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Selection of *Lactic Dahi Cultures* for the Fermentation of Soy Milk

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Abstract

Soy milk serves as an excellent medium for the growth of lactic acid bacteria. In this study, Soymilk was fermented with five dahi cultures (NCDC144, NCDC167, NCDC323, NCDC325 and ST74). Soymilk was extracted from soybeans following standard procedure. WPC70 was added @ 0.5%, 1.0% & 1.5% to the soy milk and five dahi cultures were inoculated @1%. Dahi cultures were screened on the basis of pH, acidity and lactic count in soymilk medium containing WPC70 at different levels. NCDC323 showed maximum acidity 0.74% lactic acid with minimum pH 4.08 and lactic count of log 10.87 cfu/ml under specified growth condition [incubation time (16hrs) and temperature (30C)] with supplementing WPC70 @1.5%. NCDC167, NCDC325 and NCDC74 showed considerable titratable acidity (0.69 - 0.68% lactic acid respectively), pH (4.09 - 4.11y) and lactic counts (8.08 - 10.87log cfu/ml). This study helped to develop indigenous fermented soy based products like soy dahi, and probiotic soy dahi or fermented soy beverages.

Keywords: *Dahi; Soy; Fermentation; Antimicrobial; Growth Kinetics*

Introduction

Soy bean, (Glycine max) (L) Merr. is rich in protein, fat, and carbohydrate, and has attracted much attention because of its potential health benefits [1] due to their hypolipidemic, anticholesterolemic and antiatherogenic properties as well as due to reduced allergenicity (Trindade., *et al*. 2001). Soymilk products are firmly established in modern western diets, largely due to their beneficial influence. Being free of cholesterol, gluten and lactose, soymilk is a suitable food for lactose-intolerant consumers, vegetarians and milk-allergy patients [2]. The nutritive quality of soyabean protein is at the pinnacle of food sources available from the plant World. The polyunsaturated/ saturated ratio of soymilk fatty acids is also high (1.1: 0.3) along with its concentration of folate [3]. Traditionally lactic acid bacteria are used to prepare fermented milk products from cow or buffalo milk and growth of lactic cultures in soymilk has been reported in the literature [4]. The major carbohydrates in soybeans are sucrose, raffinose and stachyose that are not digested by human beings and may cause flatulence [5]. This drawback along with the disagreeable beany flavors has often limited the consumptions of soybean as the raw food material [6]. However, fermentation of these carbohydrates convert them to easily digestible sugars. Therefore, to address those matters, fermentation of soybean products with lactic acid bacteria has been studied extensively, to develop more digestible and palatable soy foods such as fermented soybean cheese (Hang and Jackson, 1967), sour milk beverages (Yamanaka., *et al*. 1969) and soybean yogurt (Yamanaka., *et al*. 1969, Mital and Steinkraus, 1975, Nsofor., *et al*. 1992). Dahi is a traditional fermented milk curd prepared by lactic acid bacteria in India and Asean countries. In this study, dahi or curd was prepared using indigenous dahi cultures from National Collection of Dairy Cultures (NCDC), Karnal, India. We have studied the fermentation pattern of lactic dahi cultures in soymilk supplemented with whey protein concentrate as an enhancer of bacterial growth. Whey protein concentrate-70 (WPC-70) contains two main whey protein fractions (α-lactalbumin and β-lactoglobulin) which act as enhancers of microbial growth using the yogurt organisms (Bury., *et al.* 1998).

It was observed that soymilk could support the simultaneous growth of Dahi cultures (NCDC144, NCDC-167, NCDC-323, NCDC-325, ST-74). These observations further suggested the possibility and the potentiality of developing the lactic acid bacteria containing various soy milk fermented traditional food products having various health benefits. Therefore, the growth of dahi cultures, acid production and subsequent changes on pH during the fermentation of soymilk was investigated. In addition, similar investigations were also observed during the fermentation of soymilk supplementing WPC-70.

Materials and Methods

Soybean Sample

Soybeans were obtained from Local Market, Karnal, India.

Source of cultures

Bacterial strains (NCDC144, NCDC-167, NCDC-323, NCDC-325 and ST-74) used were taken from National Collection of Dairy Cultures (NCDC), Karnal, India. The cultures were maintained by biweekly transfers into sterile litmus milk or soymilk and held at 5°C between transfers. Pathogens (*S. aureus, B. cereus* ATCC13061, *E. coli* ATCC 25922, *L. monocytogenes* ATCC15303 and *Pseudomonas* sp., *Salmonella* sp and *Shigella* sp.) used for antimicrobial activity were obtained from National Collection of Dairy Cultures (NCDC), Karnal, India.

Soymilk preparation

To prepare soymilk, 100g of soybeans were soaked for 14 – 16 h in 1.0L of distilled water at room temperature (28°C) in a 2.0 L beaker. The soak water was drained from the soybeans and the beans thereafter were blanched at 98°C in boiling distilled water for 30 min. The drained beans were hand washed thoroughly to remove their testa. They were then placed in a warring blender and 600 ml of boiled distilled water at 87 – 90°C was added and then blended for 3 min. The boiled water inactivated the enzyme, lipoxygenase during blending [7]. The resulting slurry was filtered through two layers of muslin cloth and approximately 600 ml of soymilk was obtained per 100 g of soybeans in 600 ml of water.

Inoculum development

The following Dahi cultures were graciously supplied by National Collection of Dairy Cultures (NCDC), Karnal, India. Stock cultures were prepared by mixing M17-grown (Lactococci) cultures with sterile rehydrated skim milk 20% (w/w) and glycerol 20% (w/v) in a 2:5:5 ratio, placing 1 mL in Cryovials and storing at -20°C. After two successive transfers of the test organisms in M-17 broth (HiMedia, India) and incubation at 30°C for 16h, each activated culture was inoculated into M-17 broth which was then incubated at 30°C for 16h. Theses working cultures of NCDc144, NCDC-167, NCDC-323, NCDC-325 and ST-74 which were then transferred in soymilk medium to check their activity in this medium. Dahi cultures were selected because of their potential use for making traditional indigenous fermented curd to improve health by providing various health benefits like antimicrobial, anti-hypertension, anti-diarrhea etc. For the preparation of the inocula, 100 ml of sterile soy milk medium was inoculated with 1 mL of active working culture and incubated at 300C until a pH of 4.5 was reached.

Conditions

When fermentation was performed, 100 ml of sterile soymilk was placed in a 150 ml screw cap, Erlenmeyer flask and was inoculated with 1.0 ml of an inoculum of the dahi cultures. In these experiments, the initial population of each organism in the soymilk was between 3 and 4 log cfu/ml. Inoculated soymilk was incubated without shaking at 30°C for 48h. During that period, samples were taken at predetermined intervals to determine the pH and titratable acidity of and the numbers of lactic acid bacteria in the soymilk.

Fermentation of soymilk

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Microbiological and Chemical Analyses

M-17 agar (HiMedia, India) was used for the enumeration of lactic counts of dahi cultures. 1.0 ml of appropriate serial dilutions of each sample were pour-plated onto the appropriate media. After 48h of incubation at 30˚C, the colonies that appeared on the plates were counted and the cfu/ml were calculated. Antimicrobial activity of fermented soy milk was carried out following well assay method measuring zone of inhibition against gram positive and gram negative pathogens. The titratable acidity (TA) was determined by the AOAC method and expressed as % lactic acid [8]. The pH values of the samples were measured using a pH electrode and meter.

Determination of antimicrobial activity

Agar well diffusion assays were used to study the antibacterial activity of the four lactic dahi cultures strains (NCDC144, NCDC-167, NCDC-323, NCDC-325, ST-74). For this purpose, seven reference strains were used to check sensitivity to the antimicrobial substances produced by the lactic dahi cultures, these being *S. aureus*, *B. cereus* ATCC13061, *E. coli* ATCC 25922, *L. monocytogenes* ATCC15303 and *Pseudomonas* sp., *Salmonella* sp and *Shigella* sp. were cultured in Nutrient broth (Himedia, India) at 30˚C for 24h incubation. Antibacterial activity may often be due to the production of organic acids, with a consequent reduction in pH, or to the production of hydrogen peroxide. It may also be due to the production of bacteriocins or bacteriocin-like compounds. Hence, antimicrobial activity was checked by a well diffusion assay after excluding inhibition due to organic acids. One milliliter (ml) of the indicator strain (with approximately 7 X 10^5cfu/ml), cultured in Nutrient broth (Himedia, India) for 24h at 30˚C, was inoculated into 15ml of molten cooled Nutrient Agar Nutrient broth (Himedia, India) and kept at 45˚C. The resultant mixture was poured into a Petri dish. After solidification of the agar, wells (6mm in diameter) were cut into it, and 100 microlitres (µl) of supernatant of soy milk fermented with different lactic dahi cultures, was added to each well to test for antibacterial activity. The plates were kept at 3 - 4˚C for 4h to ensure diffusion of the fluid into the agar and examined for inhibition after incubation at either 30°C for 24h. Dahi cultures strains for testing were cultured @1.0% overnight in soy milk supplemented with WPC70 @ 0.5%, 1.0% and 1.5%. Cells were then removed by centrifuging at 14,000g for 5 min. The supernatant fluid was filtered through a filter with a pore size of 0.22µ (Millipore Corporation, Bedford, USA), and adjusted to pH 6.0 with sterilized 2.5M NaOH. This extract was placed into the wells.

Statistical Analysis

The mean values and the standard deviations were calculated from the data obtained with triplicate trials. Analyses of variance using ANOVA were conducted. Differences between the sample means were analyzed by Duncan's Multiple Range tests at $\alpha = 0.05$ by SPSS software.

Results and Discussions

Changes of viable count, titratable acidity (TA) and pH in soymilk during the growth of dahi cultures

Changes in lactic counts, pH and TA during the fermentation of soymilk inoculated with dahi cultures are summarized in Figure 1 and Figure 2. In general, TA increased and the pH decreased as the fermentation time increased for soymilk inoculated with dahi cultures of lactic acid bacteria [9,10]. It was observed from Figure 1, among these dahi cultures, NCDC-323 produced maximum acidity, with TA increasing from an initial 0.70 - 0.74% lactic acid after 16h of fermentation of soy milk being supplemented with WPC70 @ 0.5, 1.0 and 1.5% respectively (Figure 2). NCDC323 produced maximum acidity (0.74% lactic acid) after 16h fermentation at 300C in soy milk medium supplemented with 1.5% WPC-70. But NCDC167 and ST74 produced a good amount of acidity (0.67&0.68% lactic acidity respectively). NCDC323 was a good producer of acidity at different level of WPC70 supplementation. NCDC323 was a high acid producer as well as potential to utilize unconventional sugars (viz. stachyose, raffinose, sucrose) present in soy milk. However, NCDC323 also produced firm

curd incomparision to NCDC167 & ST74. Acid production was greater in the soy beverage, it could reflect the lower buffering capacity of the soy beverage as compared to that of milk [11].

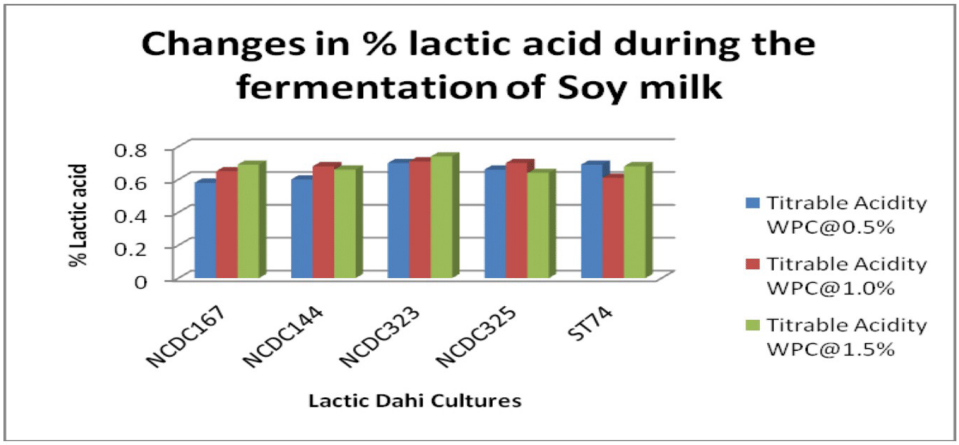

Figure 1: *Changes in % lactic acid during the fermentation of Soy milk.*

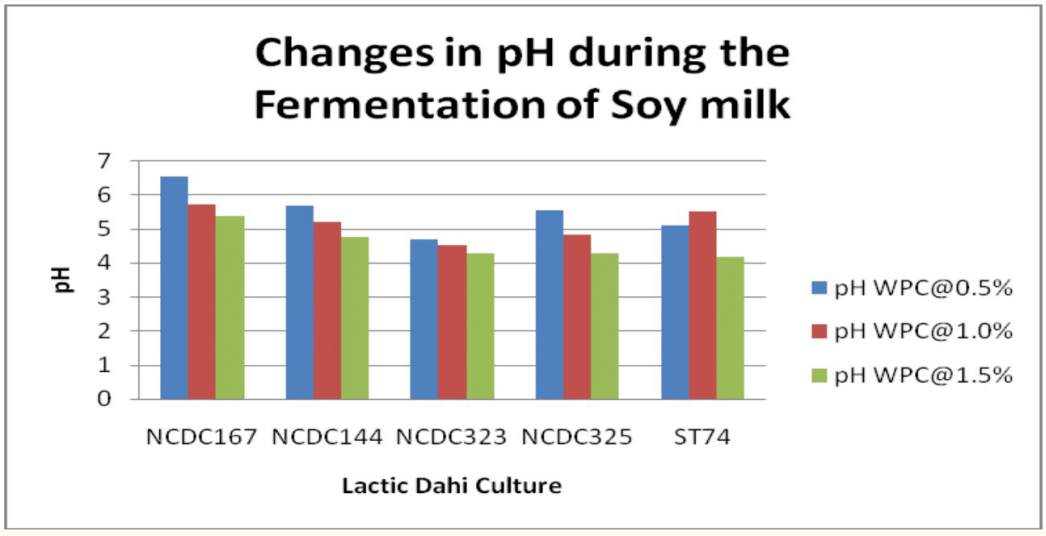

Figure 2: *Changes in pH during the fermentation of soy milk.*

From Figure 2, NCDC323 showed maximum lowering of pH (4.26) in soy milk supplemented with WPC70 @1.5% during fermentation at 300C for 16h. It was also found that NCDC325 lowered down pH upto 4.28 in the similar growth condition in comparison to NCDC167 & NCDC144. Soy milk is an excellent medium for growth of lactic acid bacteria as this medium contains different sugars like stachyose, raffinose, sucrose.

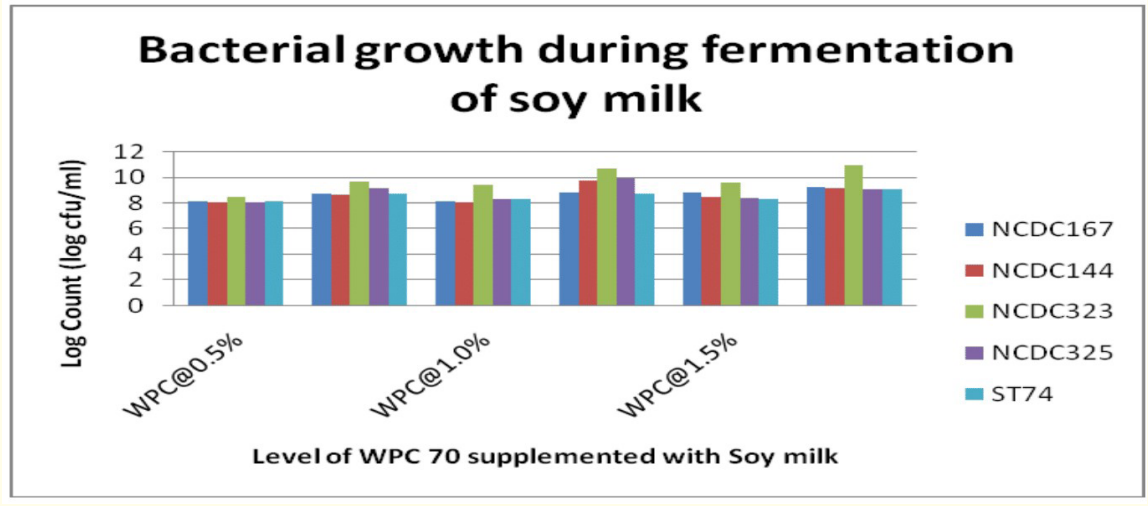

Figure 3: *Bacterial growth during fermentation of Soy milk.*

Figure 3 showed that these five lactic dahi cultures @1.0% grown well in soy milk at 300C for 16h. In this study, NCDC323 showed maximum viable count (log count 10.87 cfu/ml) during fermentation of soy milk supplemented with WPC70 @1.5%. At WPC70 @1.0%, NCDC144 also produced optimum log count 9.74 cfu/ml. NCDC323 was a suitable culture for the growth in soy milk medium. WPC70 supplementation is also an extra source of nutrient to enhance the growth of lactic dahi cultures.

Growth behaviour of Selected Dahi culture during the fermentation of soymilk

Figure 4 showed that viable number of NCDC323 increased during the fermentation of soy milk supplemented with WPC70 @1.5% at different incubation periods (0, 6, 9, 12 and 16hrs) under specific growth condition (incubation temp. 30°C). Initially, NCDC323 culture was inoculated in soy milk medium at the level of log 2.35 cfu/ml. Then, after 6 hr interval, log count was 6.98 cfu/ml and subsequently acidity increased to 0.2567%LA and pH lowered to 6.12 from 6.8. Similarly, after 9 hrs interval, log count was 8.89 cfu/ml and subsequently acidity increased to 0.46 %LA and pH lowered to 5.13 [12]. However, 16 hr incubation showed optimum log count 10.78 cfu/ml and titratable acidity 0.74%LA and pH 4.82.

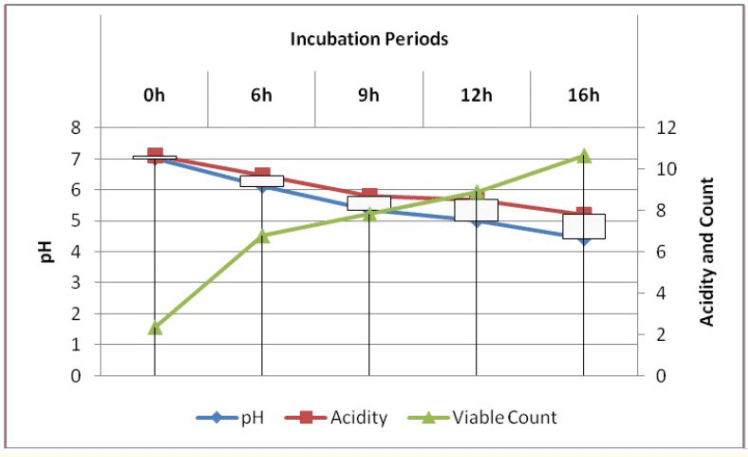

Figure 4: *Growth pattern of NCDC323 at defined incubation temperature with different incubation time intervals in soy milk medium with WPC70 @1.5%.*

Antimicrobial Activity of Fermented Soy milk with different *Lactic Dahi cultures*

Antimicrobial activity carried out in fermented soy milk with dahi cultures (NCDC-167, NCDC-323, NCDC-325 and NCDC144) against *S. aureus*, *B. cereus* ATCC13061, *E. coli* ATCC 25922, *L. monocytogenes* ATCC15303 and *Pseudomonas* sp., *Salmonella* sp and *Shigella* sp. Soy milk is fermented with inoculating dahi cultures at the rate of 1% for 24 h at 30°C. Then antimicrobial activity was checked following well assay method. It was found that NCDC-323 showed maximum zone of inhibition against *Pseudomonas* sp, *S. aureus* and *B. cereus* compared to NCDC 325 and NCDC167 as NCDC 323 produced maximum acidity and mínimum pH (Table 1). It had been showed that NCDC 323 exhibited 20.99, 22.03, 17.67 mm zone of inhibition against *S. aureus*, *B. cereus* and *Pseudomonas* sp [13-15].

Parameters	*Lactic dahi cultures*				
	NCDC167	**NCDC323**	**NCDC325**	**NCDC144**	**ST74**
Antimicrobial activity∗ against *E. coli*	16.00 ± 0.00	16.33 ± 0.33	14.00 ± 1.00	14.00 ± 1.00	15.00 ± 0.02
Antimicrobial activity∗ against *S. aureus*	17.33 ± 0.33	20.99 ± 0.07	19.33 ± 0.33	18.33 ± 0.33	16.33 ± 0.33
Antimicrobial activity∗ against *B. cereus*	15.67 ± 0.33	22.03 ± 0.09	20.33 ± 0.33	12.33 ± 0.33	15.67 ± 0.33
Antimicrobial activity∗ against *Pseudomonas*	16.00 ± 0.02	17.67 ± 0.33	15.00 ± 0.02	15.00 ± 0.00	14.00 ± 0.00
Antimicrobial activity∗ against *Shigella* sp.	15.67 ± 0.33	18.03 ± 0.09	12.33 ± 0.33	12.33 ± 0.33	17.67 ± 0.33
Antimicrobial activity∗ against *Salmonella* sp.	16.00 ± 0.02	17.67 ± 0.33	15.00 ± 0.00	15.00 ± 0.00	18.00 ± 0.03
Antimicrobial activity∗ against *L. monocytogenes*	15.00 ± 0.03	18.67 ± 0.33	16.00 ± 0.06	14.00 ± 0.00	15.67 ± 0.02

Table 1: *Antimicrobial activity against indicator organisms of dahi cultures cultured in soy milk supplemented with 1.5% WPC70 @1% for 24 hrs at 30°C incubation following well assay method.*

∗ mm, zone of clearance including well diameter 6 mm

Mean ± standard deviation; n = 4.

Conclusion

It can be concluded that NCDC 323 cultures showed maximum acid production by metabolizing soy sugars stachyose, raffinose or sucrose which causes flatulence or discomforts in human consumption. Similarly, NCDC 323 was also lowered down the pH at optimum level during fermentation of soy milk to firm the soy curd. Antimicrobial activity was found maximum in case of NCDC323 (22.03 ± 0.09 mm) and NCDC 325 (20.33 ± 0.33 mm) against *B. cereus*. WPC 70 supplemented with soy milk at the rate of 1.5% enhanced microbial growth and also increased the total solids in the final coagulam. These cultures can be further used to manufacture soy fermented products like soy dahi, soy lassi, soy shrikhand in Indian market as a functional fermented soy based products.

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