grown in prebiotic foods enriched with micronutrients. The amount of ethanol formed during fermentation was more in barley compared to wheat and oats.

Hence, micro-encapsulation can be used as an effective tool to encapsulate both the Prebiotic foods and Probiotic bacteria especially in cold conditions as the Prebiotic foods are a good carrier of Probiotic bacteria. Thus, Prebiotic approach through diet increases resident bacteria which are beneficial to human health.

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