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# Production of the Best Natural Health Supplements Using Fruit Waste Materials

**Shweta Hardia**
Govt. Holkar Science College, Indore, India
**Dr. Sanjida Iqbal**
Govt. Holkar Science College, Indore, India

***Abstract:***
*The use of natural health supplements has become widely accepted as a natural means to promote health for both humans. The study deals with the use of fruit peels for growth of bacteria, natural health supplements called as Probiotics. Lactobacillus, which is used in food industry for fermentation, preservation and as food additives, in pharmaceutical industry as probiotic and antimicrobial substances. Different fruit peels were tested. Maximum growth was observed in papaya peel extract. Quantitative analysis of papaya peel extract was found to be having sugar concentration of 20.23mg/ml.Various factors affecting growth of Lactobacillus in PPE media were determined and it was found that optimum pH was 7.0,temperature was 37$^0$C and the organism was found to survive at 2.0% NaCl and 0.3% MB. On comparing the results of PPE with MRS, the growth was found to be about 1/5$^{th}$ in PPE than in MRS media.*

***Key words:*** *Probiotics, medium optimization, microbial β-galactosidase, thermotolerant, Contamination check.*

## 1. Introduction

Developing probiotic food and feed is a key research and development area for future functional food markets. Probiotic foods are defined as "foods containing live microorganisms which actively enhance health of consumers by improving the balance of microflora in the gut when ingested live in sufficient numbers" [(1)]. In animal feeds it refers to "live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance" [(2)]. The use of probiotics to improve productivity in livestock is currently generating a great deal of interest. As microbial feed additives, probiotics offer potential as an alternative to antibiotic growth promotors, the use of which the European Union wants to phase out by 2006 [(3)]. They have been reported to have many beneficial effects when used in animal feeds as a means of controlling pathogen carriage, which include competitive exclusion of pathogens [(4,5)] and improved digestion and absorption of nutrients [(4,6,7)]. These have a positive effect on the growth rate and feed conversion. Therefore, the use of probiotic feed additives is of interest as a cost-effective alternative to controlling animal disease and improving breeding performance [(8)]. However, there is considerably no paper on the economic production of probiotics using waste materials. This paper discusses the selection criteria, desirable characteristics and production probiotic strain with suitable biological characteristics.

## 2. Materials and Methods

Selection of appropriate probiotic strain. The probiotic bacteria most commonly studied include the members of the genera *Lactobacillus, Bifidobacterium, Saccharomyces, Escherichia coli* and *Enterococcus* strains. In our studies we have selected six probiotic strains namely *Lactobacillus plantarum, Bacillussubtilis, Bifidobacterium bifidum,Enterococcus faecium, Saccharmyces cerevisiae* and *Escherichia coli*.These strains are then screened to select most appropriate strain for our studies. For screening we have fixed sixteen characters which are :-

- Probiotic property
- Natural presence in fermented food products which are consumable
- Normal constituent of human digestive system
- Ease in isolation and identificaltion
- Growth at wide range of temperature and pH
- Provision to exclude competing bacteria
- Growth on agricultural waste

- Ability to survive in human gastrointestinal tract
- Ability to produce antimicrobial substances
- Genome sequenced
- Sensitivity to common antibiotics
- Aerotolerancy
- Organotropic nutrition
- Non pathogenic
- Ability to produce lactic and acetic acid to control intestinal pH
- Important research going on the strain.

On the basis of above primary screening we have selected *Lactobacillus acidophillus* for our studies. *Bacillus subtilis* was not selected because it is not the normal constituent of human digestive system. *Bifidobacterium bifidum* is not taken under studies because it is anaerobic organism. *Saccharmyces cerevisiae* is not selected because it is not generally used as probiotic and it has several other industrial applications. *Escherichia coli* is an opportunistic pathogen and therefore it is also discarded for further studies. *Enterococcus faecium* is generally not used as probiotic.

- **Growth and Maintenance Medium:** MRS Media (Hi Media) having composition Protease peptone (10.00 $gl^{-1}$),Yeast extract(5.00 $gl^{-1}$),Beef extract (10.00 $gl^{-1}$),Dextrose (20.00 $gl^{-1}$) Polysorbate 80 (1.00 $gl^{-}$) Ammonium citrate( 2.00 $gl^{-1}$ ),Sodium acetate ( 5.00 $gl^{-1}$), Magnesium sulfate(0.10 $gl^{-1}$), Manganese sulfate ( 0.05 $gl^{-1}$),Dipotassium phosphate (2.00 $gl^{-1}$) Final pH (at 25ºC) 6.0 ± 0.2.
- **Fruit Wastes:** The fruit peels wastes were collected from house. They were washed to remove the dust particles for using, dried in open air, and then ground to prepare the production media. Banana peels, Orange peels, and Papaya peels were collected, dried and prepared in the form of ground preparations.
- **Preparation of Production Media:** All the fruit peels were crushed and 10 % concentration of them were used for production media. All of them were filter sterilized.

**3. Selection of Media**

Two tubes of each media prepared in the above steps were used for the analysis .One of them were inoculated with the freshly grown culture of Lactobacillus acidophilus while the other tube of each media as control (without inoculation).Both the tubes were incubated at $37^0$C for 24 hours. After incubation the tubes were observed for the presence of growth using following methods

Turbidimetric analysis of growth.

Each tube of different media were observed spectroscopically at 600nm and compared with their respective controls.

Standared plate count for the analysis of growth.

0.1 ml culture from the grown culture tube was inoculated on the MRS agar plate. Each plate was incubated at $37^0$C for 24 hours. After incubation the CFU/ml were counted.

- Qualitative analysis of the selected media.
- Qualitative analysis was performed for sugar and proteins by Benedict's test and Biuret test respectively.
- Quantitative analysis of sugar.
- Quantitative analysis of sugar was performed using cole's method.

**4. Determination of different factors affecting growth on PPE Media**

- **Effect of pH: -** Four test tubes containing 10ml of PPE Media were taken and their pH was adjusted to 2, 5, 7 & 9 and labeled accordingly. Each one of them were inoculated with the freshly grown culture of *Lactobacillus acidophilus* and incubated at $37^0$C for 24 hours. Growth was analysed using Turbidimetric analysis and Standared plate count method.
- **Effect of Temperature: -** Four test tubes containing 10ml of PPE Media were taken and each one of them were inoculated with the freshly grown culture of *Lactobacillus acidophilus* and incubated at different temperatures (80C, 250C, 370C & $45^0$C) for 24 hours. Growth was analysed using Turbidimetric analysis and Standard plate count method.
- **Effect of salt concentration: -** Four test tubes containing 10ml of PPE Media were taken and their salt concentration was adjusted to (0.8%, 1.0%1.5% & 2.0%) and labeled accordingly. Each one of them were inoculated with the freshly grown culture of *Lactobacillus acidophilus* and incubated at $37^0$C for 24 hours. Growth were analysed using Turbidimetric analysis and Standared plate count method.
- **Effect of Methylene blue concentration: -** Four test tubes containing 10ml of PPE Media were taken and their Methylene blue concentration was adjusted to ( 0.1%,0.2%,0.3% & 0.4% ) and labeled accordingly. Each one of them were inoculated with the freshly grown culture of *Lactobacillus acidophilus* and incubated at $37^0$C for 24 hours. Growth was analysed using Turbidimetric analysis and Standared plate count method.

**5. Comparison of growth on MRS Media and PPE Media**

PPE Agar Media was prepared by adding 1.5gm of agar-agar powder to 100ml of PPE Extract and were sterilized by autoclaving. Similarly MRS Agar Plate was prepared. Each one of them were inoculated with the 0.1ml freshly grown culture of *Lactobacillus acidophilus* and incubated at $37^0$C for 24 hours. After incubation the CFU/ml were counted.

- **Studies on the contamination of PPE Media:-**Prepared PPE Agar plates were kept in incubater without inoculation for 5 days and observed for the colonies grown on the plates by observing morphological characteristics and Microbiological studies.

**6. Results and Discussion**

*Lactobacillus acidophilus* was selected as the best health care supplement and an attempt has been made for its cultivation on fruit waste. Different fruit pee1s extracts were tested for growth and papaya peels extract has shown the highest growth and sugar concentration of about 20.23mg/ml. Various factors affecting growth of Lactobacillus in PPE media were determined and it was found that optimum pH was 7.0, temperature was $37^0$C and the organism was found to survive at 2.0% NaCl and 0.3% Methylene blue. On comparing the results of PPE with MRS (the growth media for *Lactobacillus)*, the growth was found to be about $1/5^{th}$ in PPE than in MRS media. An attempt was made to check for contamination of PPE which showed the growth of Aspergillus, Alternaria, Penicillum, yeast and Actinomycetes.

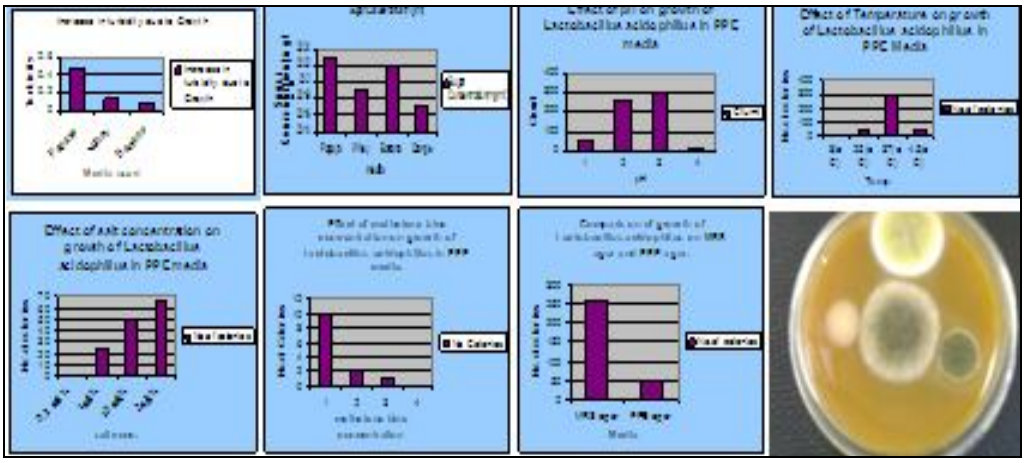

*Figure 1*

**7. Conclusion**

The results of the studies shows that as probiotics have multifactorial effects on digestive, urogenital and immune system of the body with negligible side effects, they can be used as the best health care supplement. An effective and economical method for their production can be designed by using fruits wastes such as papaya peel extract as the growth media for Lactobacillus. The biochemical properties of the organism remains unaffected while grown on PPE Media and therefore the organisms can be used as better health care supplement and can work for the betterment of mankind.

This idea can also be useful in reducing environmental pollution by converting the fruit wastes to useful products.

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