In situ production of pediocin PA-1 like bacteriocin by different genera of lactic acid bacteria in soymilk fermentation and evaluation of sensory properties of the fermented soy curd

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Abstract The lactic acid bacteria (LAB) are found to produce bacteriocins with enhanced nutritive properties in the fermented foods. In the present study, the ability of LAB cultures (*Pediococcus acidilactici* NCIM 5424, *Enterococcus faecium* NCIM 5423 and *Lactobacillus plantarum* Acr2) to produce pediocin PA-1 like bacteriocin was evaluated during soymilk fermentation. The isolates *E. faecium* NCIM 5423 and *Lb. plantarum* Acr2 were able to produce bacteriocin as well as ferment soymilk within 6 h of incubation. Upon plating the cultures *E. faecium* NCIM 5423 and *Lb. plantarum* Acr2 in soymilk were found to be viable even after 15 days of storage at 4 °C. No significant variation was observed in the viable counts of *E. faecium* NCIM 5423 and *Lb. plantarum* Acr2 ($P>0.05$). The effect of crude bacteriocin on *Listeria* cells was evidenced through scanning electron microscope (SEM) photographs wherein cell membrane damage was observed. On co-cultivation of *E. faecium* NCIM 5423 and *Lb. plantarum* Acr2 individually with *Listeria monocytogenes* ScottA a decrease in the *Listeria* count was observed within 24 h of incubation. However, during co-cultivation of ScottA with *P. acidilactici* NCIM 5424, no significant difference was observed in the viable counts ($P>0.05$). The pH, titratable acidity, pediocin activity, anti-oxidant property and sensory attributes

for *E. faecium* NCIM 5423 were studied. It was observed that *E. faecium* NCIM 5423 fermented soymilk had an acceptable sensory score during storage period. Hence, such culture can be an ideal starter for development of functional foods with longer shelf life.

Keywords Lactic acid bacteria · Pediocin PA-1 like bacteriocin · Soymilk fermentation · Anti-listerial activity · Co-cultivation · Sensory analysis

Introduction

Soybeans (*Glycine max*) have been used in several developing countries as an important nutritional component with beneficial effects (hypolipidemic, anticholesterolemic, antiatherogenic and anti-allergic properties) on the health of the consumers and are widely used in the preparation of yogurt-like products (Lopez-Lazaro and Akiyama 2002). Soy products are found to be rich in isoflavones, dietary fibre, oligosaccharides, proteins, trace minerals and vitamins, which have tremendous influence on host's health (Wang et al. 2006). Many soy products have limited use by the consumers because of their undesirable off-flavours (Favaro Trindade et al. 2001). The fermentation of soymilk by LAB has been found to reduce beany flavour and anti-nutritional factors like phytic acid with good acceptance of the soy end products (Raghavendra et al. 2011). Hence, the consumption of soymilk and their products is beneficial to the ecosystem of the gastro intestinal tract (GIT) of humans, which helps in increasing the population of probiotic bacteria and reducing the population of pathogenic bacteria.

Listeria monocytogenes has the ability to grow in foods with low pH, storage temperatures from 1 to 45 °C (Kouakou

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et al. 2010). Lui and Lin (2008) have described the crucial concern of *L. monocytogenes* contamination in soymilk products and could not successfully prevent its growth. Hence, controlling the growth of *Listeria* in food systems has become necessary. Several of the class IIa bacteriocins, especially pediocin PA-1 are found to destroy the *Listeria* with their antagonistic effects and are widely used in fermentation of dairy, food and meat processing industries (Papagianni and Anastasiadou 2009). The pediocin production in milk is achieved either by genetic modification or co-cultivation with other LAB to increase the shelf life of the fermented product (Somkuti and Steinberg 2003, 2010). However, there is a limited usage of such genetically modified organisms as a starter culture in food systems. The pediocin producing *Pediococcus acidilactici* has very limited usage in dairy milk products due to its non-lactose fermenting ability (Somkuti and Steinberg 2010).

Enterococcus and *Lactobacillus* sp. have been used in several of the dairy products as adjuvant starters, as a protective culture in fermented foods or as a probiotic strain (Franz et al. 1999; Ewe et al. 2010). *E. faecium* plays an important role in dairy products because of its specific biochemical properties like proteolysis, lipolysis and hence contributes to the production of flavour and taste in the end product (Giraffa 2003). Since *E. faecium* has several applications in the food industry, use of such cultures with bacteriocin production ability will ensure the additional safety measures of the finished product especially with regard to *Listeria* contamination. The present study focussed on the application of intergeneric pediocin PA-1 bacteriocin producing bacteria like *Enterococcus faecium* for the fermentation of soymilk to obtain a yogurt with enhanced microbial quality and safety. Sensory evaluation of finished product is also reported.

Materials and methods

Microorganisms and growth conditions

The bacteria *Pediococcus acidilactici* K7 (NCIM 5424), and *Enterococcus faecium* Acr4 (NCIM 5423), which were reported to have plasmid encoded pediocin PA-1 like bacteriocin were used for the fermentation of the soymilk (Devi and Halami 2011, 2012). The above cultures were isolated from fermented cucumber and carrot, respectively. The cultures were grown in MRS broth (HiMedia, Mumbai, India) at 37 °C under static condition for 16–18 h. The culture maintenance and growth conditions were followed, as described by Devi and Halami (2011). The food-borne pathogen *Listeria monocytogenes* ScottA (obtained from Dr. AK Bhunia, Purdue University, USA) grown in BHI medium (HiMedia) at 37 °C under shaking conditions was used for the anti-bacterial assays.

Haemolytic activity and antibiotic sensitivity tests

Production of haemolysins by the selected strains was determined by streaking the actively growing cells on nutrient broth containing 5 % (v/v) human blood. The blood agar plates are incubated at 37 °C for 24 h and observed for no zone of clearance. Different antibiotics discs of 1 mg/mL concentration of erythromycin, vancomycin, streptomycin, tetracycline, ampicillin, gentamycin, kanamycin, cefalaxine, chloramphenicol and norfloxacin (Himedia) was prepared to study the antibiotic sensitivity test. The freshly grown test cultures were swabbed on the MRS agar plates and the antibiotic-impregnated discs were placed after 15 mins. The zone of inhibition was determined after 24 h of incubation at 37 °C. The result was recorded as sensitive when zone of inhibition was observed.

Preparation of soymilk

The whole soy beans obtained from local market, were soaked overnight in distilled water. The outer seed coat was removed and blended in a grinder using distilled water to get a thick paste. The soymilk was extracted and sterilized as described by Rekha and Vijayalakshmi (2008).

Preparation of inoculum and fermentation of soymilk

One mL each of the freshly grown test cultures in MRS broth was taken and the cells were harvested by centrifugation at 8,000 rpm for 10 min at 4 °C. The collected cell pellet was washed twice and suspended in the initial volume with sterile distilled water. An inoculum volume of 0.5 % and 1 % (v/v) of cell suspension containing 10^6 cfu/mL and 10^8 cfu/mL was inoculated into sterile soymilk and incubated at 37 °C.

Physiochemical properties of fermented soymilk

The pH measurements were recorded using a digital pH meter after the fermentation of soymilk. The total titratable acidity (TA) of the fermented soymilk was determined by a standard method (AOAC 1990). The total carbohydrate content in the fermented sample was determined as described by Dubois et al. (1956). Similarly, the reducing sugar content of fermented samples was determined as described by Miller (1959). The TA, total carbohydrate content and reducing sugars were determined after 24 h of incubation of the soymilk with the bacteriocin producers. The texture and viscosity were determined as described by Early (1998). Based upon the results obtained for the above properties, further characterization of the fermented sample was performed to determine moisture, fat and ash content as described by Rekha and Vijayalakshmi (2008). All the experiments were performed in triplicates and the mean values are represented.

For anti-listerial and antioxidant activity, the whey was separated from the homogenized fermented soymilk, followed by centrifugation at 8,000 rpm at 4 °C for 10 min. The obtained whey was filtered through Whatmann filter paper no.1 and stored at -20 °C until use. Anti-listerial activity present in the whey was determined by spot-on-lawn assay against *L. monocytogenes* ScottA as described by Devi and Halami (2011). The activity was expressed as arbitrary units (AU) per mL, which is defined as the highest dilution of test sample exhibiting the zone of inhibition against indicator organism. Antioxidant property was measured by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) as described by Williams et al. (1995).

Viability of LAB in fermented soymilk

During storage, an aliquot of the fermented sample was withdrawn on day 1, 5, 10 and 15. Appropriate dilutions were made and plated on MRS agar, and incubated at 37 °C for 16–24 h. After the incubation time, the colonies present were counted, and the viability of LAB was expressed in cfu/mL.

Effect of bacteriocin on *L. monocytogenes* ScottA

Co-cultivation of ScottA with *E. faecium* NCIM 5423, *Lb. plantarum* Acr2 and *P. acidilactici* NCIM 5424 was performed by inoculation to the sterilized soymilk. A freshly grown culture of ScottA (10^3 cfu/mL) and pediocin-like bacteriocin producers (10^6 cfu/mL with 6,400 AU/mL of bacteriocin activity) were grown in BHI and MRS medium respectively, and used for inoculation. The inoculated soymilk samples were incubated at 37 °C for 24 h. An aliquot of sample was drawn after every 2 h and appropriate dilutions were made and plated on Listeria Oxford media (HiMedia) for the viability of *Listeria* cells.

Simultaneously, the bacteriocin was extracted using chloroform (SRL, Mumbai, India) as described by Burianek and Yousef (2000). The crude bacteriocin (2 µL, 200 AU/mL) was added to freshly grown culture of *L. monocytogenes* ScottA (10^6 cfu/mL) and observed for its morphological changes by scanning electron microscope. The samples were withdrawn after 30 min, 1 h, 2 h, 4 h and 6 h of incubation. Post incubation, the cells were centrifuged and fixed with 2 % glutaraldehyde solution (SRL) for 4 h and dried with ethanol gradient of 10–100 % and observed under the scanning electron microscope (SEM, LEO-435 VP, Cambridge, UK) after gold coating (McDougall et al. 1994).

Survival of *L. monocytogenes* in the presence of bacteriocin producers in soymilk

The soymilk samples were inoculated with 1 % inoculum (v/v) of the test cultures and incubated at 37 °C for 4 h. The freshly

grown *L. monocytogenes* ScottA was inoculated at a concentration of 10^3 cfu/mL to the soymilk pre-inoculated with LAB. The incubation process was continued for the fermentation of soymilk. The samples were withdrawn at 0, 2, 4, 6, 8, and 24 h. Appropriate diluted samples were plated on Listeria Oxford agar medium for the *Listeria* count and MRS media for LAB count. The plates were incubated at 37 °C for 16–24 h and the obtained colonies were counted and expressed in cfu/mL.

Sensory analysis of the fermented soymilk

The sensory evaluation was performed by Quantitative Descriptive Analysis (QDA) to assess the quality of the samples (Stone and Sidel 1998). Evaluation was carried out in ‘Sensory booths’ under standard conditions. A suitable score card was developed using ‘Free-choice profiling’ method selecting suitable terminologies. Samples were presented in porcelain dishes coded with 3-digit random numbers, to the panellists ($n=10$). The scale was anchored at 1.25 cm on either end, representing ‘Detection threshold’ and ‘Saturation threshold’, respectively. The scores given for all the attributes for each sample was tabulated. The mean value was calculated for each attribute of a sample, representing the panel’s judgement of the sensory quality of the product. This involved listing of appropriate terminology describing individual quality attributes of the product. Using this scorecard, panellists were adequately trained to detect subtle differences in the perceived intensity of the attributes.

Statistical analysis

Statistical analysis of the data was performed by one-way analysis of variance (ANOVA) and correlational analysis by Microsoft Excel (Albright et al. 1999). All the experiments were performed in triplicates ($n=3$) and represented as mean $\pm$ standard deviation (SD). The statistical analysis of the obtained sensory data was carried out using Duncan’s Multiple Range Test (DMRT) (Duncan 1955) using Statistica ‘99. The values of sensory analysis are expressed in mean $\pm$ SD ($n=10$). Significance for all the experiments was set at $P<0.05$. The sensory analysis was performed for 1st, 7th and 15th day fermented samples.

Results and discussion

Production of pediocin PA-1 like bacteriocin in soymilk fermentation

In this study, the ability to produce pediocin PA-1 like bacteriocin in soymilk by *P. acidilactici* NCIM 5424, *E. faecium* NCIM 5423 and *Lb. plantarum* Acr2 was demonstrated. These isolates are regarded as non-pathogenic, as no zone of

clearance was observed on blood agar plates. Moreover, the selected cultures were found to be sensitive to several antibiotics tested. It was observed that the selected isolate *E. faecium* NCIM 5423 and *Lb. plantarum* Acr2 were able to ferment the soymilk with 1 % inoculum at 37 °C within 4 and 6 h of incubation, respectively. Whereas, *P. acidilactici* NCIM 5424 was unable to ferment the soymilk. Similarly others have also reported the inability of *P. acidilactici* to ferment milk samples (Somkuti and Steinberg 2010). Božanić et al. (2011) observed the fermentation of soymilk by *Lb. casei*, *Lb. acidophilus*, which lasted for 12–17 h at 37 °C, whereas fermentation by *Bifidobacterium animalis* subsp. *lactis* Bb12 was observed within 4 h of incubation at 42 °C. Besides the stability of pediocin PA-1 bacteriocin at wide pH and temperature, an acceptable survival rate and stability under GIT conditions in an in vitro model was observed, indicating its probiotic nature (Papagianni and Anastasiadou 2009; Kheadr et al. 2010; Devi and Halami 2011). Hence, present studied cultures producing pediocin PA-1 like bacteriocin may have many beneficial effects on the GIT of humans.

The fermented product was subsequently assessed for pediocin PA-1 like bacteriocin production in the whey against *L. monocytogenes* ScottA. The whey of the fermented soymilk samples was found to have an increased pediocin PA-1 like bacteriocin activity from the initial 6,400 AU/mL to 25600 AU/mL after 4 h of fermentation for *E. faecium* NCIM 5423 and *Lb. plantarum* Acr2, suggesting the production of the pediocin like bacteriocin in soymilk by these organisms. Whereas, the *P. acidilactici* NCIM 5424 did not produce any activity against ScottA even after 24 h of incubation. Laukova and Czikkova (1999) had reported 200 AU/mL of enterocin CCM 4231 production by *E. faecium* within 24 h of incubation in soymilk. In contrast, Todorov and Dicks (2005) observed no bacterial growth and bacteriocin production in soymilk by two strains of *Lb. rhamnosus*, suggesting the specific requirement of nutrients for growth and production of bacteriocin. This may be the reason for the inability of the present test culture *P. acidilactici* NCIM 5424 to ferment soymilk.

Quality parameters of fermented soymilk

The obtained quality parameters of the fermented soymilk after 24 h of incubation are enlisted in Table 1. The pH of the 4 h fermented soymilk was found to be more acidic (pH 4.5) in the case of *E. faecium* NCIM 5423 which was comparable with the highest TA (0.36 %) and it was found to maintain the same pH till 24 h of incubation. The pH and TA of the 4 h fermented soymilk of *E. faecium* NCIM 5423 was increased, when compared to the other samples. However, the pH and TA was decreased when compared to the 24 h fermented soymilk by probiotic LAB, as reported by Rekha and Vijayalakshmi (2008). Total sugars and reducing sugars was lower in *E. faecium* NCIM 5423 fermented samples as compared to that

Table 1 Quality parameters of the fermented soymilk by LAB cultures

Tests	Bacterial cultures	
	<i>E. faecium</i> NCIM 5423	<i>Lb. plantarum</i> Acr2
Fermentation duration (h)	4	6
Flavour	++	++
Colour	Creamy white	Creamy white
Viscosity	+++	++
Whey production	++	++
Solidity	+++	+
Pediocin activity (AU/mL)	25600	25600
pH	4.5±0.1	4.7±0.1
Titrate acidity (%)	0.3±0.02	0.2±0.01
Total sugars (mg/mL)	11.3±0.01	17.5±0.1
Reducing sugars (mg/mL)	6.0±0.1	7.4±0.1
Antioxidant property (%)	76.8±0.1	70.9±0.1

++ (good), +++ (excellent), AU/mL- Arbitrary units per mL, Values are the mean ± SD ($n=3$ trials) for pH, titrate acidity, total sugars, reducing sugars and antioxidant property

of *Lb. plantarum* Acr2, indicating the utilization of sugars by the organism. Ewe et al. (2010) reported that the concentration of the carbohydrates in soymilk was found to be lower after 24 h of fermentation by LAB, suggesting the hydrolysis/utilization of the carbohydrates present in the product.

It was observed that the soymilk fermented by *E. faecium* NCIM 5423 has good viscosity, texture and firmness when compared to the fermented soymilk by *Lb. plantarum* Acr2. Similarly, Rossi et al. (1999) have reported fermentation of soymilk with *E. faecium* CRL183 with good viscosity, consistency and texture for its use as a probiotic product. The DPPH scavenging activity was observed to be 76.8 % and 70.9 % for *E. faecium* NCIM 5423 and *L. plantarum* Acr2, respectively. From the results of this study, the highest level of antioxidant activity was observed in the whey of the fermented soymilk by *E. faecium* NCIM 5423, suggesting its better adaptability to the soymilk substrates when compared to *Lb. plantarum* Acr2. Similarly, Osuntoki and Korie (2010) reported that *Lb. plantarum* strains isolated from dairy and non-dairy fermented foods produced very low levels of DPPH radical scavenging activity, suggesting lesser adaptability in the fermented milk. Hence, the soy product obtained by the *E. faecium* NCIM 5423 was found to have a better viscosity, solidity, TA and antioxidant property when compared to the *Lb. plantarum* Acr2 fermented soymilk product.

Viability of cultures during storage at 4 °C

The survival of the pediocin PA-1 like bacteriocin producing LAB was observed during storage at 4 °C. The viable count of the cultures *P. acidilactici* NCIM 5424, *E. faecium* NCIM

Table 2 Survival of the pediocin PA-1 like bacteriocin producing bacteria (counts in log cfu/mL) during storage period at 4 °C in soymilk

Bacterial cultures	Storage period (days)			
	1	5	10	15
<i>P. acidilactici</i> NCIM 5424	8.4±0.01 ^a	5.2±0.02 ^b	3.0±0.08 ^c	1.6±0.01 ^a
<i>E. faecium</i> NCIM 5423	8.6±0.01 ^a	8.5±0.01 ^a	8.5±0.01 ^a	8.5±0.01 ^a
<i>Lb. plantarum</i> Acr2	8.4±0.01 ^a	8.4±0.02 ^b	8.4±0.01 ^a	8.4±0.008 ^a

Values are the mean ± SD ($n=3$ trials), and the alphabetic superscripts (a,b,c) represented in the same columns followed by different letters were significantly different $P<0.05$

5423 and *Lb. plantarum* Acr2 in the soymilk was determined on day 1, 5, 10 and 15 during storage at 4 °C. Table 2 represents the mean ± SD log values of the viable count of tested LAB in soymilk. There was no significant difference in viable counts between *E. faecium* NCIM 5423 and *Lb. plantarum* Acr2 ($P>0.05$), as compared to *P. acidilactici* NCIM 5424. During the storage period, the viable count of the isolate *P. acidilactici* NCIM 5424 significantly reduced ($P<0.05$) by 6–7 logs, whereas, *E. faecium* NCIM 5423 and *Lb. plantarum* Acr2 maintained their viability during storage period.

The soymilk was reported to contain very low levels of carbohydrates, fat, fatty acid, while lactose was absent, etc. (Hajirostamloo 2009). As reported earlier by Papagianni and Anastasiadou (2009), the *P. acidilactici* strains require an

adequate amount of carbohydrate for their growth and metabolism, and their survival rate was found to be lower in the presence of low levels of carbohydrate source. This may be the reason behind the reduction of viable count of *P. acidilactici* NCIM 5424 in the present study. The *E. faecium* 5423 and *Lb. plantarum* Acr2 were able to grow luxuriantly with an average cfu/mL of 10^8 . The wider adaptability of *E. faecium* NCIM 5423 to extreme environmental conditions like pH, temperature and salt was reported by Devi and Halami (2011). Somkuti and Steinberg (2010) also observed similar results for *P. acidilactici* in the fermentation of commercial milk samples. Similarly, the viable counts of LAB starter cultures were found to be maintained at 4 °C for 21 days of storage of fermented milk (Saccaro et al. 2009). As these

Fig. 1 Effect of bacteriocin produced by *E. faecium* NCIM 5423 on *L. monocytogenes* ScottA. SEM images show the cells of *L. monocytogenes* ScottA, untreated (a), treated with bacteriocin for 2 h (b), 4 h (c) and 6 h (d) at 37 °C. (Magnification of 40,000 X)

Table 3 Inhibition of *L. monocytogenes* ScottA in the presence of pediocin PA-1 like bacteriocin producing cultures (counts in log cfu/mL)

Bacterial cultures	Co-cultivation time (h)				
	0	2	4	6	8
<i>P. acidilactici</i> NCIM 5424	3.2±0.03 ^a	3.2±0.05 ^b	2.3±0.02 ^a	2.3±0.03 ^a	2.2±0.03 ^a
<i>E. faecium</i> NCIM 5423	3.3±0.04 ^b	3.1±0.02 ^a	1.7±0.14 ^c	0.9±0.04 ^b	-
<i>Lb. plantarum</i> Acr2	3.2±0.04 ^b	3.3±0.04 ^b	2.9±0.05 ^b	1.8±0.09 ^c	0.3±0.12 ^c

Values are the means ± SD ($n = 3$ trials) and the alphabetic superscripts (a,b,c) represented in the same columns followed by different letters were significantly different $P < 0.05$, - no growth of *L. monocytogenes* ScottA was observed

bacteria (*E. faecium* NCIM 5423 and *Lb. plantarum* Acr2) are found to grow under low levels of carbohydrates present in the soy milk, they were able to survive during the storage period of 15 days at 4 °C.

Inhibition of *L. monocytogenes* ScottA with bacteriocin producing LAB

The effect of the bacteriocin on the ScottA cells was studied to determine their survivability under in vitro conditions. The inhibition of *L. monocytogenes* ScottA and the mode of action of the pediocin producers on *Listeria* cells were determined by co-cultivation in the presence of pediocin producers. The results indicated a reduction in *Listeria* cell count from 10^3 cfu/mL to a negligible number (10^1 cfu/mL) within 8 h of incubation in the presence of bacteriocin producers in BHI broth. No growth was observed for the *Listeria* after 24 h of incubation with the *E. faecium* NCIM 5423, *P. acidilactici* NCIM 5424 and *Lb. plantarum* Acr2. Similarly, the crude bacteriocin preparation of *E. faecium* NCIM 5423, *P. acidilactici* NCIM 5424 and *Lb. plantarum* Acr2 on ScottA reduced the *Listeria* count. Figure 1 shows images obtained from scanning electron microscopy of *L. monocytogenes* ScottA treated with *E. faecium* NCIM 5423 at 0, 2, 4, 6 h of incubation. Membrane damage as indicated by shrinkage or disruption of cell was observed by the end of 6 h incubation with crude bacteriocin. Similar results were also obtained for *P. acidilactici* NCIM 5424 and *Lb. plantarum* Acr2. Several reports have suggested cell disruption as a mode of action on the food-borne pathogenic organisms by the bacteriocin Klayraung and Okonogi (2009). The production of highly active bacteriocins can be useful in controlling the *Listeria* or other food spoilage organisms in any food system. Hence, such cultures with pediocin PA-1 like bacteriocin production can be used in several food systems to improve their shelf-life.

Survival of *L. monocytogenes* ScottA on co-cultivation with LAB starter cultures in soymilk

In the present study, the survival of *L. monocytogenes* ScottA by the pediocin PA-1 like bacteriocin producers

under in situ conditions was observed. The mean ±SD log values during co-cultivation of the bacteriocin producers and *L. monocytogenes* ScottA are represented in Table 3. It was observed that during co-cultivation of ScottA with *P. acidilactici* NCIM 5424, no significant difference was observed in the viable counts ($P > 0.05$). Whereas, in case of *E. faecium* NCIM 5423 and *Lb. plantarum* Acr2, significant reduction was observed in the viable count of ScottA (upto 2–3 logs) during co-cultivation in the soy milk fermentation. The reduction can be correlated with the viable count of LAB, as the bacteriocin producing cultures maintained their viability during the storage period of 15 days. The *P. acidilactici* NCIM 5424 which was unable to grow in the soymilk could not reduce the *Listeria* efficiently, whereas, *E. faecium* NCIM 5423 and *Lb. plantarum* Acr2 reduced the *Listeria* count to a negligible number within 8 h of incubation in soymilk. The strong anti-listerial peptide produced by these bacteria was responsible for the decline in the *Listeria* count (Papagianni and Anastasiadou 2009). The *E. faecium* NCIM 5423 reduced the *Listeria* count more expeditiously within 4 h when compared to *Lb. plantarum* Acr2.

Table 4 Sensory quality scores of soymilk fermented with *E. faecium* NCIM 5423

Sensory parameters	Storage at 4 °C (in days)		
	1	7	15
Colour	8.9±0.9 ^b	9.1±1.1 ^b	7.9±1.2 ^a
Body	9.8±0.8 ^c	5.1±1.2 ^b	3.8±1.0 ^a
Aroma	5.3±1.3 ^a	6.9±1.1 ^b	7.2±1.2 ^b
Pulsy	6.3±0.9 ^b	5.6±0.8 ^a	5.9±0.7 ^b
Fermented	3.7±1.1 ^a	5.8±1.0 ^b	5.9±1.0 ^b
Sourish	2.4±0.8 ^a	3.7±0.9 ^b	3.0±0.9 ^a
Mouthcoating	2.9±1.1 ^a	4.7±1.2 ^b	4.2±1.4 ^b
Sourish	4.0±1.0 ^a	3.9±1.0 ^b	4.0±0.9 ^a
After taste	4.1±1.1 ^a	4.5±1.3 ^a	5.6±1.2 ^b
Overall Quality	8.4±1.2 ^b	8.0±1.3 ^b	7.6±1.4 ^a

The values are represented as mean ± SD ($n = 10$), different alphabetic superscripts (a, b and c) represented in the same columns indicate significantly different $P \leq 0.05$

Somkuti and Steinberg (2010) observed the inhibition of the *L. monocytogenes* ScottA by the pediocin producer *P. acidilactici* PAF on co-cultivation with *Streptococcus thermophilus* and *Lb. delbruekii* ssp. *bulgaricus* in milk. However, the LAB including *S. thermophilus*, *Lb. delbruekii* subsp. *bulgaricus*, *Lb. rhamnosus*, *Lb. jonsoni* are commonly used in the fermentation of soymilk to improve the flavour, texture, aroma, etc. (Donkor et al. 2007). Hence, bacteria which have the ability to survive in soymilk, produce pediocin like bacteriocin, ferment the soymilk efficiently, inhibit *Listeria*, extend the shelf-life of the end product with desirable sensory properties are of interest.

Sensory attributes of the soy yogurt fermented with *E. faecium* NCIM 5423

As the *P. acidilactici* NCIM 5424 was unable to ferment the soymilk, further studies were not performed with this culture. The incubation time after LAB inoculation required for setting the curd with good texture was considered as main criteria for fermentation. Thus, further studies on *E. faecium* NCIM 5423 were carried out. However, the quality parameters of the *E. faecium* NCIM 5423 were found to be more desirable than *Lb. plantarum* Acr2. The fermented soymilk formed by the *E. faecium* NCIM 5423 was observed to have a fat content of 24.2 % and moisture content of 87.1 %. A negligible amount of ash (0.021 g/100 g) was also recorded.

Sensory analysis was carried out to assess the quality parameters of the *E. faecium* NCIM 5423 fermented soymilk. The attributes used for fermented soymilk were colour – buff; consistency – set, mouth coating; aroma – beany, pulsey, fermented; taste – sour; aftertaste – beany and overall quality. The sensory analysis of fermented soymilk scored high points for consistency, color and taste. Table 4 gives the sensory scores of soy curd fermented with *E. faecium* NCIM 5423 on 1st, 7th and 15th day. It can be seen from Table 4 that there was no significant difference in the overall quality scores for the samples fermented on day 1 and 7 and both are found to be acceptable. It was observed that sample of day 7 was thick when compared to the samples of day 1 and 15, while no significant difference was seen between samples of day 7 and 15 in ‘beany’ aroma, ‘pulsey’ aroma, and ‘fermented’ aroma. However, slight increase in perception of sour taste was observed in sample of day 15, as fermentation time increased. It was interesting to note that the storage period did not adversely affect the acceptability of the fermented soymilk by *E. faecium* NCIM 5423 ($P>0.05$). Hence, the soymilk fermented by *E. faecium* NCIM 5423 in the present study had good stability with anti-listerial property and can be used in the dairy industry as a bio-preservative agent or as live probiotic starter bacteria.

Conclusion

The development of a good quality soy curd with intergeneric pediocin PA-1 like bacteriocin producers was achieved with *E. faecium* NCIM 5423. The curd formed after fermentation by the isolate NCIM 5423 was found to have good firmness, anti-oxidant property and acceptable sensory score. The bacteriocin produced in situ was able to inhibit *Listeria* in fermented soymilk while maintaining the viability of the bacteriocin producing organism. The use of such LAB with bacteriocin producing ability as a starter culture has the dual advantage of obtaining an acceptable fermented curd and extended shelf life of the soy product. The bacterial cultures, used in this study can be used as bio-preservative agents in diverse food systems due to its adaptability and broad spectrum of activity. A comparative study of the sensory as well as nutritive properties of the selected intergeneric pediocin producing LAB in the fermentation of different milk products like cow milk, buffalo milk, skim milk etc., will be elucidated in future studies.

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References

- Albright SC, Winston WL, Zappe C (1999) Data analysis and decision making with Microsoft excel. Brooks/Cole Publishing Company, Pacific Grove
- AOAC (1990) Official methods of analysis, 15th edn. Association of official analytical chemists, Washington D.C, ISBN 2-93 558 442-0
- Božanić R, Lovković S, Jelčić I (2011) Optimizing fermentation of soymilk with probiotic bacteria. Czech J Food Sci 29:51–56
- Burianek LL, Yousef AE (2000) Solvent extraction of bacteriocins from liquid culture. Lett Appl Microbiol 31:193–197
- Devi SM, Halami PM (2011) Detection and characterization of pediocin PA-1/AcH like bacteriocin producing lactic acid bacteria. Curr Microbiol 63:181–185
- Devi SM, Halami PM (2012) Detection of mobile genetic elements in pediocin PA-1 like producing lactic acid bacteria. J Basic Microbiol 52:1–7. doi:10.1002/jobm.201200079
- Donkor ON, Henriksson A, Vasiljević T, Shah NP (2007) α -Galactosidase and proteolytic activities of selected probiotic and dairy cultures in fermented soymilk. Food Chem 70:375–381
- Dubois M, Giles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugar and related substances. Anal Chem 28:350–356
- Duncan DB (1955) Multiple range test and multiple F tests. Biometrics 11:1–42
- Early RE (1998) Technology of dairy products, 2nd edn. Blackie academic and professional, London, p 446
- Ewe J, Wan-Abdullah W, Liong M (2010) Viability and growth characteristics of *Lactobacillus* in soymilk supplemented with B-vitamins. Int J Food Sci Nutr 61:87–107

- Favaro Trindade CSF, Terzi SC, Trugo LC, Della Modesta RC, Couri S (2001) Development and sensory evaluation of soymilk based yogurt. *Arch Latinoam Nutr* 51:100–104
- Franz CMAP, Holzapfel WH, Stiles ME (1999) Enterococci at the crossroads of food safety? *Int J Food Microbiol* 47:1–24
- Giraffa G (2003) Functionality of enterococci in dairy products. *Int J Food Microbiol* 88:215–222
- Hajirostamloo B (2009) Comparison of nutritional and chemical parameters of soymilk and cow milk. *World Acad Sci Eng Technol* 57:436–438
- Kheadr E, Zihler A, Dabour N, Lacroix C, Le Blay G, Fliss I (2010) Study of the physicochemical and biological stability of pediocin PA-1 in the upper gastrointestinal tract conditions using a dynamic in vitro model. *J Appl Microbiol* 109:54–64
- Klayraung S, Okonogi S (2009) Antibacterial and antioxidant activities of acid and bile resistant strains of *Lactobacillus fermentum* isolated from miang. *Braz J Microbiol* 40:757–766
- Kouakou P, Ghalfi H, Dortu C, Evrard P, Thonart P (2010) Combined use of bacteriocin-producing strains to control *Listeria monocytogenes* regrowth in raw pork meat. *Int J Food Microbiol* 45:937–943
- Laukova A, Czikkova S (1999) The use of enterocin CCM 4231 in soymilk to control the growth of *Listeria monocytogenes* and *Staphylococcus aureus*. *J Appl Microbiol* 87:182–186
- Lopez-Lazaro M, Akiyama M (2002) Flavonoids as anticancer agents: structure-activity relationship study. *Curr Med Chem Anti-Cancer Agents* 2:691–714
- Lui J, Lin T (2008) Survival of *Listeria monocytogenes* inoculated in retail soymilk products. *Food Control* 19:862–867
- McDougall LA, Holzapfel WH, Schillinger U, Feely DE, Rupnow JH (1994) Scanning electron microscopy of target cells and molecular weight determination of a bacteriocin produced by *Lactococcus lactis* D53. *Int J Food Microbiol* 24:95–308
- Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Anal Chem* 31:426–428
- Osuntoki A, Korie I (2010) Antioxidant activity of whey from milk fermented with *Lactobacillus* species isolated from Nigerian fermented foods. *Food Technol Biotechnol* 48:505–511
- Papagianni M, Anastasiadou S (2009) Pediocins: the bacteriocins of pediococci. Sources, production, properties and applications. *Microb Cell Factories* 8:1–16
- Raghavendra P, UshaKumari SR, Halami PM (2011) Phytate-degrading *Pediococcus pentosaceus* CFR R123 for application in functional foods. *Benefic Microbes* 2:57–61
- Rekha CR, Vijayalakshmi G (2008) Biomolecules and nutritional quality of soymilk fermented with probiotic yeast and bacteria. *Appl Biochem Biotechnol* 151:452–463
- Rossi EA, Vendramini RC, Carlos IZ, Pei YC, de Valdez GF (1999) Development of a novel fermented soymilk product with potential probiotic properties. *Eur Food Res Technol* 209:305–307
- Saccaro DM, Tamime AY, Pilleggi AOPS, Oliveira MN (2009) The viability of three probiotic organisms grown with yoghurt starter cultures during storage for 21 days at 4 °C. *Int J Dairy Technol* 62:397–404
- Somkuti GA, Steinberg DH (2003) Pediocin production by recombinant lactic acid bacteria. *Biotechnol Lett* 25:473–477
- Somkuti GA, Steinberg DH (2010) Pediocin production in milk by *Pediococcus acidilactici* in co-culture with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. *J Ind Microbiol Biotechnol* 37:65–69
- Stone H, Sidel JL (1998) Quantitative descriptive analysis: development, applications and future. *Food Technol* 52:48–52
- Todorov SD, Dicks LMT (2005) Growth parameters influencing the production of *Lactobacillus rhamnosus* bacteriocins ST461BZ and ST462BZ. *Ann Microbiol* 55:283–289
- Wang YC, Yu RC, Chou CC (2006) Antioxidative activities of soymilk fermented with lactic acid bacteria and bifidobacteria. *Food Microbiol* 18:128–135
- Williams WB, Cuvelier M, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *Lebensm Wiss Technol* 28:25–30