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**RESEARCH ARTICLE**

# PREPARATION OF ACIDOPHILUS SOY MILK AND ITS THERAPEUTIC EFFECT AGAINST GASTROINTESTINAL PATHOGENS

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**ABSTRACT**

This study evaluates the therapeutic effect of acidophilus soy milk against intestinal infection causing pathogens. *Lactobacillus acidophilus* strains were isolated from curd and identified based on the colony morphology and biochemical profile. Using *Lactobacillus acidophilus* fermented soy milk was prepared. Its antimicrobial efficiency was identified against five pathogens such as *Escherichia coli, Salmonella typhi, Shigella dysentriae, Vibrio cholera* and *Staphylococcus aureus*. Among these five pathogens *Vibrio cholerae* was effectively inhibited by acidophilus soy milk.



## INTRODUCTION

Soy protein is the best among the plant protein and is inferior to animal protein only in being deficient in sulphur containing amino acids. It can be used as substitute of cow milk by people with intolerance and for infants and children who are allergic to human or cow milk (Johnson *et al.,* 1992). To make it more palatable, attempts are now being made to remove beany flavour of soy milk. Many approaches have been used like heat treatment or during initial processing to destroy lipoxidase, which causes the production of undesirable flavours during oxidation of lipids (Mital and Steinkraus, 1976).

Fermentation technology, which can be used to improve the flavour (Wang *et al.,* 1974). Lactic acid bacteria play an important role in food fermentation processes. *Lactobacillus acidophilus*, non-pathogenic and a member of the normal intestinal micro flora is widely used in fermented dairy products and is considerable industrial medical interest because it has been reported to aid in the reduction of the levels of harmful bacteria and yeast in the small intestine and to produce laccase, an enzyme which is important for the digestion of milk (Deraz *et al.,* 2007). Many strains belonging to the *L.acidophilus* groups have been reported to produce antimicrobial compounds which show a great antagonistic activity against both Gram positive and Gram negative bacteria (Alla *et al.,* 2003). The present study was planned to isolate the *Lactobacillus acidophilus* from curd and to prepare soy based fermented product that can be used to evaluate the antibacterial potentialities against gastro intestinal pathogens.

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## MATERIALS AND METHODS

***Isolation of strains:*** One ml/gm of curd was transferred to 100 ml of MRS broth for enrichment for 48 hrs and using streak plate method cultures were transferred to the plate containing media MRS (de Man Rogosa and Sharpe) agar medium. The plates were incubated at 37˚ for 24 hrs. Well isolated colonies were picked up for further analysis.

***Identification:*** The strain was identified based on the morphology and biochemical profile.

***Preparation of Soymilk:*** Hundred grams of soybeans were first washed and soaked overnight in distilled water. After decanting the water, the soaked soybeans were mixed with distilled water of 10 times their weight and the mixed in a blender for 3 minutes. The resultant slurry was filtered through a double layered cheese cloth to yield soy milk was pasteurized (Wang *et al.,* 2002).

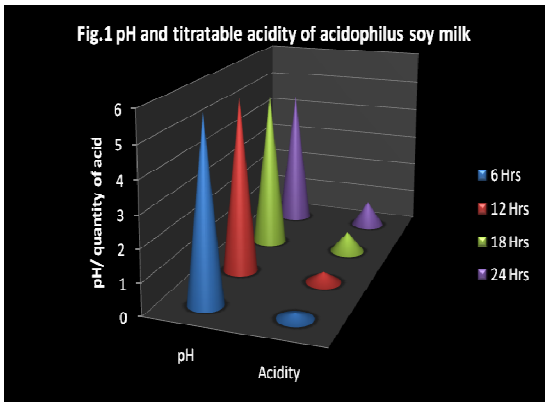
***Fermentation:*** The culture was inoculated (around 2%) in a respective containers and incubated at 39° - 45°C for 3 - 4 h.
***Estimation of pH and titratable acidity:*** The pH values were determined using a digital pH meter. Two drops of 1%

phenolphthalein was added with 5 ml of fermented milk, mixed well and titrated with 0.1N NaOH until the appearance of pink colour. Total acidity was calculated using formula. Acidity (%) = volume of titrate (ml) × Normality of NaOH×64×100/volume of sample ×1000.

***Antibacterial activity:*** Antibacterial activity of acidophilus soymilk was tested by the well diffusion method (Bauer *et al.,* 1966) using five pathogens such as *Escherichia coli, Salmonella typhi, Shigella dysentriae, Vibrio cholera* and *Staphylococcus aureus.* The pathogens were procured from IMTECH, Chandigarh. Bacterial strains were suspended in a peptone broth and incubated at 37˚C for 24 hours. Then the cultures were spreaded over the Mueller Hinton agar respectively; the wells were made with the aid of flamed cork borer on the surface of the agar plates. Cell free extracts of fermented products obtained by centrifugation of the samples at 4500 rpm for 10 min and were passed through 0.45 µm membrane filter (Padmanabha Reddy *et al.,* 2006). Approximately 0.1 ml of cell free extract was transferred to the wells respectively, incubated at 37 °C for 24 – 48 h.

## RESULT AND DISCUSSION

Based on the colony morphology (white, rounded, smooth colonies on MRS agar), cell wall type (Gram positive), non motile, catalase and oxidase negative isolates were purified and maintained as LAB. Table.1 showed the biochemical profile, which determines the isolates were *Lactobacillus acidophilus* ( John G Holt *et al.,* 1982).



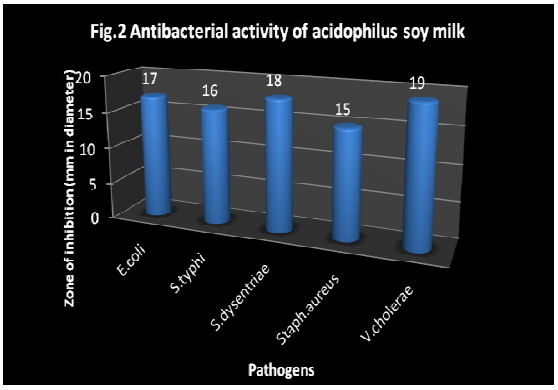




**Table 1. Characteristics of isolated strain**

| Biochemical tests | S1 |
|---|---|
| Gram Staining | +ve, rod |
| Motility | - |
| Catalase | - |
| Oxidase | - |
| *Carbohydrate fermentation* | |
| Arabinose | + |
| Cellobiose | + |
| Fructose | + |
| Esculin | + |
| Galactose | + |
| Glucose | + |
| Inulin | + |
| Lactose | + |
| Maltose | + |
| Mannose | + |
| Melibiose | - |
| Raffinose | - |
| Rhamnose | - |
| Ribose | - |
| Salicin | + |
| Sorbitol | + |
| Sucrose | + |
| Trehalose | + |
| Xylose | + |

**S1= *Lactobacillus acidophilus***

By using the isolated strain, acidophilus soy milk was prepared. Soymilk preparation was adopted by the method of Wang *et al.,* (2002). With different interval during fermentation were prescribed for the measurement of pH and titratable acidity. Fig. 1 showed that when incubation increased, pH of the fermented product was decreased, in contrast titratable acidity reach optimum level. Sarker and Banerjee (1996) revealed antibacterial activity of *Lactobacillus* against a variety of Gram positive and Gram negative bacteria. There are six gastrointestinal pathogens were selected in this study. Maximum zone of inhibition was noted in *Vibrio cholera* and followed by *Shigella dysentrieae* and then *Escherichia coli.* Minimum inhibition was noted in *Staphylococcus aureus.* This result supports the previous findings.

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