

Original Research Article

Enhancement of growth and bioactivities of probiotic bacterium *Streptococcus thermophilus* as influenced by lactalbumin hydrolysate and inulin supplementation in skimmed milk medium

ABSTRACT

Aims: To determine the probiotic potential of *Streptococcus thermophilus* and effects of lactalbumin hydrolysate (whey protein) and inulin (prebiotics) supplementation in the production medium on the growth and bioactivity profile of *S. thermophilus* under different pH and temperature conditions.

Place and Duration of Study: The research work was carried out in Microbiology laboratory, Department of Biomedical Sciences, Sri Ramachandra University, India between May 2015 and August 2015

Methodology: *S. thermophilus* NCDC 74 was initially screened for better growth in three production media. The parameters examined in the study were specific growth rate and yield coefficient, anti-oxidant, anti-adhesive and anti-pathogenic activities, antibiotic and drug resistance and acidic pH tolerance. The collected data were analysed with IBM.SPSS statistics software 23.0 Version.

Results: The skimmed milk medium showed maximum growth of 8.56×10^{10} log CFU/ml compared to two synthetic media with lactose and sucrose as carbon sources which contained 2.34×10^{10} log CFU/ml and 1.19×10^{10} log CFU/ml respectively. The initial pH of 6.5 and temperature of 42°C have been found to be optimum for the bacterial activities. The specific growth rate and yield coefficient were positively influenced in a significant manner by the experimental groups, except that inulin addition increased only the microbial load and specific growth rate was 0.26 hr^{-1} which was the same as that of control. The experimental conditions also enhanced the anti-oxidant, anti-adhesive, acidic pH tolerance abilities of *S. thermophilus*. *S. thermophilus* exhibited maximum anti-adhesive activity of 56.5% against *Candida albicans* at 42°C compared to its activity of 26.1% and 33.9% against *Salmonella typhi* and *staphylococcus aureus* respectively. *S. thermophilus* was resistant to amphotericin and omeprazole, but sensitive to amoxicillin, ampicillin and ciprofloxacin. The experimental conditions influenced the anti-pathogenic activity of *S. thermophilus* against *Salmonella typhi* positively.

Conclusion: Scale up of the production conditions would result in large scale production of highly efficient probiotic strain, to exert increased beneficial actions in human and applications in food and pharmaceutical industries.

Keywords: Probiotics; Gut microbiota; *Streptococcus thermophilus*; Skimmed milk medium; Inulin; Lactalbumin hydrolysate.

1. INTRODUCTION

The term 'probiotic' was coined and defined by Lilly and Stillwell (1965) as a 'substance secreted by a microbe to trigger the growth of another'. World Health Organisation and the Food and Agriculture Organization of the United Nations have defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host." [1,2]. The human gastrointestinal tract accommodates several hundred bacterial species including *Lactobacillus* spp, *Bifidobacterium* spp which maintain the health status of the host through various proposed mechanisms which include anti-pathogenic activity against harmful microbes in the gut through release of anti-microbial agents like bacteriocins; induction of immune responses; interference in microbial metabolism, improved epithelial barrier function etc [3,4]. However ageing, physical dysfunctions, antibiotic and immunosuppressive therapies, dietary and environmental influences lead to dysbiosis by altering the gut microbiota composition. This clearly demands the usage of probiotics as supplements to compensate for the imbalance of the gut microbiota and retain health status. For an organism to be categorized as probiotic, the organism should possess certain features which include tolerance to acidic pH and bile salt environment; non-pathogenic or non-toxic in nature, viability under storage conditions, beneficial to humans [5].

Streptococcus thermophilus is a lactic acid bacterium which has been used as a starter culture for yogurt and cheese production since a very long time and has been proven to be an efficient probiotic bacterium [6,7]. *S. thermophilus* happens to be a positive remedy for rotavirus induced diarrhea, antibiotic associated diarrhea [8], bacterial vaginosis while it shows increased efficiency for certain conditions like lactose intolerance, cholesterol assimilation [9] compared to other species. Therapeutic effects of *S. thermophilus* on chronic gastritis [10] and mucositis in rats [9] have been proven. *S. thermophilus* as a probiotic is not just restricted to gut, but also effects nasal canal and skin. *S. thermophilus* has been proven to act as topical probiotic [11] which enhances body's ceramide levels in stratum corneum and to reduce the colonization of pathogens in human nasal canals. It has also been reported that the milk fermented by *S. thermophilus* has anti-mutagenic property [12,13]. *S. thermophilus* releases amphiphilic polymer compounds called biosurfactants which tend to change the properties of surface of gut like surface energy, which eventually results in the anti-adhesion of pathogenic microbes, mainly from the surface in wound region and anti-adhesive property against several bacterial and fungal species isolated from voice prostheses made of silicone rubber has been reported [14,15].

Prebiotics are defined as non-digestible substances that induce the growth of lactic acid bacteria, thereby confer health benefits to host. Inulin is one of the commonly used prebiotic sources, which belong to class of dietary fibers called fructans. Whey protein isolated from whey is the mixture of alpha-lactalbumin and beta-lactoglobulin. Despite reports on the influence of inulin and lactalbumin hydrolysate on the growth of *S. thermophilus*, studies have not been done to examine their effects on

various bio-activities of *S. thermophilus*. This study aims to understand the significance and reaction of inulin and lactalbumin hydrolysate on growth and strain specific probiotic traits of *S. thermophilus*.

2. MATERIAL AND METHODS

2.1 Bacterial strain and growth conditions

S. thermophilus NCDC 74 used in the study was obtained from National Collection of Dairy Cultures (NCDC). The strain was routinely grown in LM17 broth (M1029 - Himedia laboratories Pvt Ltd.) and incubated at 37°C during the experiments and stock culture of the strain was sub cultured on LM17 agar on regular intervals. The inocula used in the experiments were prepared according to 0.5 McFarland Standard.

2.2 Selection of the production medium

Three media formulations were considered for selection of optimal growth and the medium which showed better growth among the three media was used for further experimental studies. The media used were as follows

1. Enriched milk medium composed of skimmed milk powder, 100g L⁻¹; yeast extract, 50g L⁻¹; peptone 10g L⁻¹;
 2. Synthetic media with two different carbon sources composed of yeast extract, 6g L⁻¹; peptone, 6g L⁻¹; beef extract, 3.3g L⁻¹; potassium di-hydrogen phosphate (KH₂PO₄), 1.5g L⁻¹; sodium acetate, 1g L⁻¹; ascorbic acid, 0.5g L⁻¹; magnesium sulphate (MgSO₄.7H₂O), 0.5g L⁻¹; manganese sulphate (MnSO₄.7H₂O), 0.4g L⁻¹;
- Lactose and Sucrose were added in the concentration of 30g L⁻¹ as sole carbon source separately for two media.

The media were set to pH 7 and autoclaved before use. The media were inoculated with fresh inoculum separately and kept for overnight incubation at 37°C. Serial dilution of the media was performed and the samples were enumerated by pour plate technique.

2.3 Experimental groups in the study

2.3.1 Control conditions

- pH-7
- Temperature-37°C

2.3.2 Experimental conditions

- Supplementation of production medium with
 1. 1% Lactalbumin Hydrolysate (61302 - Sigma Aldrich) –Whey protein source
 2. 1% Inulin (GRM 103- Himedia laboratories Pvt Ltd.) – Prebiotic source
- Varying culture conditions:
 3. pH-6.5
 4. pH-7.5
 5. pH-8

6. Incubation temperature-42°C

2.4 Determination of specific growth rate

The medium inoculated with *S. thermophilus* was checked for colony growth by pour plate technique for every 1.5 hours. The maximal specific growth was calculated using the formula:

$$\mu_{max} = \frac{(\ln X_2 - \ln X_1)}{(t_2 - t_1)}$$

Where X_1 and X_2 are biomass (log CFU/ml) at time t_1 and t_2 of exponential phase respectively. Maximum specific growth rate of the organism was determined by the method described by Ali and Hasnia (2008) [16]. The results were expressed in hr^{-1} .

2.5 Determination of yield coefficient

The lactic acid content in the medium was checked by volumetric titration using 0.11N NaOH. The results were expressed in g L^{-1} . The yield coefficient was calculated according to the formula described by Stanbury [17]:

$$Y_p = \frac{dp}{dx}$$

Where Y_p is the yield of product (g L^{-1} lactic acid) in terms of biomass.

Note: The lactic acid equivalent to 0.11N NaOH is 0.01g

2.6 Preparation of Cell Free Fraction (CFF)

The media were withdrawn at their respective late exponential phases of their growth and subjected to centrifugation at 10,000 rpm for 10 minutes at 4°C to obtain CFF (Cell Free Fraction) [18].

2.7 Anti-oxidant activity

Anti-oxidant activity of CFF was evaluated using DPPH free radical scavenging assay as described in [18] with slight modifications. 500µl of CFF was added with 2ml of DPPH (2,2-Diphenyl-1-picryl hydrazyl) at concentration of 3mg/50ml methanol. Control was made using 2ml of DPPH solution added to 500µl of methanol. The samples were mixed and incubated in the dark for 30 minutes. Absorbance was measured at 517nm using spectrophotometer. The free radical scavenging activity was measured in terms of percentage (%) using the formula

$$\% \text{ Free radical scavenging activity} = \frac{(A_{517} \text{ Control} - A_{517} \text{ Test sample})}{A_{517} \text{ Control}} \times 100$$

The concentration of anti-oxidant was calculated from the standard graph of ascorbic acid equivalent.

2.8 Anti-pathogenic activity

Anti-pathogenic activity of CFF was tested against *Salmonella typhi* (clinical isolate), qualitatively by agar well diffusion method and also quantitatively by the method described by Gudina et al. [19]. 100µl of CFF was added onto a column of 96-well plate, which was inoculated with 20µl of *S. thermophilus* culture. Well containing only *S. thermophilus* culture was used as control. The plate was incubated overnight at 37°C. The absorbance was measured at 600nm using spectrophotometer. The growth inhibition percentage was calculated using the formula

$$\% \text{ Growth inhibition} = \frac{(A_{600} \text{ Control} - A_{600} \text{ Test sample})}{A_{600} \text{ Control}} \times 100$$

2.9 Anti-adhesive activity

The anti-adhesive property of CFF was tested against clinical isolates of *Salmonella typhi*, *Staphylococcus aureus*, *Candida albicans* according to the method described by Gudina et al., [19]. 200µl of CFF was added to 96-well plate and incubated overnight at 4°C. After washing twice with PBS, 200µl of *Salmonella typhi*, *Staphylococcus aureus*, *Candida albicans* cultures were added to the well and plate was incubated at 4°C for 5 hours. By washing the well with PBS, unattached organisms were removed while adhered organisms were fixed using methanol for 15 minutes. The plate was then emptied and dried followed by staining using crystal violet dye for 5 minutes. The excess stain was removed by rinsing under running tap water. The plates were air-dried and 33%glacial acetic acid was added to resolubilise the dye bound to adhered organisms. Wells containing only *Salmonella typhi*, *Staphylococcus aureus*, *Candida albicans* cultures were used as control. The absorbance was measured at 595nm spectrophotometrically. The anti-adhesion percentage was calculated using the formula

$$\% \text{ Antiadhesion} = \frac{(A_{600} \text{ Control} - A_{600} \text{ Test sample})}{A_{600} \text{ Control}} \times 100$$

2.10 Antibiotic and drug resistance

Antibiotic and drug resistance ability of *S. thermophilus* was tested using agar well diffusion method [20]. Commercially available antibiotics and drug (Amoxicillin, Ampicillin, Amphotericin, Ciprofloxacin and Omeprazole) were obtained and diluted to different concentrations (125, 250, 500, 1000 µg/ml). The medium containing *S. thermophilus* was retrieved at late exponential – early stationary phase and used to prepare lawn culture on M17 agar plate. Drug preparations were loaded onto the wells created in the agar plate and plates were incubated overnight at 37°C. The zone of inhibition was measured.

2.11 Acidic pH tolerance

The pH tolerance ability of *S. thermophilus* was tested for pH 2 and 4 as described in [21] with slight modifications. *S. thermophilus* was retrieved from the medium at late exponential – early stationary phase and inoculum from the medium was transferred to fresh M17 media set to pH 2 and 4 separately. The M17 media were incubated overnight at 37°C and enumerated using pour plate technique.

2.12 Statistical analysis

The collected data were analysed with IBM.SPSS statistics software 23.0 Version. To describe about the data descriptive statistics frequency analysis, percentage analysis were used for categorical variables and the mean & S.D were used for continuous variables. For the multivariate analysis the Kruskal Walli's test was used followed by Mann-Whitney U test. In the above statistical tool the probability value .05 was considered as significant level.

3. RESULTS AND DISCUSSION

3.1 Selection of the production medium

The production medium contains basic essential nutrients which bacteria utilize to obtain energy for their growth and various metabolic and physiological activities of bacteria. The composition of production medium is specific to each bacterium depending on the ability of bacteria to utilize the nutrients in the medium. Out of the three media screened for optimum growth, skimmed milk medium (SKM) showed maximum growth followed by synthetic medium containing lactose as sole carbon source. Yeast extract and peptone in SKM are responsible for drastic growth as nitrogen influence has significant effect on growth of *S. thermophilus* [22]. The synthetic medium containing sucrose showed minimal growth comparatively, but considered to have good bacterial counts in general. The growth of *S. thermophilus* in SKM was 3.5 and 8.5 times more than lactose and sucrose medium respectively as shown in Table 1 which is similar to the results obtained in [23]. The affinity of *S. thermophilus* towards lactose and sucrose is linked to the presence of genes coding for enzymes and membrane proteins which are involved in metabolism of sugars and their transport into the cells [24]. The pH of the medium significantly dropped over time as a result of production of lactate or other acids. SKM was finally selected as production medium to carry out further studies.

Table 1: Growth of *S. thermophilus* in three media (culture conditions: 37°C, pH 7)

Medium	CFU / ml
Synthetic medium with lactose carbon source	2.34×10^{10}
Synthetic medium with sucrose carbon source	1.19×10^{10}
Enriched skimmed milk medium	8.56×10^{10}

3.2 Specific growth rate and yield coefficient determination

The specific growth rate and yield coefficient determination reflects the ability of *S. thermophilus* to utilise nutrients and multiply generations while simultaneously secreting various products during the different phases of their growth. The experimental conditions proposed in the study positively affected the growth rate and lactic acid production efficiency of *S. thermophilus* in a significant manner ($P \leq .01$). The varying pH, temperature conditions and addition of lactalbumin hydrolysate and inulin resulted in altered growth curve patterns of *S. thermophilus* as shown in Figure 1.1-1.3. Compared to control, the specific growth rate and yield coefficient were found to be maximum at pH – 6.5 and 42°C, while a gradual decrease in growth rate and yield coefficient was observed with the increase in initial pH. Specific growth rate at 42°C was approximately 3.5 times higher than control (Figure 1.4, 1.5). Addition of inulin increased only the microbial load over time compared to control, while specific growth rate was not significantly increased ($P > .05$), which is attributed to the ability of *S. thermophilus* to consume complex fibers at slower rate over time. However, inulin addition results in

better yield coefficient while lactalbumin hydrolysate addition resulted in significant increase in both the parameters proving that influence of nitrogen source is vital for rapid growth of *S. thermophilus*. Lactalbumin hydrolysate contains hydrophilic amino acids which are highly beneficial for bacterial growth [25]. Cell development and acid production are enhanced by addition of organic nitrogen sources compared to inorganic nitrogen sources [26].

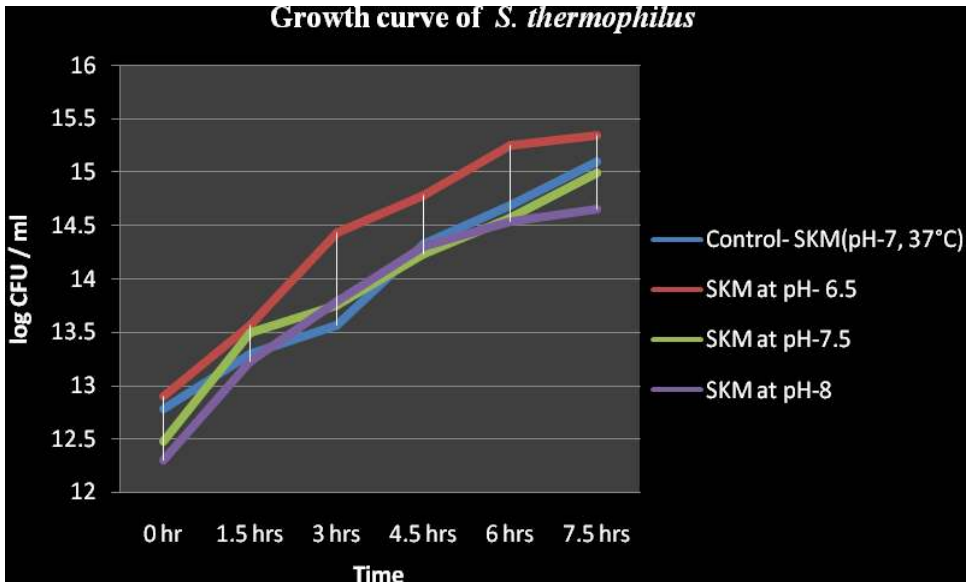


Figure 1.1: Effect of varying pH conditions on growth of *S. thermophilus*. The temperature was maintained at 37°C for the study groups. The log CFU values were recorded for regular intervals of 1.5 hrs for different study groups maintained at pH 6.5, 7.5 and 8. The control group was maintained at pH 7

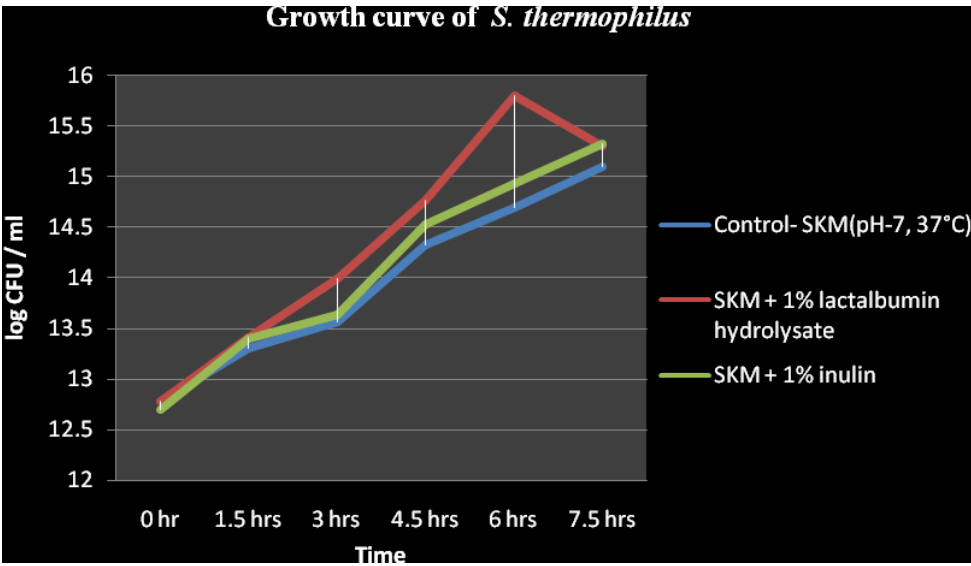


Figure 1.2: Effect of 1% lactalbumin hydrolysate and 1% inulin on growth of *S. thermophilus*. The temperature and pH were maintained at 37°C and 7 for all the study groups. The log CFU were recorded for

regular intervals of 1.5 hrs for study groups SKM + 1% lactalbumin hydrolysate and SKM + 1% inulin and control group was SKM without supplementation.

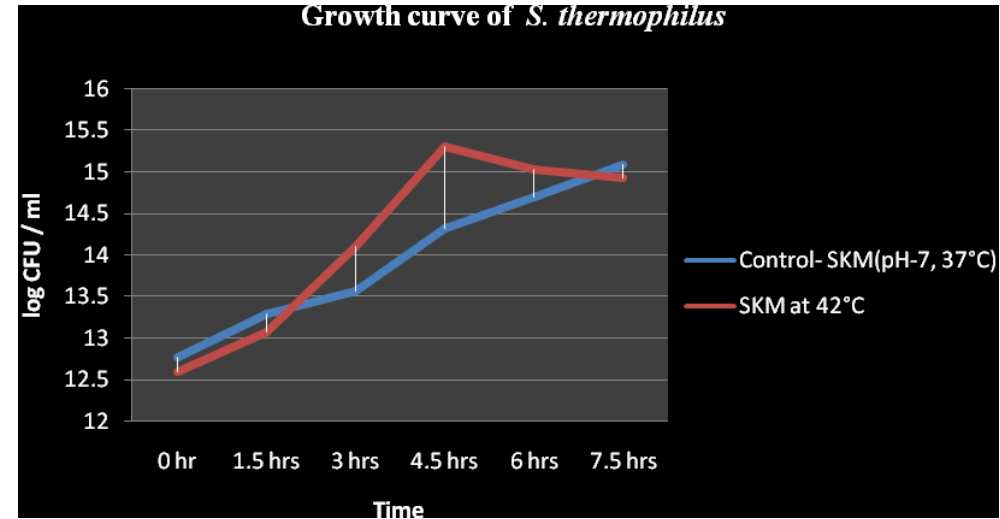


Figure 1.3: Effect of temperature on growth of *S. thermophilus*. The log CFU were recorded for control group (SKM at 37°C) and study group (SKM at 42°C). Both groups were maintained at pH 7

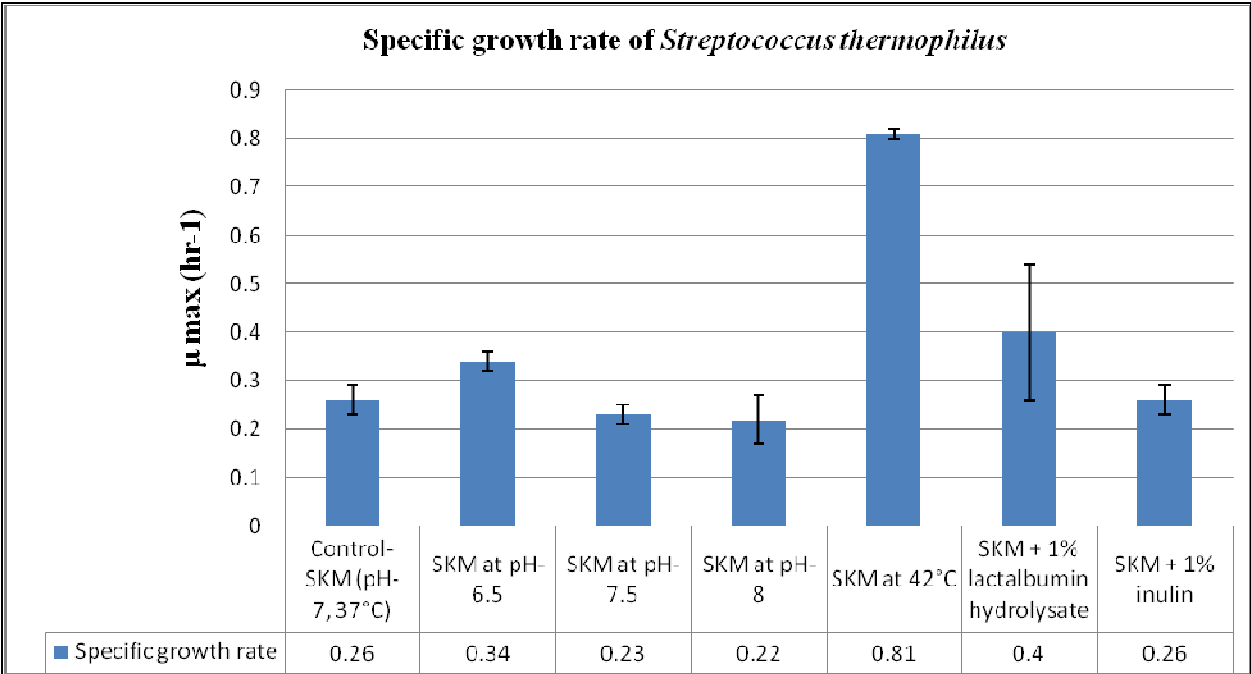


Figure 1.4: Specific growth rate (hr^{-1}) of *S. thermophilus*. SKM with initial pH 6.5, 7.5, 8 were maintained at 37°C. SKM supplemented with 1% lactalbumin hydrolysate and 1% inulin were maintained at 37°C and in itial pH 7. SKM with incubation temperature at 42°C was m aintained with initial pH 7

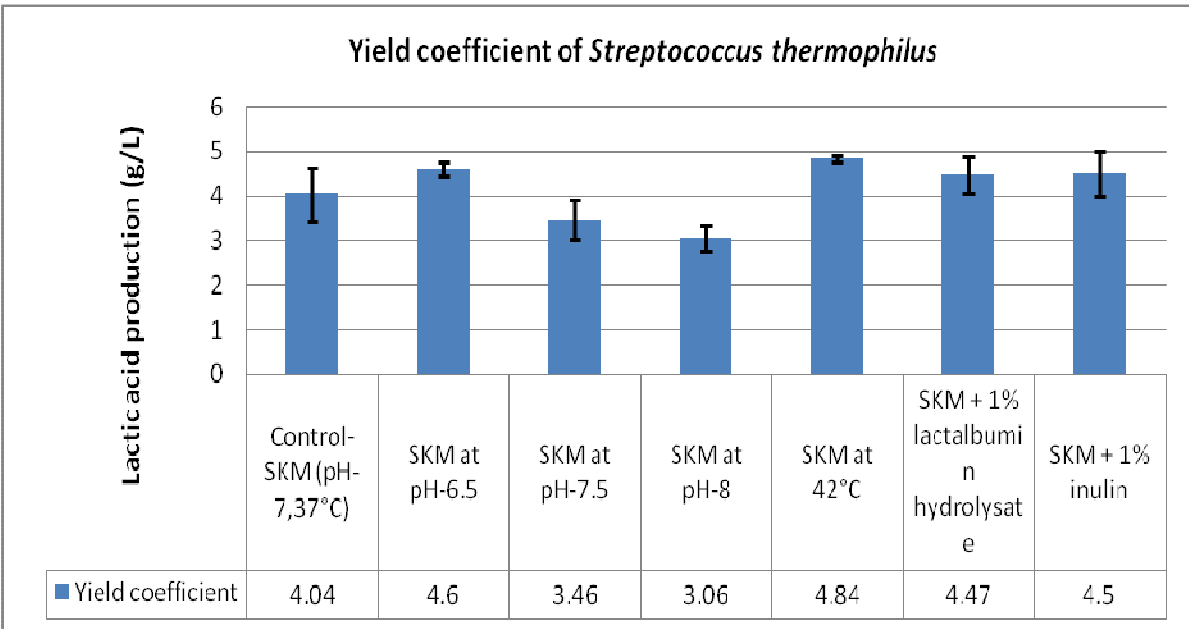


Figure 1.5: Yield coefficient of *S. thermophilus*. SKM with initial pH 6.5, 7.5, 8 were maintained at 37°C). SKM supplemented with 1% lactalbumin hydrolysate and 1% inulin were maintained at 37°C and initial pH 7. SKM with incubation temperature at 42°C was maintained with initial pH 7

3.3 Anti-oxidant property of *S. thermophilus*

Ability to scavenge free radicals and reducing ability against oxidative effects of reactive oxygen species are certain important mechanisms for antioxidant activity. The CFF of *S. thermophilus* culture was tested for ability to scavenge free radicals by hydrogen donating ability under proposed experimental conditions. At 42°C, the anti-oxidant activity was found to be significantly increased with free radical scavenging activity of 93.5 %. However initial pH of 6.5 resulted in the maximum anti-oxidant activity of 94.7 % ($P \leq 0.01$) and the corresponding anti-oxidant concentration was 86 µg/µl as ascorbic acid equivalent. Increase in initial pH resulted in decreased anti-oxidant potential as shown in Table 2. Inulin and lactalbumin hydrolysate in the medium resulted in equal and slightly higher anti-oxidant activity of 89.2% ($P > 0.05$) and 89.9% ($P \leq 0.05$) respectively compared to control with scavenging activity of 89.1%. Amaretti A et al., [27] also proved that *S. thermophilus* possessed very high anti-oxidant potential. Sulphonation of polysaccharides produced by the lactic acid bacteria enhances anti-oxidant potential of the bacteria as sulphated exopolysaccharides produced by *S. thermophilus* showed better free radical scavenging activity compared to control exopolysaccharides and the free radical scavenging activity of *S. thermophilus* was concentration dependent [28].

Table 2: Free radical scavenging activity of *S. thermophilus* cell free fraction

Experimental groups	% free radical scavenging activity	Concentration of ascorbic acid equivalent in the sample (µg/µl)
Control- SKM(pH-7, 37°C)	89.1 ± 0.2	78
SKM at pH- 6.5 (37°C)	94.7 ± 0.6	86
SKM at pH-7.5 (37°C)	88.3 ± 0.4	76
SKM at pH-8 (37°C)	81.6 ± 1.0	68
SKM at 42°C(pH -7)	93.5 ± 0.2	85
SKM + 1% lactalbumin hydrolysate (37°C, pH-7)	89.9 ± 0.9	80
SKM + 1% inulin (37°C, pH -7)	89.2 ± 0.5	78

3.4 Anti-adhesive property of *S. thermophilus*

Biosurfactants released by *S. thermophilus* are glycolipids which tend to modify the surface features of gut resulting in anti-adhesion of several bacterial and fungal species [15]. The anti-adhesive property of CFF was tested against *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans* (Figure 2). The biosurfactants released by *S. thermophilus* during their late exponential-early stationary phase is responsible for anti-adhesion against several bacterial and fungal species. Maximum anti-adhesion was observed against *Candida albicans* compared to other two species which favors the results obtained by Henk *et al.*, [14]. The anti-adhesive ability of *S. thermophilus* was drastically increased at 42°C ($P \leq 0.01$) with activity of 26.1 %, 33.9 % and 56.5 % compared to control with activity of 16.2 %, 10.7 %, 18.5 % against *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans* respectively. Initial pH of 6.5 enhanced the activity while lactalbumin hydrolysate and inulin supplementation in the medium also increased the anti-adhesive activity of *S. thermophilus* with greater efficiency shown against *Candida albicans*. In addition to their anti-adhesive activity in the gut, pulmonary biosurfactants released by *S. thermophilus* play a major role against inflammatory lung diseases and other lung infections by inhibiting the bacterial colonization in the target sites [15].

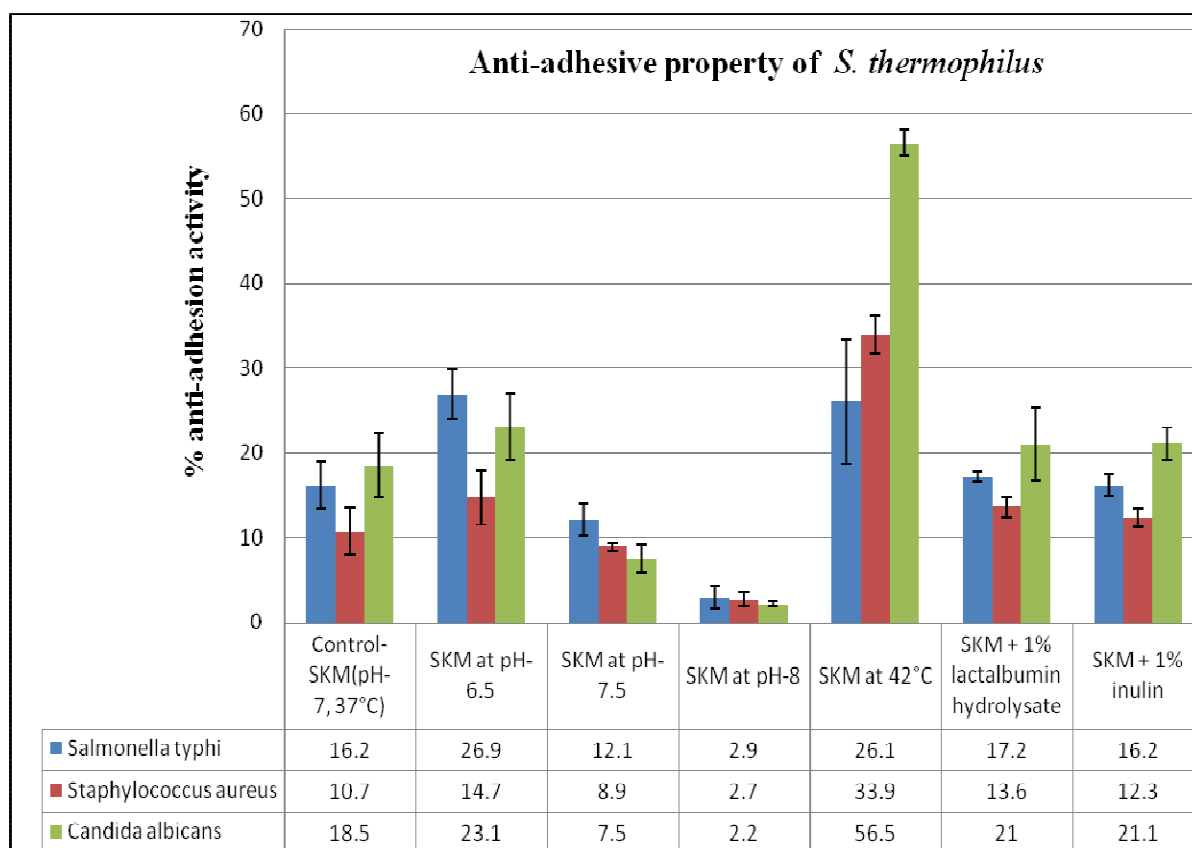


Figure 2: Anti-adhesive property of *S. thermophilus* CFF under different experimental conditions. SKM with initial pH 6.5, 7.5, 8 were maintained at 37°C. SKM supplemented with 1% lactalbumin hydrolysate and 1% inulin were maintained at 37°C and initial pH 7. SKM with incubation temperature at 42°C was maintained with initial pH 7

3.5 Anti-pathogenic property of *S. thermophilus*

The anti-pathogenic activity of *S. thermophilus* is exerted by secretion of bacteriocins or anti-microbial peptides which inhibit the growth of harmful bacteria [9,29]. Anti-pathogenic effect of *S. thermophilus* against *E. coli*, *Salmonella* and *Pseudomonas* spp has been proved [30]. The CFF of *S. thermophilus* culture was tested for anti-pathogenic activity against *Salmonella typhi*. Qualitative analysis using agar well diffusion method proved that *S. thermophilus* was able to produce the zone of inhibition against the pathogen as shown in Figure 3. However quantitative analysis of % anti-pathogenic activity revealed that experimental conditions proposed involved in the study had no much special influence on the anti-pathogenic activity resulting in same level or slightly higher level activity compared to control of 97.5 % ($P>.05$) except that the activity progressed in slightly decreasing fashion with increasing initial pH (Table 3). The bacteriocins lead to loss of cell viability through permeabilization of cell membranes of the organisms [31]. The peptides released as a result of enzymatic fractionation of milk proteins by *S. thermophilus* tend to exhibit many activities of which anti-microbial activity is of utmost significance as probiotics in human gut and in preservation against spoilage bacteria [32,33]. Anti-microbial activity of peptides is rapid at low pH when there is excess

amount of lactic acid produced as a result of fermentation and anti-microbial efficacy is retained for a longer time at low pH conditions [32,33].



Figure 3: Qualitative agar well diffusion method

Table 3: Anti-pathogenic activity of *S. thermophilus* CFF under different experimental conditions as % growth inhibition against *Salmonella typhi*

Experimental groups	% growth inhibition
Control- SKM(pH-7, 37°C)	97.5 ± 0.3
SKM at pH- 6.5 (37°C)	97.7 ± 0.4
SKM at pH-7.5 (37°C)	97.3 ± 0.3
SKM at pH-8 (37°C)	96.2 ± 0.2
SKM at 42°C(pH -7)	97.6 ± 0.3
SKM + 1% lactalbumin hydrolysate (37°C, pH-7)	97.9 ± 0.5
SKM + 1% inulin (37°C, pH -7)	97.7 ± 0.5

3.6 Antibiotic and drug resistance of *S. thermophilus*

The usage of antibiotics leads to the development of antibiotic resistant strains. *S. thermophilus* was tested for the ability to resist antibiotics and drugs which are commonly used for gastrointestinal problems. The study included drugs like ampicillin, amoxicillin, amphotericin, ciprofloxacin and omeprazole. The analysis was done using agar well diffusion method. *S. thermophilus* was resistant to amphotericin and omeprazole while the same was susceptible to other antibiotics (Figure 4). The resistance ability to commonly used antibiotics suggests that the bacteria are able to thrive in the antibiotic environment resulting in continuous evolution of strains with resistance potential against the exposed antibiotics. The antibiotic resistance ability of *S. thermophilus* is mostly plasmid mediated while no correlation between antibiotic resistance pattern and plasmid occurrence has been proved in some strains of *S. thermophilus* [34]. The resistance to amphotericin which is a commonly used antifungal agent and the enhanced anti-adhesive potential against *Candida albicans* can be combinatorially exploited for increased benefits in humans. However experimental conditions either slightly decreased the sensitivity of *S. thermophilus* or had equal effect against the antibiotics compared to control (Table 4). However, large scale production of probiotics for therapeutic purposes should not involve designing the probiotics to possess resistance than is needed for the intended purpose [34].

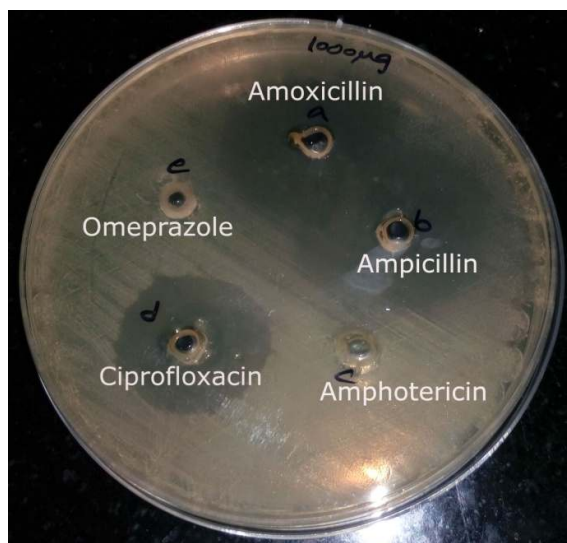


Figure 4: Zone of inhibition observed against 5 different antibiotics / drug by *S. thermophilus* using agar well diffusion method. The antibiotics used were amoxicillin, ampicillin, amphotericin, ciprofloxacin. The drug used was omeprazole.

Table 4: Antibiotic resistance of *S. thermophilus* – zone of inhibition measured in cms. SKM with initial pH 6.5, 7.5, 8 were maintained at 37°C. SKM supplemented with 1% lactalbumin hydrolysate and 1% inulin were maintained at 37°C and initial pH 7. SKM with incubation temperature at 42°C was maintained with initial pH 7. The strain was resistant to amphotericin and omeprazole

Experimental groups	Amoxicillin				Ampicillin				Ciprofloxacin			
	125µg	250µg	500µg	1000µg	125µg	250µg	500µg	1000µg	125µg	250µg	500µg	1000µg
Control- SKM(pH-7, 37°C)	2.3	2.5	2.7	2.9	2.5	2.6	2.9	3	1.7	2	2	2.4
SKM at pH- 6.5	2.3	2.4	2.7	2.8	2.4	2.6	2.8	3	1.8	2.1	2.2	2.4
SKM at pH-7.5	2.2	2.4	2.5	2.7	2.3	2.5	2.7	2.8	1.4	1.6	1.7	2
SKM at pH-8	2.1	2.3	2.5	2.7	2.3	2.4	2.6	2.7	1.3	1.6	1.8	2.1
SKM at 42°C	2.2	2.3	2.6	2.8	2.3	2.4	2.5	2.8	1.4	1.6	1.9	2.2
SKM + 1% lactalbumin hydrolysate	2.1	2.3	2.5	2.8	2.4	2.5	2.7	2.8	1.5	1.7	1.9	2.2
SKM + 1% inulin	2.2	2.4	2.6	2.8	2.4	2.6	2.7	2.9	1.4	1.8	2	2.3

3.7 Acidic pH tolerance of *S. thermophilus*

The ability of bacteria to survive and grow at low pH conditions is an important criterion to be considered as effective probiotics which exert therapeutic effects in gut when taken as supplements and acidic pH tolerance of *S. thermophilus* has been proved in various *in vitro* [21] and *in vivo* studies [6,7] which proved the viability of *S. thermophilus* in human feces from subjects consuming yogurt samples and the data obtained in this study also supported those results. Initial pH of 6.5 and temperature of 42°C significantly increased the acidic pH tolerance potential of *S. thermophilus*. Supplementation of medium with inulin and lactalbumin hydrolysate also significantly enhanced the tolerance ability. The ability of *S. thermophilus* to survive at low pH of 2 and 4 suggests that the bacterium has a huge potential as probiotics in human gut when consumed as supplements (Table 5). Microencapsulation of bacteria using alginate beads increased the survival of *S. thermophilus* in gut conditions effectively [35] while xanthan gum and carrageenan gum [36] are also effective encapsulating materials. Hence the use of encapsulation techniques to protect the bacteria during transit in human gut is a promising approach.

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Table 5: CFU/ml enumerated in media set at pH 2 and 4

Experimental groups	CFU / ml	
	pH-2	pH-4
Control- SKM(pH-7, 37°C)	3.22×10^9	1.87×10^{10}
SKM at pH- 6.5 (37°C)	3.35×10^9	1.93×10^{10}
SKM at pH-7.5 (37°C)	2.78×10^9	1.67×10^{10}
SKM at pH-8 (37°C)	9.3×10^8	1.51×10^{10}
SKM at 42°C(pH -7)	3.91×10^9	1.91×10^{10}
SKM + 1% lactalbumin hydrolysate (37°C, pH-7)	3.76×10^9	1.92×10^{10}
SKM + 1% inulin (37°C, pH -7)	3.47×10^9	1.89×10^{10}

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4. CONCLUSION

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The results obtained in the study proved that culture conditions pH-6.5 and 42°C and supplementation of additional nutrients like 1% inulin and 1% lactalbumin hydrolysate **in the skimmed milk medium** enhance the bioactivity of *S. thermophilus* like specific growth rate, yield coefficient, probiotic traits which can be exploited in the production of probiotic supplements and other applications in food and pharmaceutical industries owing to its novel industrial importance. These results may be scaled up for industrial scale production of *S. thermophilus* with increased bioactivity resulting in an efficient strain production.

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CONSENT

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Not applicable

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ETHICAL APPROVAL

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Not applicable

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