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Comparative evaluation of growth kinetics, acidification and technological characteristics of *Lactiplantibacillus plantarum* NCDC25, *Lacticaseibacillus rhamnosus* NCDC24 and *Streptococcus thermophilus* NCDC293 during soymilk fermentation

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Abstract
The present study was undertaken to evaluate the growth kinetics, acidification behaviour and technological characteristics of selected cultures in soymilk fermentation with a view to identifying suitable cultures for fermented soy yoghurt manufacture. Three cultures, namely *Streptococcus thermophilus* NCDC293, *Lactiplantibacillus plantarum* NCDC25 and *Lacticaseibacillus rhamnosus* NCDC24, were evaluated under standardized fermentation conditions (37 ± 1°C for 12 h). Growth performance was assessed through viable cell counts, specific growth rate and generation time, while acidification behaviour was monitored by changes in pH and titratable acidity. Technological suitability was further evaluated based on acetaldehyde and exopolysaccharide (EPS) production.
All cultures exhibited active growth in soymilk, achieving final populations above 8.8 log CFU/mL. *S. thermophilus* NCDC293 showed the highest viable count (9.28 log CFU/mL), specific growth rate (0.312 h^{-1}) and the shortest generation time (2.22 h). The culture also demonstrated superior acidification, reducing the pH from 6.80 to 4.45 and increasing titratable acidity to 0.82% lactic acid after 12 h of fermentation. Furthermore, *S. thermophilus* NCDC293 produced significantly higher levels of acetaldehyde (42.50 ppm) and EPS (98.60 mg/L) compared with the probiotic cultures. *L. plantarum* NCDC25 exhibited intermediate performance, whereas *L. rhamnosus* NCDC24 showed comparatively lower but acceptable technological characteristics. Comparative evaluation using normalized technological performance scores confirmed the superior overall performance of *S. thermophilus* NCDC293.
The results indicate that *S. thermophilus* NCDC293 is the most suitable starter culture for fermented soy yoghurt manufacture, while *L. plantarum* NCDC25 and *L. rhamnosus* NCDC24 may be employed as probiotic adjunct cultures to enhance the functional value of fermented soy products.

Keywords: Soymilk fermentation, soy yoghurt, probiotic cultures, *streptococcus thermophiles*, growth kinetics, acidification, exopolysaccharides

Introduction
The increasing consumer demand for plant-based foods has stimulated significant interest in fermented soy products as nutritious and sustainable alternatives to conventional dairy products. Soybeans are rich sources of high-quality protein, essential amino acids, polyunsaturated fatty acids, dietary fibre, vitamins and bioactive phytochemicals, making them an excellent raw material for the development of functional foods (Messina, 2016; Friedman and Brandon, 2001) [11, 21]. Fermentation further enhances the nutritional and sensory quality of soy products by improving digestibility, reducing anti-nutritional factors and generating desirable flavour compounds (Marco *et al*., 2021) [19].
Fermented soy foods have been consumed for centuries in Asian countries and include products such as tempeh, miso, natto, soy yoghurt and fermented soymilk beverages. These products are gaining increasing popularity worldwide because of their health-promoting properties and suitability for lactose-intolerant, vegan and health-conscious consumers (Granato *et al*., 2010; Ashaolu, 2020) [4, 12].

During fermentation, microbial activity modifies the composition of soymilk through the production of organic acids, enzymes and bioactive metabolites, resulting in improved sensory acceptability and enhanced nutritional value (Champagne *et al*., 2018) [7].

One of the major challenges associated with soymilk-based products is the presence of a characteristic beany flavour, which often limits consumer acceptance. Fermentation has been recognized as an effective strategy for reducing undesirable flavour compounds while simultaneously generating pleasant aromatic compounds such as acetaldehyde, diacetyl and acetoin (Donkor *et al*., 2007) [9]. Furthermore, fermentation contributes to protein coagulation, texture development and increased product stability, thereby facilitating the production of yoghurt-like soy products with desirable rheological characteristics (Bedani *et al*., 2013) [5].

Recent advances in functional food research have highlighted the importance of fermented soy products as carriers of probiotic microorganisms. The combination of soy-derived bioactive compounds and probiotic cultures can provide synergistic health benefits, including modulation of intestinal microbiota, improvement of digestive health, reduction of serum cholesterol and enhancement of immune function (Marco *et al*., 2021; Nagpal *et al*., 2012) [19, 22]. Consequently, the development of fermented soy products containing technologically efficient and probiotic cultures has become an important area of research in food biotechnology and functional food development.

1.2 Soymilk as a carrier for probiotic cultures

Soymilk has emerged as an attractive non-dairy substrate for the delivery of probiotic microorganisms owing to its favourable nutritional composition and widespread consumer acceptance. It contains high-quality proteins, essential amino acids, unsaturated fatty acids, oligosaccharides, minerals and bioactive compounds that can support the growth and metabolic activity of probiotic cultures during fermentation (Champagne *et al*., 2018; Granato *et al*., 2010) [7, 12]. In addition, the absence of lactose makes soymilk particularly suitable for individuals with lactose intolerance, milk protein allergy and those following vegan diets.

Compared with dairy milk, soymilk provides a unique environment for microbial growth due to its distinct carbohydrate and protein composition. Several lactic acid bacteria and probiotic strains have demonstrated the ability to utilize soybean-derived nutrients efficiently and produce desirable metabolites during fermentation (Donkor *et al*., 2007) [9]. The fermentation process enhances nutrient bioavailability, improves protein digestibility and contributes to the degradation of anti-nutritional factors such as trypsin inhibitors and oligosaccharides responsible for gastrointestinal discomfort (Friedman & Brandon, 2001) [11].

The suitability of soymilk as a probiotic carrier depends largely on its ability to support microbial growth and maintain high viable cell counts throughout fermentation and storage. Previous studies have reported that selected strains of Lactobacillus, Lacticaseibacillus, Lactiplantibacillus, Bifidobacterium and *Streptococcus thermophilus* can achieve cell populations exceeding 10^7-10^8 CFU/mL in fermented soymilk, which is generally considered adequate for delivering probiotic health benefits (Bedani *et al*., 2013; Nagpal *et al*., 2012) [5, 22]. The growth of these microorganisms results in the production of organic acids, flavour compounds and exopolysaccharides, which improve the sensory quality and functional attributes of fermented soy products.

Another important advantage of soymilk is the presence of soybean oligosaccharides and phytochemicals that may exert prebiotic effects and support the survival of probiotic microorganisms. Fermentation also promotes the conversion of isoflavone glycosides into their biologically active aglycone forms, thereby enhancing antioxidant activity and potential health benefits (Messina, 2016) [21]. Consequently, fermented soymilk products have attracted considerable attention as functional foods capable of combining the nutritional benefits of soybeans with the therapeutic potential of probiotic cultures.

Despite these advantages, substantial differences exist among probiotic cultures in their ability to grow, acidify and produce metabolites in soymilk. Therefore, evaluating the fermentation performance of selected cultures under standardized soymilk conditions is essential for identifying strains suitable for the development of high-quality fermented soy yoghurt products.

1.3 Growth and survival of probiotic cultures in plant-based substrates

The successful development of probiotic foods depends largely on the ability of probiotic microorganisms to grow, survive and remain metabolically active in the selected food matrix. Traditionally, dairy products have been used as carriers for probiotic cultures because of their balanced nutrient composition and favourable physicochemical properties. However, increasing consumer demand for plant-based foods has encouraged the exploration of non-dairy substrates as alternative vehicles for probiotic delivery (Marco *et al*., 2021) [19].

Plant-based substrates differ considerably from dairy systems in terms of carbohydrate composition, protein structure, buffering capacity and availability of growth-promoting factors. These differences can significantly influence the growth kinetics, metabolic activity and survival of probiotic microorganisms during fermentation and storage (Champagne *et al*., 2018) [7]. Consequently, the selection of probiotic cultures capable of efficiently utilizing plant-derived nutrients has become an important consideration in the development of fermented plant-based products.

Among plant-based substrates, soymilk has received considerable attention because of its high protein content and favourable nutritional profile. Nevertheless, the absence of lactose and the presence of anti-nutritional compounds may affect the growth behaviour of certain probiotic strains. Previous studies have demonstrated that probiotic bacteria exhibit strain-dependent responses when cultivated in plant-based matrices, resulting in substantial differences in growth rate, acidification capacity and final viable cell populations (Bedani *et al*., 2013; Donkor *et al*., 2007) [5, 9]. Therefore, the performance of probiotic cultures must be evaluated individually before their application in fermented soy products.

The maintenance of adequate viable cell counts is essential for achieving the desired health benefits associated with probiotic consumption. International recommendations generally suggest that probiotic foods should contain viable populations of at least 10^6-10^7 CFU/g or mL at the time of

consumption to exert beneficial physiological effects (Nagpal *et al*., 2012) [22]. The ability of probiotic cultures to attain and maintain such populations depends on their adaptability to the fermentation environment, tolerance to acidic conditions and capacity to utilize available nutrients efficiently.

In addition to survival, the metabolic activity of probiotic cultures during fermentation is equally important. Active growth results in the production of organic acids, flavour compounds and bioactive metabolites that contribute to product quality and functionality. Growth kinetics parameters such as specific growth rate and generation time provide valuable information regarding microbial adaptation and fermentation efficiency in plant-based substrates (Holzapfel & Schillinger, 2002) [14]. Cultures exhibiting rapid growth and high viability are generally preferred for industrial fermentation processes because they facilitate efficient acidification and improve product consistency.

Therefore, understanding the growth and survival characteristics of probiotic cultures in soymilk is essential for selecting strains capable of producing fermented soy products with desirable technological, sensory and functional attributes. Comparative evaluation of microbial growth performance can provide valuable insights into the suitability of different cultures for fermented soy yoghurt manufacture.

1.4 Importance of acidification and flavour development

Acidification is one of the most important biochemical processes occurring during the fermentation of soymilk and plays a critical role in determining the quality, safety and acceptability of fermented soy products. During fermentation, lactic acid bacteria metabolize
available carbohydrates and produce organic acids, primarily lactic acid, resulting in a progressive decrease in pH and a corresponding increase in titratable acidity (Tamime & Robinson, 2007) [28]. The acidification process contributes to microbial stability by inhibiting the growth of spoilage and pathogenic microorganisms while simultaneously promoting the coagulation of soy proteins necessary for the formation of yoghurt-like products.

The rate and extent of acidification are important technological characteristics of starter and probiotic cultures. Rapid acid production shortens fermentation time, improves manufacturing efficiency and ensures the development of the desired texture and consistency in fermented products (Donkor *et al*., 2007) [9]. Cultures exhibiting efficient acidification can achieve the target pH required for protein gel formation and product stabilization, thereby improving the overall quality of fermented soy yoghurt. Differences among microbial cultures in acidification performance are often associated with variations in carbohydrate utilization, metabolic activity and growth kinetics.

In addition to acid production, flavour development is a major determinant of consumer acceptance of fermented soy products. Soymilk possesses a characteristic beany flavour arising from lipid oxidation products and volatile compounds generated during soybean processing. This flavour is often perceived as undesirable and can limit consumer acceptance of soy-based foods (Friedman & Brandon, 2001) [11]. Fermentation has been widely recognized as an effective approach for improving flavour quality through the production of desirable volatile compounds and the reduction of off-flavours.

Among the flavour compounds generated during fermentation, acetaldehyde is considered one of the most important contributors to the characteristic aroma of yoghurt and fermented milk products. Acetaldehyde imparts a fresh, pleasant and mildly fruity flavour that enhances sensory acceptability and helps mask the beany notes of soymilk (Donkor *et al*., 2007) [9]. Other metabolites such as diacetyl, acetoin and organic acids also contribute to the overall flavour profile of fermented soy products. The ability of microbial cultures to produce these compounds varies considerably among strains and directly influences the sensory characteristics of the final product.

Therefore, evaluation of acidification behaviour and flavour-related metabolite production is essential for identifying cultures suitable for fermented soy yoghurt manufacture. Cultures capable of rapid acidification and efficient flavour development are particularly desirable because they contribute simultaneously to product safety, texture formation and sensory quality. Consequently, assessment of pH reduction, titratable acidity development and acetaldehyde production provides valuable information regarding the technological suitability of probiotic cultures for soymilk fermentation.

1.5 Role of EPS in fermented soy products

Exopolysaccharides (EPS) are high-molecular-weight polysaccharides synthesized and secreted by certain lactic acid bacteria during growth and fermentation. These biopolymers have attracted considerable attention in the food industry because of their ability to improve the texture, rheological properties and stability of fermented products without the need for additional stabilizers or thickeners (Ruas-Madiedo & de los Reyes-Gavilán, 2005) [26]. In fermented soy products, EPS production is particularly important because plant-based substrates generally lack the casein network present in dairy systems, making texture development more challenging.

The synthesis of EPS during fermentation contributes significantly to the viscosity and mouthfeel of fermented soy products. EPS molecules interact with proteins and water, resulting in improved water-holding capacity, reduced syneresis and enhanced gel stability (Patel & Prajapati, 2013) [24]. These properties are especially desirable in soy yoghurt manufacture, where achieving a smooth and creamy texture comparable to conventional dairy yoghurt remains a major technological objective.

In addition to their technological benefits, exopolysaccharides may contribute to the functional properties of fermented foods. Several studies have reported that microbial EPS can exhibit biological activities such as antioxidant, immunomodulatory and prebiotic effects, thereby enhancing the health-promoting potential of fermented products (Welman & Maddox, 2003) [29]. Furthermore, EPS may improve the survival of probiotic cultures by providing protection against environmental stresses encountered during processing and storage.

The ability to produce EPS is highly strain-dependent, and considerable variation exists among lactic acid bacteria with respect to both the quantity and composition of the polysaccharides produced (Ruas-Madiedo & de los Reyes-Gavilán, 2005) [26]. Consequently, EPS production has become an important criterion in the selection of starter and

probiotic cultures for fermented food applications. Cultures capable of producing higher amounts of EPS are generally preferred because they contribute to improved texture, product stability and consumer acceptability.

In fermented soymilk systems, EPS-producing cultures can partially compensate for the absence of dairy proteins and enhance the structural characteristics of the final product. Therefore, the evaluation of EPS production provides valuable information regarding the technological suitability of probiotic cultures for fermented soy yoghurt manufacture. Cultures exhibiting superior EPS synthesis may facilitate the development of fermented soy products with improved consistency, reduced syneresis and enhanced sensory quality.

1.6 Research gap

Although considerable research has been conducted on fermented soy products and probiotic soymilk beverages, most studies have primarily focused on product development, sensory evaluation and the survival of probiotic cultures during storage. Comparatively fewer investigations have examined the fermentation performance of individual probiotic and starter cultures in soymilk under standardized conditions. The growth behaviour, acidification capacity and metabolite production of microbial cultures are highly strain-dependent and may vary substantially even among closely related species (Donkor *et al*., 2007; Bedani *et al*., 2013) [5, 9].

Previous studies have demonstrated the potential of various lactic acid bacteria and probiotic strains for soymilk fermentation; however, direct comparative information on growth kinetics, generation time, acidification characteristics, acetaldehyde production and exopolysaccharide synthesis under identical fermentation conditions remains limited. Such information is essential for selecting cultures capable of producing fermented soy products with desirable technological and sensory attributes. Furthermore, most published studies have focused on commercial probiotic cultures, whereas comparatively little information is available regarding the performance of cultures maintained in the National Collection of Dairy Cultures (NCDC), which are widely used in dairy and fermented food research in India.

The successful manufacture of fermented soy yoghurt depends on the selection of cultures that can efficiently adapt to the soymilk environment, achieve high viable cell populations, rapidly acidify the substrate and produce metabolites contributing to flavour and texture development. However, comprehensive comparative evaluations integrating microbial growth characteristics, acidification behaviour and metabolite production of selected NCDC cultures in soymilk are scarce in the literature. In particular, information regarding the fermentation performance of *Streptococcus thermophilus* NCDC293, *Lactiplantibacillus plantarum* NCDC25 and *Lacticaseibacillus rhamnosus* NCDC24 in standardized soymilk systems is limited.

Therefore, there is a need for a systematic investigation of the growth kinetics, acidification profiles, acetaldehyde production and exopolysaccharide synthesis of selected cultures during soymilk fermentation. Such information would facilitate the identification of cultures possessing superior technological characteristics and provide a scientific basis for the development of high-quality fermented soy yoghurt products.

1.7 Objective

The present investigation was undertaken to evaluate the fermentation performance of selected probiotic and starter cultures in soymilk with a view to identifying cultures suitable for fermented soy yoghurt manufacture. The specific objectives of the study were:

1. To investigate the growth characteristics of *Streptococcus thermophilus* NCDC293, *Lactiplantibacillus plantarum* NCDC25 and *Lacticaseibacillus rhamnosus* NCDC24 during soymilk fermentation. 2. To determine the growth kinetics of the selected cultures in terms of viable cell count, specific growth rate and generation time. 3. To evaluate the acidification behaviour of the cultures by monitoring changes in pH and titratable acidity during fermentation. 4. To assess the production of acetaldehyde as an indicator of flavour development in fermented soymilk. 5. To quantify exopolysaccharide (EPS) production and evaluate its contribution to the technological suitability of the cultures. 6. To compare the overall technological performance of the selected cultures and identify the most suitable culture(s) for the manufacture of fermented soy yoghurt.

The study was designed to provide a comprehensive assessment of microbial growth, acidification efficiency and metabolite production under standardized soymilk fermentation conditions, thereby generating information useful for the development of high-quality fermented soy products.

2. Materials and Methods

2.1 Cultures

Three cultures exhibiting superior technological and probiotic characteristics in preliminary screening studies were selected for evaluation in soymilk fermentation. These included *Lactiplantibacillus plantarum* NCDC25, *Lacticaseibacillus rhamnosus* NCDC24 and *Streptococcus thermophilus* NCDC293. The cultures were obtained from the National Collection of Dairy Cultures (NCDC), ICAR-National Dairy Research Institute, Karnal, India. *Streptococcus thermophilus* NCDC293 was selected as a starter culture, whereas *Lactiplantibacillus plantarum* NCDC25 and *Lacticaseibacillus rhamnosus* NCDC24 were selected as probiotic cultures based on their superior technological and probiotic characteristics observed during preliminary screening studies. (Holzapfel & Schillinger, 2002; Tamime & Robinson, 2007) [14, 28].

The selected cultures represented distinct functional groups relevant to fermented soy products. *S. thermophilus* is a well-established starter culture known for its rapid growth and acidification ability, whereas *L. plantarum* and *L. rhamnosus* are recognized probiotic species capable of surviving under gastrointestinal conditions and producing beneficial metabolites (Nagpal *et al*., 2012; Marco *et al*., 2021) [19, 22]. The three cultures were selected on the basis of their superior performance in earlier screening experiments involving growth kinetics, acidification characteristics, exopolysaccharide production and probiotic functionality.

Stock cultures were maintained in sterile reconstituted skim milk and stored under refrigerated conditions (4 ± 1°C). Prior to use, the cultures were activated by two successive transfers in sterilized skim milk and incubated at 37°C for 18-24 h to obtain actively growing cells. The activated cultures were subsequently used as inocula for soymilk fermentation experiments. Fresh cultures containing approximately 10^8-10^9 CFU/mL were employed to ensure

uniform inoculation and reproducibility of fermentation performance.

The purity and viability of the cultures were verified periodically by microscopic examination and viable count determination before their use in experimental trials. All fermentation experiments were conducted using freshly activated cultures to minimize variability arising from differences in physiological state and microbial activity.

2.2 Preparation of Soymilk

Soymilk was prepared from soybean seeds of the variety Indira Soya-9 following the standardized procedure developed during preliminary optimization studies. The soybean seeds were cleaned to remove foreign matter, damaged seeds and other impurities. The cleaned seeds were soaked in potable water at room temperature (28 ± 2°C) for 12 h to facilitate hydration and softening. After soaking, the water was drained and the hydrated seeds were thoroughly washed with fresh water.

The soaked soybeans were ground with water using a soybean-to-water ratio of 1:8 (w/v), which had previously been identified as the optimum ratio for obtaining maximum soymilk yield and desirable compositional characteristics. The resulting slurry was filtered through a double-layer muslin cloth to separate the insoluble residue (okara) from the liquid extract. The filtrate obtained was designated as raw soymilk (Liu, 1997; Nelson *et al*., 1976) [17, 23].

The raw soymilk was heated to boiling and maintained for 15 min with continuous stirring to inactivate lipoxygenase and other heat-labile anti-nutritional factors, reduce the characteristic beany flavour and improve microbiological safety. The heat-treated soymilk was then cooled to room temperature and standardized to contain 12-14% total solids and 3-4% sucrose. The standardized soymilk was subsequently pasteurized at 90°C for 10 min and cooled to the fermentation temperature (37 ± 1°C) before inoculation with the selected cultures.

The prepared soymilk served as the fermentation substrate for evaluating the growth characteristics, acidification behaviour, acetaldehyde production and exopolysaccharide synthesis of the selected cultures. All soymilk preparations were carried out under hygienic conditions to minimize contamination and ensure reproducibility of the experimental results.

2.3 Fermentation Conditions

Standardized soymilk prepared as described above was cooled to 37 ± 1°C and aseptically inoculated with 2% (v/v) of the respective activated culture. Separate fermentation trials were conducted for *Lactiplantibacillus plantarum* NCDC25, *Lacticaseibacillus rhamnosus* NCDC24 and *Streptococcus thermophilus* NCDC293. The inoculated soymilk was thoroughly mixed to ensure uniform distribution of microbial cells throughout the fermentation medium.

Fermentation was carried out at 37 ± 1°C for 12 h under static incubation conditions. Samples were withdrawn at intervals of 0, 3, 6, 9 and 12 h for the determination of viable cell counts, pH and titratable acidity. At the end of the fermentation period, the fermented soymilk samples were analyzed for growth kinetics, acetaldehyde production and exopolysaccharide (EPS) synthesis.

The fermentation temperature and inoculum level were selected based on previous studies demonstrating optimum growth and acidification of lactic acid bacteria in soymilk systems (Donkor *et al*., 2007; Champagne *et al*., 2018) [7, 9]. All fermentations were conducted in triplicate to ensure reproducibility of the results. Following fermentation, the samples were immediately cooled to 4 ± 1°C to arrest further microbial activity prior to analysis.

The fermentation performance of the selected cultures was evaluated by comparing microbial growth, acidification behaviour, flavour compound production and EPS synthesis under identical soymilk fermentation conditions.

2.4 Determination of Viable Counts

The growth of the selected cultures during soymilk fermentation was monitored by determining viable cell counts at 0, 3, 6, 9 and 12 h of incubation. Fermented soymilk samples were aseptically withdrawn at each sampling interval and subjected to serial decimal dilution using sterile 0.85% physiological saline solution.

Appropriate dilutions were pour-plated in duplicate using de Man, Rogosa and Sharpe (MRS) agar for *Lactiplantibacillus plantarum* NCDC25 and *Lacticaseibacillus rhamnosus* NCDC24, whereas *Streptococcus thermophilus* NCDC293 was enumerated using M17 agar. The inoculated plates were incubated at 37°C for 48 h under appropriate growth conditions. Colonies developing on plates containing 30-300 colonies were counted, and the results were expressed as colony-forming units per millilitre (CFU/mL) of fermented soymilk (Harrigan, 1998; APHA, 2001) [3, 13].

The viable counts obtained were converted to logarithmic values (log CFU/mL) prior to statistical analysis. Growth curves were constructed using the viable count data generated during fermentation. These data were subsequently used for the calculation of growth kinetic parameters, including specific growth rate (μ) and generation time (g), as described by Adams and Moss (2008) [1].

All microbiological analyses were performed in triplicate, and the mean values were used for subsequent calculations and statistical evaluation.

2.5 Growth Kinetic Analysis

The growth performance of the selected cultures in soymilk was evaluated using viable count data obtained during fermentation. Growth kinetic parameters, namely specific growth rate (μ) and generation time (g), were calculated from the exponential phase of microbial growth. These parameters provide quantitative information regarding the adaptation and proliferation of microorganisms in the fermentation medium and are widely used for assessing the suitability of starter and probiotic cultures for fermented food manufacture (Adams & Moss, 2008; Madigan *et al*., 2018) [1, 18].

The specific growth rate (μ) was calculated using the following equation:

$$\mu = (\ln N_2 - \ln N_1) / (t_2 - t_1)$$

Where:

- N_1 = viable cell count at time (t_1)
- N_2 = viable cell count at time (t_2)
- ($t2$ - $t1$) = time interval during exponential growth (h)

The generation time (g), defined as the time required for the microbial population to double, and was calculated using the equation:

$g = \ln 2 / \mu$

Where: g = generation time (h)
- μ = specific growth rate (h−1)
- ln 2 = natural logarithm of 2 (0.693)

Growth rate and generation time were calculated individually for each culture using viable count data obtained during fermentation. Higher values of specific growth rate and lower values of generation time were considered indicative of superior adaptation and growth performance in soymilk. These parameters were subsequently used to compare the fermentation efficiency of *Streptococcus thermophilus* NCDC293, *Lactiplantibacillus plantarum* NCDC25 and *Lacticaseibacillus rhamnosus* NCDC24.
All calculations were performed using mean viable count values obtained from three independent replications, and the resulting growth kinetic parameters were subjected to statistical analysis.

2.6 pH Measurement
The pH of the fermenting soymilk was determined at 0, 3, 6, 9 and 12 h of fermentation using a digital pH meter (Model Elico LI-120 or equivalent). Prior to measurement, the instrument was calibrated using standard buffer solutions of pH 4.0 and 7.0 according to the manufacturer's instructions. Approximately 25 mL of the sample was transferred into a clean beaker, and the pH electrode was immersed directly into the sample. The pH value was recorded after stabilization of the reading (AOAC, 2019) [2].
Monitoring pH changes during fermentation provides an indication of acid production and metabolic activity of the cultures. The reduction in pH results primarily from the formation of lactic acid and other organic acids during microbial fermentation and is considered an important indicator of fermentation efficiency and product quality (Tamime & Robinson, 2007) [28]. The pH data obtained at different fermentation intervals were used to compare the acidification behaviour of the selected cultures in soymilk.
All measurements were carried out in triplicate, and the mean values were used for statistical analysis.

2.7 Titratable Acidity
Titratable acidity of the fermenting soymilk was determined at 0, 3, 6, 9 and 12 h of fermentation following the standard AOAC method (AOAC, 2019) [2]. A known quantity (10 mL) of the sample was transferred to a conical flask and diluted with an equal volume of distilled water. The mixture was titrated against standardized 0.1 N sodium hydroxide (NaOH) solution using phenolphthalein as an indicator until the appearance of a faint pink colour that persisted for approximately 30 s.
Titratable acidity was expressed as percentage lactic acid (% LA) and calculated using the following equation:

$\text{Titratable Acidity (\% LA)} = (V \times N \times 0.09 \times 100) / W$

Where:
- (V) = volume of 0.1 N NaOH used for titration (mL)
- (N) = normality of NaOH
- (0.09) = equivalent weight factor of lactic acid (g meq^{-1})
- (W) = weight or volume of sample taken for analysis

Titratable acidity serves as an important indicator of acid production during fermentation and reflects the metabolic activity of lactic acid bacteria. The increase in acidity during fermentation is primarily attributed to the conversion of fermentable carbohydrates into lactic acid and other organic acids. Therefore, titratable acidity, together with pH, was used to evaluate the acidification performance of the selected cultures in soymilk (Tamime & Robinson, 2007) [28].
All determinations were carried out in triplicate, and the mean values were used for statistical analysis.

2.8 Acetaldehyde Estimation
Acetaldehyde production by the selected cultures was determined at the end of fermentation (12 h) using the colorimetric method described by Lees and Jago (1969) [15] with suitable modifications. Fermented soymilk samples were distilled, and the acetaldehyde present in the distillate was reacted with p-hydroxydiphenyl reagent under acidic conditions to develop a characteristic colour. The intensity of the colour produced was measured spectrophotometrically at 560 nm using a UV-Visible spectrophotometer.
A standard curve was prepared using known concentrations of acetaldehyde, and the concentration of acetaldehyde in the samples was calculated from the corresponding absorbance values. The results were expressed as parts per million (ppm) of acetaldehyde.
Acetaldehyde is recognized as one of the principal flavour compounds responsible for the characteristic aroma of yoghurt and fermented milk products. In fermented soy products, acetaldehyde contributes to flavour improvement by imparting a pleasant yoghurt-like aroma and reducing the perception of undesirable beany flavour. Therefore, acetaldehyde production was used as an important technological criterion for evaluating the suitability of cultures for fermented soy yoghurt manufacture (Tamime & Robinson, 2007; Donkor *et al*., 2007) [9, 28].
All analyses were performed in triplicate, and the mean values were used for statistical evaluation.

2.9 Exopolysaccharide Determination
Exopolysaccharide (EPS) production by the selected cultures was determined at the end of fermentation (12 h) following the method of Cerning (1990) [6] with slight modifications. Fermented soymilk samples were heated at 100°C for 15 min to inactivate microbial enzymes and then centrifuged at 10,000 × g for 15 min at 4°C to remove bacterial cells and insoluble materials. The supernatant obtained was collected and treated with an equal volume of chilled trichloroacetic acid (TCA, 20%, w/v) to precipitate proteins. The mixture was kept at 4°C for 12 h and subsequently centrifuged at 10,000 × g for 15 min.
The protein-free supernatant was mixed with three volumes of chilled absolute ethanol and incubated at 4°C overnight to precipitate exopolysaccharides. The precipitated EPS was recovered by centrifugation, dissolved in distilled water and quantified by the phenol-
sulphuric acid method using glucose as the standard (Dubois *et al*., 1956) [10]. Absorbance was measured at 490 nm using a UV-Visible spectrophotometer, and EPS concentration was calculated from a standard glucose calibration curve. The results were expressed as milligrams per litre (mg/L).

Exopolysaccharide production was considered an important technological attribute because EPS contributes to viscosity enhancement, improved texture, water-holding capacity and reduced syneresis in fermented products. Therefore, EPS synthesis by the selected cultures was evaluated as an indicator of their suitability for fermented soy yoghurt manufacture (Ruas-Madiedo & de los Reyes-Gavilán, 2005) [26].

All analyses were performed in triplicate, and the mean values were used for statistical evaluation.

2.10 Statistical Analysis

All experiments were conducted in triplicate using a Completely Randomized Design (CRD). The data obtained for viable counts, growth rate, generation time, pH, titratable acidity, acetaldehyde production and exopolysaccharide synthesis were expressed as mean ± standard deviation. Statistical analysis of the experimental data was performed using OPSTAT statistical software developed by Chaudhary Charan Singh Haryana Agricultural University, Hisar, India.

Analysis of variance (ANOVA) was carried out to determine the significance of differences among treatments. Time-course data (viable counts, pH and titratable acidity) were analyzed as factorial experiments considering culture and fermentation time as sources of variation. The significance of treatment effects was evaluated at the 5% probability level ($P \leq 0.05$). Standard Error of Mean (SEm±), Critical Difference (CD) and Coefficient of Variation (CV %) were calculated wherever applicable. When the calculated F-value was significant, treatment means were compared using the Critical Difference (CD) test at the 5% level of significance (Sheoran *et al*., 1998) [27].

The statistical analysis was used to assess differences among cultures with respect to growth characteristics, acidification behaviour, acetaldehyde production and exopolysaccharide synthesis during soymilk fermentation. Results were considered statistically significant when $P \leq 0.05$.

For graphical comparison of technological characteristics, the measured parameters were normalized to a 0-1 scale. Generation time was reverse-normalized because lower values indicate superior growth performance.

3. Results and Discussion

3.1 Growth Characteristics of Selected Cultures in Soymilk: The growth behaviour of *Streptococcus thermophilus* NCDC293, *Lactiplantibacillus plantarum* NCDC25 and *Lacticaseibacillus rhamnosus* NCDC24 in soymilk during 12 h of fermentation is presented in Table 1 and Figure 1. All three cultures exhibited active growth in soymilk, indicating that the substrate provided sufficient nutrients to support microbial proliferation. However, significant differences ($P \leq 0.05$) were observed among the cultures after 3 h of fermentation, as reflected by the CD values reported in Table 1.

At the beginning of fermentation, all cultures showed similar viable counts of approximately 6.08 log CFU/mL. During fermentation, microbial populations increased progressively, reaching final counts ranging from 8.83 to 9.28 log CFU/mL after 12 h. The highest viable count was recorded for *S. thermophilus* NCDC293 (9.28 log CFU/mL), followed by *L. plantarum* NCDC25 (9.00 log CFU/mL) and *L. rhamnosus* NCDC24 (8.83 log CFU/mL). The superior growth of *S. thermophilus* NCDC293 indicates its greater adaptation to soymilk and more efficient utilization of available nutrients.

The growth curves (Figure 1) revealed that all cultures entered an active exponential growth phase between 3 and 9 h of fermentation. *S. thermophilus* NCDC293 showed the steepest increase in viable count, rising from 6.93 log CFU/mL at 3 h to 8.90 log CFU/mL at 9 h. In comparison, *L. plantarum* NCDC25 and *L. rhamnosus* NCDC24 attained viable counts of 8.54 and 8.34 log CFU/mL, respectively, at the same fermentation period. The differences among cultures became increasingly pronounced as fermentation progressed, suggesting variations in metabolic efficiency and adaptation to the soybean matrix.

The final viable counts obtained in the present study exceeded the minimum recommended level of 10^6-10^7 CFU/mL generally required for probiotic and fermented foods, indicating that all cultures remained viable at technologically desirable levels. Similar observations have been reported by Nagpal *et al*. (2012) [22] and Bedani *et al*. (2013) [5], who observed substantial growth of starter cultures in soy yoghurt, and by Bedani *et al*. (2013) [5], who demonstrated successful proliferation of probiotic bacteria in fermented soy products.

The observed growth performance suggests that soymilk can serve as an effective substrate for both starter and probiotic cultures. Nevertheless, *S. thermophilus* NCDC293 exhibited the greatest growth potential, which may be attributed to its superior carbohydrate metabolism and rapid adaptation to fermentation conditions. The higher population achieved by this culture is advantageous for acid development, flavour formation and overall fermentation efficiency.

The statistical analysis further confirmed the reliability of the results. Coefficient of variation (CV) values ranged from 0.098 to 0.265%, indicating excellent experimental precision. Significant F-values observed from 3 h onwards demonstrated that culture type exerted a significant influence on microbial growth during fermentation.

Overall, the results indicate that all three cultures were capable of sustained growth in soymilk; however, *S. thermophilus* NCDC293 showed the most favourable growth characteristics, followed by *L. plantarum* NCDC25 and *L. rhamnosus* NCDC24. These findings support the potential use of NCDC293 as a primary starter culture for fermented soy yoghurt manufacture, while NCDC25 and NCDC24 may serve as suitable probiotic adjunct cultures.

Table 1. Viable counts (log CFU/mL) of selected cultures during soymilk fermentation

Culture	0 h	3 h	6 h	9 h	12 h
Streptococcus thermophilus NCDC293	6.08 ± 0.00	6.93 ± 0.01	8.00 ± 0.01	8.90 ± 0.01	9.28 ± 0.01
Lactiplantibacillus plantarum NCDC25	6.08 ± 0.00	6.80 ± 0.01	7.74 ± 0.02	8.54 ± 0.01	9.00 ± 0.01
Lacticaseibacillus rhamnosus NCDC24	6.08 ± 0.00	6.71 ± 0.01	7.56 ± 0.01	8.34 ± 0.01	8.83 ± 0.01
SEm±	0.003	0.010	0.012	0.011	0.011
CD ($P \leq 0.05$)	NS	0.035	0.042	0.038	0.038
CV (%)	0.098	0.249	0.265	0.216	0.205

Note: Values are expressed as mean ± standard deviation of three replications. Means within a column differing by more than the CD value are significantly different at $P \leq 0.05$. NS = Non-significant; SEm± = Standard Error of Mean; CD = Critical Difference; CV = Coefficient of Variation.

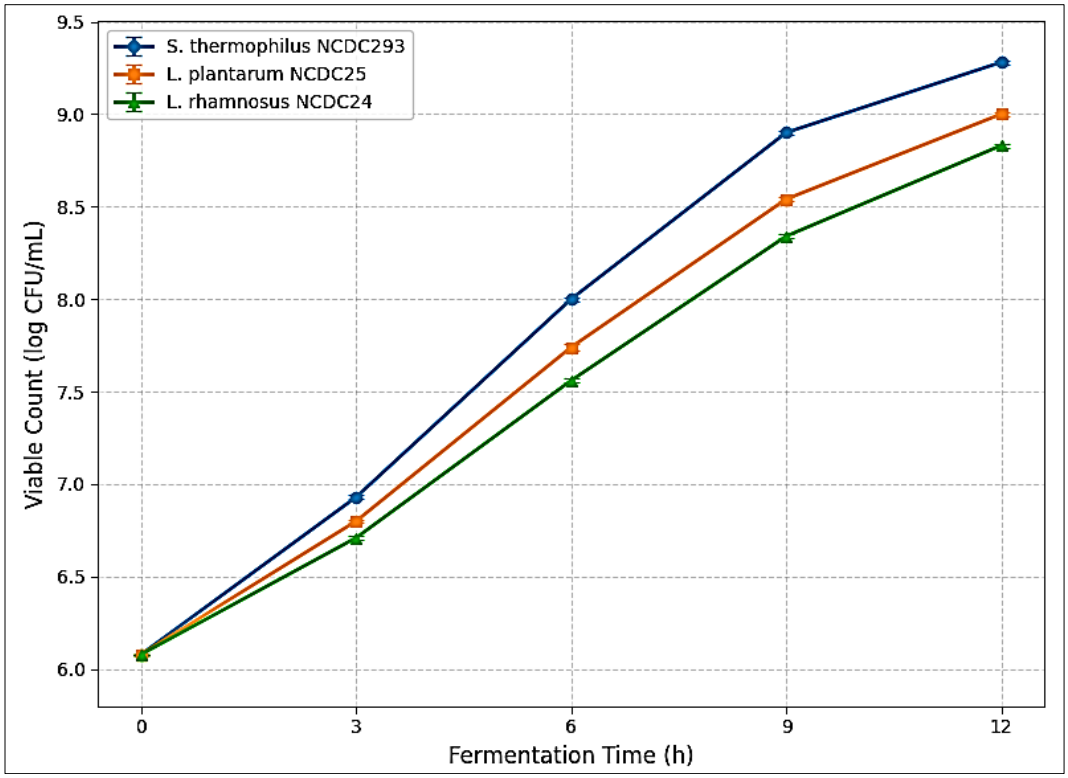


Fig 1: Growth curves of *Streptococcus thermophilus* NCDC293, *Lactiplantibacillus plantarum* NCDC25 and *Lacticaseibacillus rhamnosus* NCDC24 during soymilk fermentation. Values represent mean ± SD (n = 3)

3.2 Growth Kinetics

Growth kinetic parameters were calculated from viable count data obtained during the exponential growth phase (3-9 h).

The growth kinetic parameters of the selected cultures, namely *Streptococcus thermophilus* NCDC293, *Lactiplantibacillus plantarum* NCDC25 and *Lacticaseibacillus rhamnosus* NCDC24, are presented in Table 2 and Figure 2. Growth kinetics were evaluated in terms of specific growth rate (μ) and generation time (g), which provide quantitative measures of microbial adaptation and proliferation in soymilk.

Significant differences ($P \leq 0.05$) were observed among the cultures for both growth rate and generation time. The highest specific growth rate was recorded for *S. thermophilus* NCDC293 (0.312 h^{-1}), followed by *L. plantarum* NCDC25 (0.273 h^{-1}) and *L. rhamnosus* NCDC24 (0.248 h^{-1}). The differences among cultures exceeded the critical difference (CD = 0.004), indicating statistically significant variation in their growth performance. The corresponding coefficient of variation (CV = 0.721%) demonstrated high precision of the experimental observations.

Generation time showed an inverse relationship with growth rate. *S. thermophilus* NCDC293 exhibited the shortest generation time (2.22 h), indicating rapid cell division and efficient utilization of nutrients present in soymilk. In contrast, *L. plantarum* NCDC25 and *L. rhamnosus* NCDC24 required 2.54 and 2.79 h, respectively, to complete one generation cycle. The significantly lower generation time of NCDC293 confirms its superior growth efficiency and adaptation to the fermentation environment.

The growth kinetic profile observed in Figure 2 further supports the viable count data presented in Section 3.1. Cultures exhibiting higher specific growth rates attained higher final populations within the fermentation period. The rapid growth of *S. thermophilus* NCDC293 may be attributed to its strong fermentative metabolism and efficient utilization of available carbohydrates, leading to accelerated biomass production. Similar observations have been reported by Donkor *et al*. (2007) [9], who found that starter cultures with higher growth rates exhibited superior fermentation performance in soy yoghurt systems.

The relatively lower growth rate and longer generation time of *L. rhamnosus* NCDC24 suggest a slower adaptation to the soybean substrate. Nevertheless, the culture maintained satisfactory growth and achieved a final population exceeding 10^8 CFU/mL, indicating its suitability as a probiotic adjunct culture. Likewise, *L. plantarum* NCDC25 demonstrated intermediate kinetic characteristics, combining good growth performance with probiotic potential.

Growth kinetics are important technological indicators because faster-growing cultures generally produce acid more rapidly, shorten fermentation time and improve manufacturing efficiency. Therefore, the superior growth rate and lower generation time observed for *S. thermophilus* NCDC293 support its selection as the primary starter culture for soy yoghurt manufacture. The probiotic cultures NCDC25 and NCDC24, although slower growing, remained highly viable and may be used as adjunct cultures to enhance the functional value of fermented soy products.

Overall, the kinetic analysis clearly demonstrated the superior growth performance of *S. thermophilus* NCDC293, followed by *L. plantarum* NCDC25 and *L. rhamnosus* NCDC24. These findings are consistent with the viable count results and confirm the suitability of the selected cultures for fermented soy yoghurt production.

Table 2. Growth rate and generation time of selected cultures in soymilk

Culture	Growth Rate (μ h^{-1})	Generation Time (h)
Streptococcus thermophilus NCDC293	0.312 ± 0.001	2.22 ± 0.01
Lactiplantibacillus plantarum NCDC25	0.273 ± 0.001	2.54 ± 0.01
Lacticaseibacillus rhamnosus NCDC24	0.248 ± 0.001	2.79 ± 0.01
SEm±	0.001	0.006
CD (P≤0.05)	0.004	0.020
CV (%)	0.721	0.398

Note: Values are expressed as mean ± standard deviation of three replications. Means differing by more than the CD value are significantly different at P≤0.05. SEm± = Standard Error of Mean; CD = Critical Difference; CV = Coefficient of Variation.

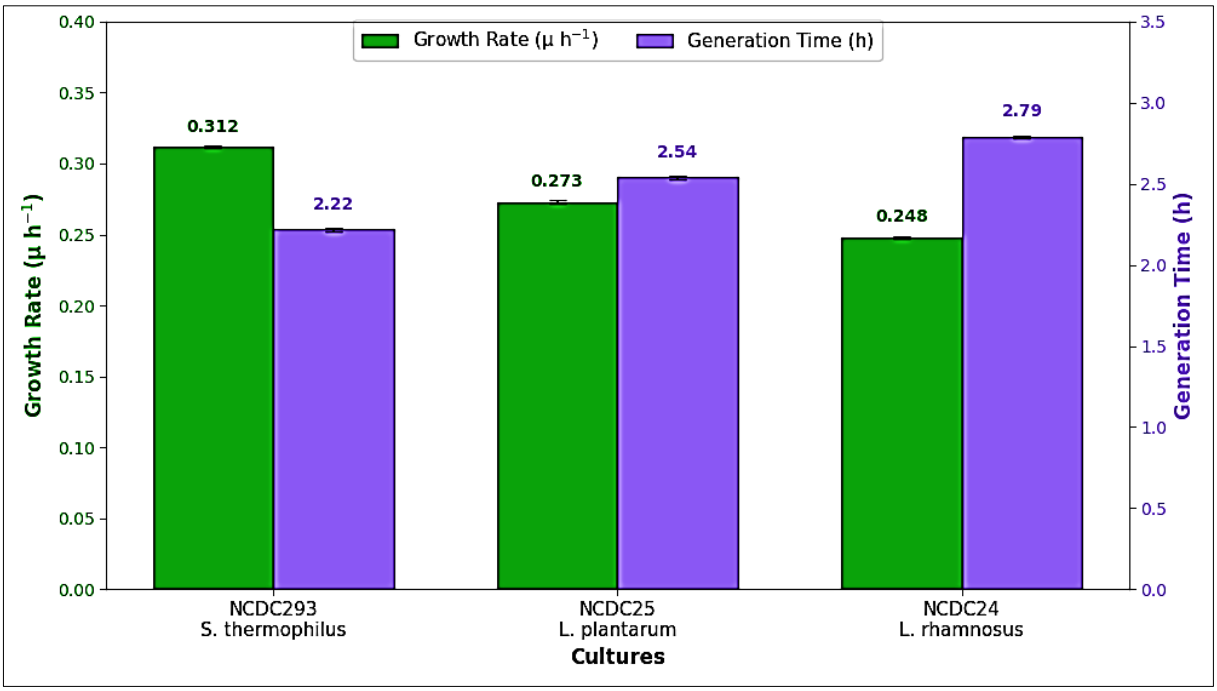


Fig 2: Growth rate and generation time of *Streptococcus thermophilus* NCDC293, *Lactiplantibacillus plantarum* NCDC25 and *Lacticaseibacillus rhamnosus* NCDC24 in soymilk

3.3 Acidification Behaviour

The acidification behaviour of *Streptococcus thermophilus* NCDC293, *Lactiplantibacillus plantarum* NCDC25 and *Lacticaseibacillus rhamnosus* NCDC24 during soymilk fermentation is presented in Table 3 and Figure 3. Acidification was evaluated through changes in pH and titratable acidity, two important indicators of fermentation efficiency and metabolic activity of lactic acid bacteria.

All cultures exhibited a progressive decrease in pH accompanied by a corresponding increase in titratable acidity throughout the fermentation period. The initial pH of soymilk was approximately 6.80 for all treatments. As fermentation progressed, the pH declined significantly (P≤0.05), while titratable acidity increased due to the production of organic acids, primarily lactic acid.

Among the cultures evaluated, *S. thermophilus* NCDC293 demonstrated the most rapid acidification. The pH decreased from 6.80 at the start of fermentation to 4.45 after 12 h, representing the greatest reduction among the tested cultures. Simultaneously, titratable acidity increased from 0.153 to 0.820% lactic acid. In comparison, *L. plantarum* NCDC25 achieved a final pH of 4.65 and titratable acidity of 0.720%, whereas *L. rhamnosus* NCDC24 showed the least acidification, reaching a final pH of 4.85 and acidity of 0.640%.

The pH profiles shown in Figure 3 clearly indicate that acid production accelerated after 3 h of fermentation, corresponding to the exponential growth phase of the cultures described earlier. The decline in pH was particularly pronounced between 3 and 9 h, suggesting active carbohydrate metabolism and organic acid synthesis during this period. The inverse relationship between pH and titratable acidity observed throughout fermentation confirms that acid accumulation was responsible for the reduction in pH.

The superior acidification capacity of *S. thermophilus* NCDC293 is consistent with its higher viable count and growth rate observed in Sections 3.1 and 3.2. Faster growth generally results in greater substrate utilization and increased production of acidic metabolites. The ability of NCDC293 to rapidly reduce pH is technologically advantageous because it shortens fermentation time, improves product safety by inhibiting undesirable microorganisms and contributes to the development of desirable texture in fermented products (Tamime & Robinson, 2007) [28].

The slower acidification observed in *L. plantarum* NCDC25 and *L. rhamnosus* NCDC24 may be attributed to differences in carbohydrate metabolism and adaptation to the soybean matrix. Nevertheless, both probiotic cultures produced sufficient acidity to achieve pH values below 5.0 and acidity levels above 0.60% lactic acid, indicating satisfactory fermentation performance. Similar acidification patterns in fermented soy products have been reported by Donkor *et al.* (2007) [9], who observed progressive decreases in pH and

corresponding increases in titratable acidity during soy yoghurt manufacture.

Statistical analysis confirmed significant differences among cultures from 3 h onward for both pH and titratable acidity. The critical difference values were 0.041 for pH and 0.020 for titratable acidity, indicating that the observed differences in acidification performance were statistically meaningful. The low coefficient of variation values observed in the present study further demonstrate the reliability and reproducibility of the experimental data. Overall, the results indicate that all three cultures were capable of effective acidification of soymilk; however, *S. thermophilus* NCDC293 exhibited the strongest acidification potential, followed by *L. plantarum* NCDC25 and *L. rhamnosus* NCDC24. These findings support the suitability of NCDC293 as a primary starter culture for fermented soy yoghurt manufacture, while NCDC25 and NCDC24 may serve as probiotic adjunct cultures contributing additional functional benefits.

Table 3. Changes in pH and titratable acidity during soymilk fermentation by selected cultures

Culture	Parameter	0 h	3 h	6 h	9 h	12 h
NCDC293 (Streptococcus thermophilus)	pH	6.80 ± 0.01	6.40 ± 0.01	5.74 ± 0.01	5.04 ± 0.01	4.45 ± 0.01
	Titratable acidity (% LA)	0.153 ± 0.003	0.290 ± 0.006	0.490 ± 0.006	0.690 ± 0.006	0.820 ± 0.006
NCDC25 (*Lactiplantibacillus plantarum*)	pH	6.80 ± 0.01	6.52 ± 0.01	5.96 ± 0.01	5.32 ± 0.01	4.65 ± 0.01
	Titratable acidity (% LA)	0.153 ± 0.003	0.250 ± 0.006	0.410 ± 0.006	0.580 ± 0.006	0.720 ± 0.006
NCDC24 (*Lacticaseibacillus rhamnosus*)	pH	6.80 ± 0.00	6.62 ± 0.01	6.14 ± 0.01	5.56 ± 0.01	4.85 ± 0.01
	Titratable acidity (% LA)	0.150 ± 0.000	0.220 ± 0.006	0.350 ± 0.006	0.500 ± 0.006	0.640 ± 0.006

Note: Significant differences ($P \leq 0.05$) among cultures were observed from 3 h onward for both pH and titratable acidity. The CD (P≤0.05) values were 0.041 for pH and 0.020 for titratable acidity, indicating superior acidification by S. thermophilus NCDC293.

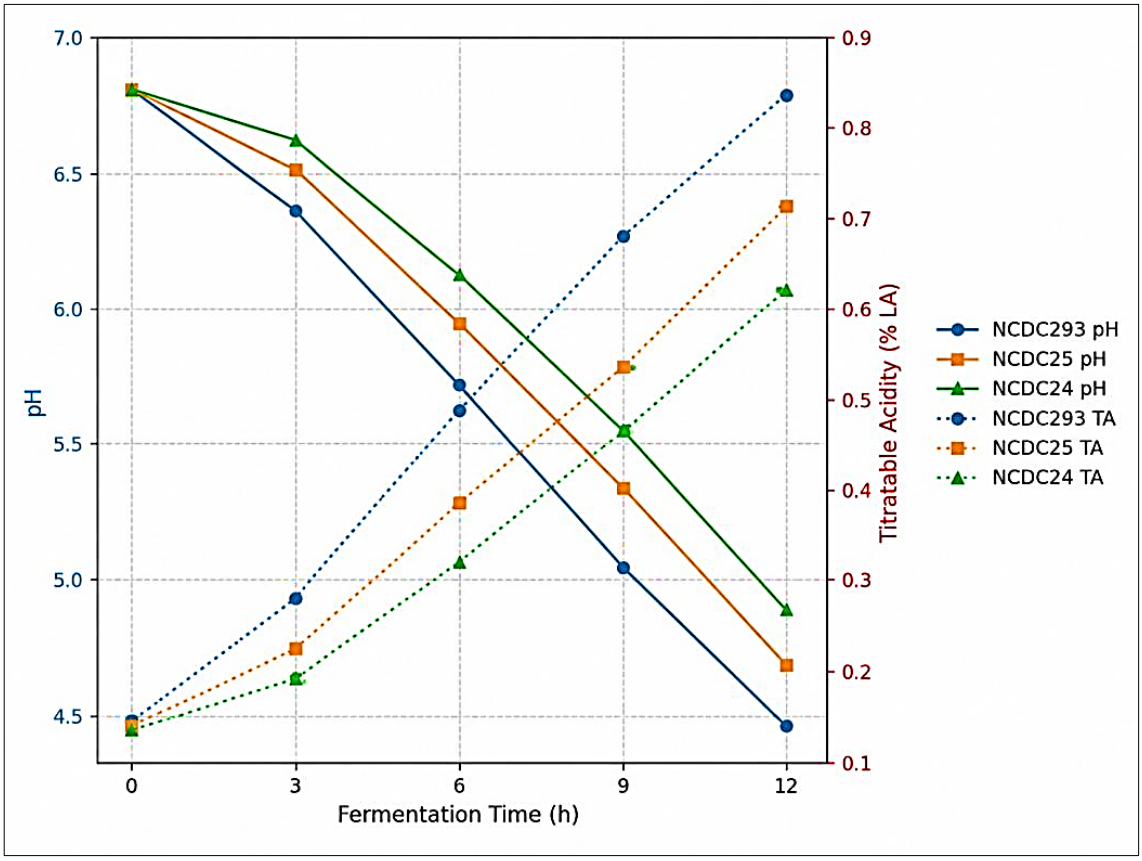


Fig 3: pH and titratable acidity profiles of selected cultures during soymilk fermentation

3.4 Acetaldehyde Production

Acetaldehyde is one of the most important flavour compounds produced during lactic fermentation and is largely responsible for the characteristic fresh, yoghurt-like aroma of fermented dairy and plant-based products. The ability of starter and probiotic cultures to synthesize acetaldehyde is therefore an important technological criterion for the development of high-quality fermented soy products. The acetaldehyde production by the selected cultures is presented in Table 4.

Significant differences (P≤0.05) were observed among the three cultures with respect to acetaldehyde production. *Streptococcus thermophilus* NCDC293 produced the highest concentration of acetaldehyde (42.50 ppm), followed by *Lactiplantibacillus plantarum* NCDC25 (35.20 ppm) and *Lacticaseibacillus rhamnosus* NCDC24 (30.80 ppm). The higher acetaldehyde production by *Streptococcus thermophilus* agrees with the findings of Marshall (1987) [20], who reported its important role in yoghurt flavour development. The observed differences exceeded the critical difference (CD = 0.530 ppm), indicating statistically significant variation among the cultures.

The superior acetaldehyde production by *S. thermophilus* NCDC293 may be attributed to its efficient metabolism of

amino acids and carbohydrates, resulting in enhanced synthesis of flavour-active compounds. This finding is consistent with its higher growth rate, viable cell count and acidification capacity observed in previous sections. Rapid growth and metabolic activity generally favour the formation of volatile compounds responsible for desirable sensory attributes in fermented products.

L. plantarum NCDC25 produced an intermediate level of acetaldehyde, suggesting a moderate capacity for flavour development in soymilk. Although lower than that of *S. thermophilus* NCDC293, the amount produced by NCDC25 was sufficient to contribute positively to the characteristic aroma of fermented soy yoghurt. Similar observations have been reported for probiotic lactobacilli, which are known to generate flavour compounds while simultaneously providing health-promoting benefits (Leroy and De Vuyst, 2004) [16].

The lowest acetaldehyde concentration was recorded for *L. rhamnosus* NCDC24. Despite its comparatively lower production, the concentration remained within the range reported for acceptable flavour development in fermented plant-based products. The lower acetaldehyde production may be related to differences in metabolic pathways and substrate utilization characteristics of this species.

The low coefficient of variation (CV = 1.212%) indicates excellent experimental precision and reproducibility of the measurements. Furthermore, the small standard deviations associated with each treatment demonstrate the consistency of acetaldehyde production across replicates.

Overall, the results indicate that *S. thermophilus* NCDC293 possessed the greatest potential for flavour development in fermented soymilk owing to its significantly higher acetaldehyde production. Consequently, NCDC293 appears particularly suitable as a starter culture for soy yoghurt manufacture, whereas *L. plantarum* NCDC25 and *L. rhamnosus* NCDC24 may serve as probiotic adjunct cultures contributing both flavour and functional properties.

Table 4. Acetaldehyde production by selected cultures in soymilk

Culture	Acetaldehyde (ppm)
Streptococcus thermophilus NCDC293	42.50 ± 0.50
Lactiplantibacillus plantarum NCDC25	35.20 ± 0.40
Lacticaseibacillus rhamnosus NCDC24	30.80 ± 0.30
SEm±	0.150
CD (P≤0.05)	0.530
CV (%)	1.212

Note: Values are expressed as mean ± standard deviation of three replications. Means differing by more than the CD value are significantly different at $P \leq 0.05$. SEm± = Standard Error of Mean; CD = Critical Difference; CV = Coefficient of Variation.

3.5 Exopolysaccharide Production

Exopolysaccharides (EPS) are extracellular polymeric substances synthesized by several lactic acid bacteria during fermentation. EPS production is considered an important technological characteristic because it improves viscosity, water-holding capacity, mouthfeel, texture and overall consumer acceptability of fermented products. In fermented soy products, EPS can partially compensate for the absence of milk proteins and contribute to the development of a smooth and stable yoghurt-like structure. The EPS production by the selected cultures is presented in Table 5.

Significant differences (P≤0.05) were observed among the three cultures with respect to EPS production. *Streptococcus thermophilus* NCDC293 produced the highest amount of EPS (98.60 mg/L), followed by *Lactiplantibacillus plantarum* NCDC25 (91.80 mg/L) and *Lacticaseibacillus rhamnosus* NCDC24 (85.40 mg/L). The differences among the cultures exceeded the critical difference value (CD = 1.223 mg/L), indicating statistically significant variation in EPS synthesis.

The superior EPS production by *S. thermophilus* NCDC293 demonstrates its excellent technological suitability for soy yoghurt manufacture. EPS-producing strains of *S. thermophilus* are widely recognized for enhancing viscosity and reducing syneresis in fermented products (Ruas-Madiedo and de los Reyes-Gavilán, 2005) [26]. The higher EPS yield observed in the present study is consistent with the superior growth performance and acidification capacity exhibited by this culture during fermentation.

L. plantarum NCDC25 showed intermediate EPS production (91.80 mg/L), indicating a substantial ability to contribute to texture development. In addition to its probiotic potential, EPS synthesis by *L. plantarum* may improve the rheological properties and stability of fermented soy products. Previous studies have reported that EPS-producing strains of *L. plantarum* can enhance viscosity and sensory quality while also offering potential health benefits associated with prebiotic-like polysaccharides.

The lowest EPS production was recorded for *L. rhamnosus* NCDC24 (85.40 mg/L). Although significantly lower than that of the other cultures, the EPS level remained considerable and could still contribute positively to product texture and stability. The lower EPS yield may be associated with strain-specific differences in carbohydrate metabolism and polysaccharide biosynthesis pathways.

The coefficient of variation (CV = 0.361%) was very low, indicating excellent experimental precision and reproducibility. Likewise, the small standard deviations observed for all treatments suggest consistent EPS production across replicates.

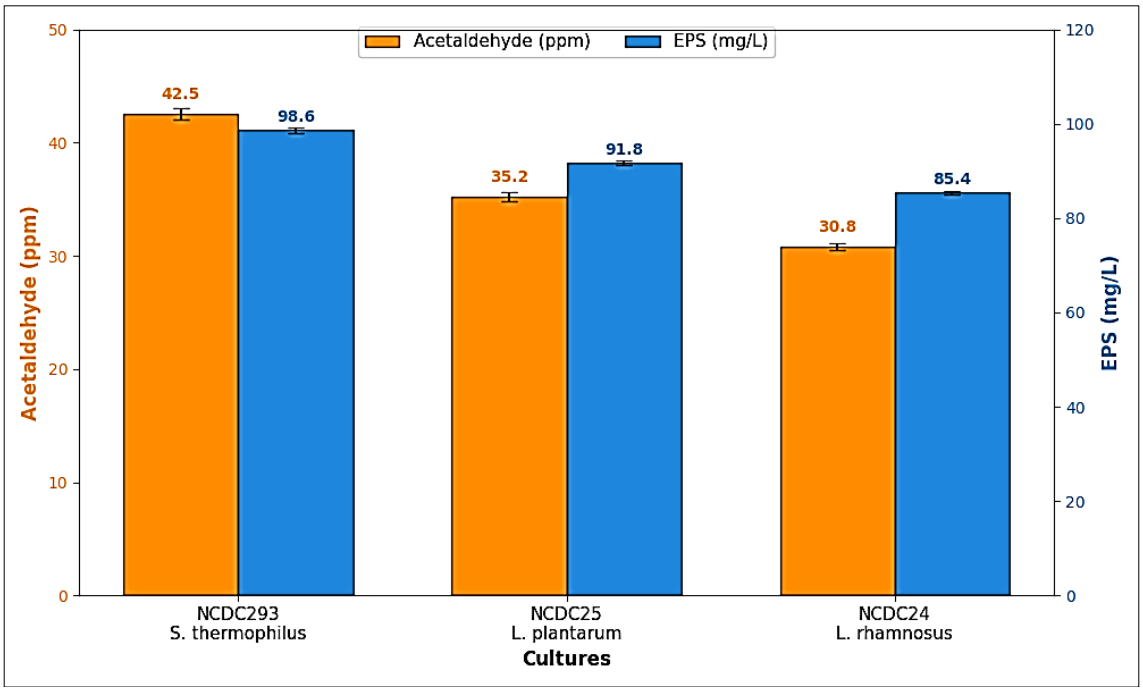
EPS production followed a trend similar to that observed for viable cell count, growth rate, acidification and acetaldehyde production, with *S. thermophilus* NCDC293 consistently outperforming the probiotic cultures. This suggests that NCDC293 possesses superior technological functionality for fermented soy yoghurt manufacture. However, the appreciable EPS production by *L. plantarum* NCDC25 and *L. rhamnosus* NCDC24 indicates their suitability as adjunct probiotic cultures capable of improving both functional and textural attributes of the final product.

Overall, the results demonstrate that *S. thermophilus* NCDC293 is the most efficient EPS-producing culture among those evaluated and therefore has the greatest potential to enhance texture, viscosity and stability in fermented soymilk. The probiotic cultures, particularly *L. plantarum* NCDC25, also exhibited promising EPS-producing ability and may be effectively combined with starter cultures in the development of high-quality fermented soy products.

Table 5: Exopolysaccharide (EPS) production by selected cultures in soymilk

Culture	EPS (mg/L)
Streptococcus thermophilus NCDC293	98.60 ± 0.60
Lactiplantibacillus plantarum NCDC25	91.80 ± 0.50
Lacticaseibacillus rhamnosus NCDC24	85.40 ± 0.40
SEm±	0.347
CD (P≤0.05)	1.223
CV (%)	0.361

Note: Values are expressed as mean ± standard deviation of three replications. Means differing by more than the CD value are significantly different at P≤0.05. SEm± = Standard Error of Mean; CD = Critical Difference; CV = Coefficient of Variation.



Note: Values are expressed as mean ± standard deviation of three replications. Means differing by more than the CD value are significantly different at $P \leq 0.05$. SEm± = Standard Error of Mean; CD = Critical Difference; CV = Coefficient of Variation.

Fig 4: Acetaldehyde and exopolysaccharide production by selected cultures in soymilk

3.6 Comparative Technological Performance

The overall technological performance of the selected cultures was evaluated using six key parameters, namely final viable count, growth rate, generation time, titratable acidity, acetaldehyde production and exopolysaccharide (EPS) production. These parameters collectively determine the suitability of a culture for fermented soy yoghurt manufacture. The comparative data are presented in Table 6, while the normalized technological performance profile is illustrated in Figure 5.

To facilitate visual comparison among cultures, the technological parameters were normalized on a common scale ranging from 0 to 1. Since a lower generation time indicates superior growth performance, this parameter was reverse-normalized before plotting the radar chart. Consequently, higher normalized scores consistently represented superior technological performance across all evaluated attributes.

Among the three cultures evaluated, *Streptococcus thermophilus* NCDC293 consistently exhibited superior performance across all technological characteristics. The culture attained

the highest final viable count (9.28 log CFU/mL), highest specific growth rate (0.312 h^{-1}), shortest generation time (2.22 h), greatest titratable acidity (0.82% lactic acid), highest acetaldehyde production (42.50 ppm) and maximum EPS production (98.60 mg/L). These results indicate excellent adaptation of NCDC293 to the soymilk environment and its superior ability to support rapid fermentation, flavour development and texture enhancement.

Lactiplantibacillus plantarum NCDC25 demonstrated intermediate technological performance, achieving a final viable count of 9.00 log CFU/mL, growth rate of 0.273 h^{-1}, generation time of 2.54 h, titratable acidity of 0.72%, acetaldehyde production of 35.20 ppm and EPS production of 91.80 mg/L. The culture exhibited satisfactory fermentation efficiency while maintaining desirable probiotic characteristics, indicating its suitability as a probiotic adjunct culture in fermented soy products.

Lacticaseibacillus rhamnosus NCDC24 recorded comparatively lower values for most technological parameters, including viable count (8.83 log CFU/mL), growth rate (0.248 h^{-1}), titratable acidity (0.64%), acetaldehyde production (30.80 ppm) and EPS production (85.40 mg/L). Nevertheless, the culture maintained viable populations exceeding 10^8 CFU/mL and demonstrated acceptable fermentation performance, supporting its potential application as a probiotic adjunct culture.

The normalized radar chart (Figure 5) clearly illustrates the differences in overall technological performance among the cultures. *S. thermophilus* NCDC293 occupied the largest area within the radar plot, reflecting its consistently superior performance across all evaluated parameters. *L. plantarum*

NCDC25 occupied an intermediate position, whereas *L. rhamnosus* NCDC24 formed the smallest profile. The larger radar area observed for NCDC293 indicates its balanced and superior performance with respect to microbial growth, acidification efficiency, flavour compound production and EPS synthesis.

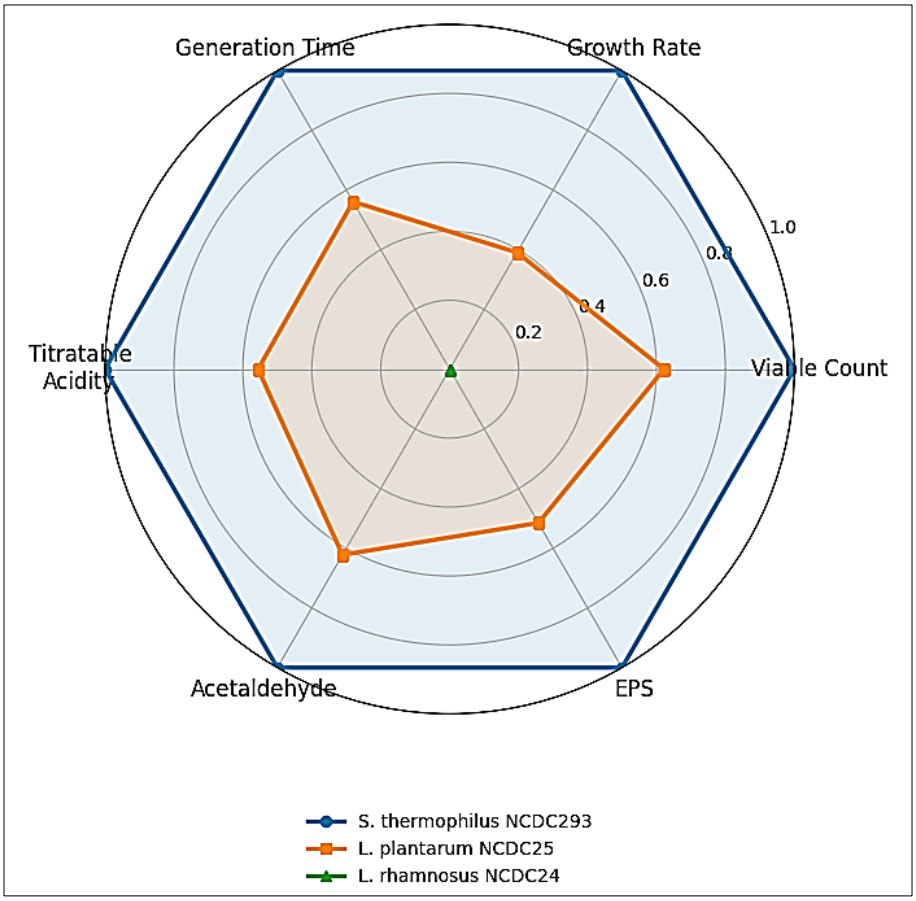
The superior technological characteristics exhibited by *S. thermophilus* NCDC293 are consistent with previous reports describing the importance of *S. thermophilus* as an efficient starter culture capable of rapid acidification, flavour development and texture enhancement in fermented products (Tamime and Robinson, 2007) [28]. Similarly, the appreciable performance of *L. plantarum* NCDC25 and *L. rhamnosus* NCDC24 supports their potential utilization as probiotic adjunct cultures for improving the functional value of fermented soy products.

Overall, the comparative analysis demonstrated that *Streptococcus thermophilus* NCDC293 possessed the most favourable technological attributes among the cultures evaluated. Its superior growth kinetics, acidification behaviour, acetaldehyde production and EPS synthesis collectively justify its selection as the most suitable starter culture for fermented soy yoghurt manufacture. Meanwhile, *Lactiplantibacillus plantarum* NCDC25 and *Lacticaseibacillus rhamnosus* NCDC24 may be effectively employed as probiotic adjunct cultures to enhance the nutritional and functional properties of the final product.

Table 6: Comparative technological characteristics of selected cultures in soymilk

Parameter	NCDC293 (S. thermophilus)	NCDC25 (L. plantarum)	NCDC24 (L. rhamnosus)
Final viable count (log CFU/mL)	9.28 ± 0.01	9.00 ± 0.01	8.83 ± 0.01
Growth rate (μ h^{-1})	0.312 ± 0.001	0.273 ± 0.001	0.248 ± 0.001
Generation time (h)	2.22 ± 0.01	2.54 ± 0.01	2.79 ± 0.01
Final pH	4.45 ± 0.01	4.65 ± 0.01	4.85 ± 0.01
Titratable acidity (% LA)	0.82 ± 0.01	0.72 ± 0.01	0.64 ± 0.01
Acetaldehyde (ppm)	42.50 ± 0.50	35.20 ± 0.40	30.80 ± 0.30
EPS (mg/L)	98.60 ± 0.60	91.80 ± 0.50	85.40 ± 0.40

Note: Values are mean ± standard deviation of three replications. Final values correspond to measurements obtained after 12 h fermentation of standardized soymilk.



Note: Generation time was reverse-normalized because lower values represent superior growth performance.

Fig 5: Radar chart showing normalized technological performance scores of *Streptococcus thermophilus* NCDC293, *Lactiplantibacillus plantarum* NCDC25 and *Lacticaseibacillus rhamnosus* NCDC24 during soymilk fermentation. Scores were normalized to a 0-1 scale, and generation time was reverse-normalized because lower values indicate superior growth performance

4. Conclusion

The present study evaluated the performance of three selected cultures, namely *Streptococcus thermophilus* NCDC293, *Lactiplantibacillus plantarum* NCDC25 and *Lacticaseibacillus rhamnosus* NCDC24, in soymilk fermentation with respect to growth characteristics,

acidification behaviour, flavour development and exopolysaccharide production. Significant differences ($P \leq 0.05$) were observed among the cultures for most of the technological parameters evaluated.

Among the cultures tested, *S. thermophilus* NCDC293 exhibited the highest technological performance. The culture achieved the greatest viable cell count (9.28 log CFU/mL), highest specific growth rate (0.312 h^{-1}), shortest generation time (2.22 h), lowest final pH (4.45), highest titratable acidity (0.82% lactic acid), greatest acetaldehyde production (42.50 ppm) and highest EPS production (98.60 mg/L). These characteristics indicate superior adaptation to the soymilk environment and enhanced capability for rapid fermentation, flavour formation and texture improvement.

L. plantarum NCDC25 demonstrated intermediate performance, achieving substantial growth (9.00 log CFU/mL), appreciable acidification (0.72% lactic acid), and considerable production of acetaldehyde (35.20 ppm) and EPS (91.80 mg/L). The culture therefore appears suitable as a probiotic adjunct capable of contributing both technological and functional benefits to fermented soy products.

Although *L. rhamnosus* NCDC24 exhibited comparatively lower values for most technological parameters, it maintained high viable populations (>8.8 log CFU/mL) and produced adequate levels of acidity, flavour compounds and EPS. These findings indicate its potential use as a probiotic adjunct culture where functional attributes are desired.

The comparative technological assessment and radar chart analysis clearly identified *S. thermophilus* NCDC293 as the most suitable culture for fermented soy yoghurt manufacture. Its superior growth kinetics, acidification efficiency, flavour-producing capacity and EPS synthesis make it an excellent starter culture for developing high-quality fermented soy products. The probiotic cultures *L. plantarum* NCDC25 and *L. rhamnosus* NCDC24 may be effectively incorporated as adjunct cultures to enhance the probiotic functionality of the final product.

Overall, the study demonstrates that soymilk can successfully support the growth and metabolic activities of selected lactic cultures and provides a suitable substrate for the development of fermented soy yoghurt. The findings contribute valuable information for the selection of starter and probiotic cultures in plant-based fermented food systems and support the growing demand for functional dairy alternatives.

Based on the results obtained, *Streptococcus thermophilus* NCDC293 is recommended as the most suitable starter culture for fermented soy yoghurt manufacture owing to its superior growth kinetics, acidification efficiency, acetaldehyde production and exopolysaccharide synthesis. *Lactiplantibacillus plantarum* NCDC25 and *Lacticaseibacillus rhamnosus* NCDC24 may be employed as probiotic adjunct cultures to enhance the functional and nutritional value of fermented soy products.

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