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Full Length Research Paper

Effect of cultural components on antimicrobial activity of bacteriocin produced by bacteria isolated from gut of poultry

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The effects of various carbon and nitrogen sources and enzymes on the antibacterial activity of *Enetrococcus faecium* bacteriocin L17 (3.5 kDa) isolated from poultry gut and confirmed by polymerase chain reaction (PCR) and 16S rDNA sequencing was carried out. The bacteriocin exhibited antibacterial activity against both gram-negative and gram-positive bacteria, as well as some funding such as Micrococcus flavus and *Candida mycoderma,* but had no effect on Lactic acid bacteria (LAB). The metabolite was stable in the presence of lipolytic or glycolytic substances and over a wide range of temperatures and its activity was also enhanced in the presence of 1 - 1.5% Tween80, 0.1% macrogol as well as various carbon (lactose) and nitrogen (lactose combined with beef extract and soy peptone) sources. Poteolytic enzymes however, completely inactivated the metabolite. These results are promising because the trend today is to employ eubiotic or symbiotic products and their use in the poultry industry could be a natural way to protect the flocks against potential pathogens.

Key words: Bacteriocin, medium composition, antimicrobial activity, poultry.

INTRODUCTION

Bacteriocins from lactic acid bacteria are peptides or proteins with antimicrobial activity against other gram-positive bacteria, including food-spoilage and/or pathogenic strains (De Vuyst et al., 1996). Because of their antimicrobial effect, bacteriocins, and/or their producing organisms, have a potential use as feed preservatives. In order to enhance and increase the productivity of bacteriocins, a better understanding of the factors affecting their production is required (Aasen et al., 2000).

Enterococci represent lactic-acid producing bacteria that can be isolated from different ecosystems (human, animals, water, soil, plants, food/feed, waste) including poultry (Strompfová and Laukova, 2007). Additionally the literature describes the characterization, isolation and genetics of bacteriocins produced by lactic acid bacteria, including *Enterococci*, especially in species related to feed industry (Strompfova et al., 2007).

While most studies of reported strains producing bacteriocins are *Lactobacillus pentosus* (Todorov and Dicks, 2004; Todorov et al., 2006), few bacteriocin-producing strains of *Enterococcus faecium* have been reported. These strains include, bacteriocins produced by *E. faecium* CRL1385 isolated from chicken (Audisio et al., 2001), the environmental strain *E. faecium* EK13 (Marekova et al., 2003), *E. faecium* GM-1 isolated from an infant (Kang et al., 2005) and *E. faecium* ALP7 isolated from shellfish (Pinto et al., 2009). In addition, fewer bacteriocin-producing strains of *E. faecium* isolated from chicken have been reported (Audisio et al., 2001; Strompfova et al., 2007). Furthermore while the screening for production of bacteriocins by animal *Enterococci* has been studied (Du Toit et al., 2000), little

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information is available about this feature in isolates obtained from chickens.

Bacteriocin production is strongly dependent on pH, nutrient sources, incubation temperature, and activity levels, and do not always correlate with cell mass or growth rate of the producer strain (Kim et al., 1997; De Rojas et al., 2004). The induction pathway may be influenced by medium and fermentation conditions, and this must be considered when interpreting the fermentation results (Nilsen et al., 1998). In some cases, higher bacteriocin activity has been recorded at suboptimal growth conditions (De Vuyst et al., 1996; Kim et al., 1997; Aasen et al., 2000; Audisto et al., 2001; Todorov et al., 2006).

Apart from studies conducted on the characterization and isolation of bacteriocin produced by *E. faecium* EK13 (Marekova et al., 2003), little is known about the growth conditions of bacteriocin produced by *E. faecium* GM-1 (Kang et al., 2005), and *E. faecium* ALP7 (Pinto et al., 2009). This also includes the understanding of the carbon and nitrogen sources required for optimal production of the bacteriocins. So, we hypothesized that the antimicrobial activity of bacteriocin produced by *E. Faecium* L17 isolated from gut of poultry could be affected by the growth condition and the optimization of the growth medium for bacteriocin L17 could be adjusted. We further investigated the optimization of the growth medium including carbon sources and nitrogen sources required to increase the levels of antimicrobial activity of this metabolite.

MATERIALS AND METHODS

Isolation and identification of bacteriocin-producing strains

E. faecium L17 used in this study was isolated from the appendix contents of poultry by aliquoting 100 µl of 5 times diluted contents of gut with 0.9% NaCl onto lactic acid bacteria selective media (Mann Rogosa Sharpe) MRS agar plates and were well diffused. The inoculated plates were incubated for 48 h at 37°C in 5% CO_2 atmosphere without agitation. Isolates were separated and identified initially at the level of genus or family based on colony morphology. Gram staining, cell morphology and sugar fermentation tests (Ling, 2003) were also conducted and each anaerobic strain was streaked on MRS agar plates two times to attain a pure culture. Before total DNA extraction, the strains were cultivated for 24 h in MRS broth at 37°C. Further identification was done by PCR with species-specific primers. The following primers were used: EntF: 5 -TAC TGA CAA ACC ATT CAT GAT- 3 and EntR: 5 -AAC TTC GTC ACC AAC GCG AAC-3 (Ke et al., 1999). Amplification of the 16S rDNA was carried out using the following protocol: primary DNA denaturation step at 94°C for 5 min, followed by 35 cycles of 30 sec at 94°C, 30 sec at 54°C, and 1 min at 72°C, with an extension of the amplified product at 72°C for 7 min. Amplification reactions were performed in a MyCycler Thermal Cycler System (Bio-Rad). Following amplification, 5 l of product was separated at 90 V for 30 min in a 1% (w/v) agarose gel in 1×TAE buffer (4.84 g Tris-base, 1.09 g glacial acetic acid, 0.29 g ethylene diaminetetra acetic acid, 1 L distilled water), and then stained with 0.5 g/ml of ethidium bromide. A 100 bp ladder (Sigma) was used to estimate the polymerase chain reaction (PCR) product size. PCR product, used as templates, were purified with the GFX PCR DNA and Band Purification kit (GE HealthCare, Amersham Biosciences, Amersham, UK) and sent to Invitrogen (Shanghai, China) for sequencing. On-line similarity searches were performed using the BLAST program in GenBank (http://www.ncbi.nlm.nih.gov).

Bacterial strains, growth condition and culture media

Bacterial strains used in this study were supplied by the following organizations: *Escherichia coli* and *Salmonella pullorum* were provided by Department of Veterinary Medicine, Heilongjiang Bayi Agricultural University; *Staphylococcus aureus* and *Streptococcus agalactiae* were supplied by Department of Veterinary Medicine, Northeast Agricultural University; and *Candida mycoderma* and *Saccharomyces cerevisiae* came from Institute of Microbiology Chinese Academy of Sciences. *Lactobacillus sakei*, *Lactobacillus curvatus, Lactobacillus plantarum* and *Lactobacillus salivarius* were obtained from clinical isolates. These strains were employed as indicator strains of antimicrobial substance production by *E. faecium* L17 (Table 1).

Detection of antimicrobial activity

Well diffusion inhibition assays were conducted by the Oxford cup test as described by Zhou et al. (2006) with the following modifications. Cell-free supernatant (CFS) harvested from MRS broths was incubated for 48 h at 37°C in 5% CO_2 atmosphere (until approx. 10^6 cfu/ ml). Cells were removed from the culture by centrifugation for 25 min at 4500 g and 4°C. Supernatants were filter-sterilized using 0.45 µm microfilters and adjusted to pH6.0 with 1 M NaOH to prevent the inhibitory effect of lactic acid. 100 µl of indicator strain culture (10^8 cfu/ml) was sprayed uniformly on the cultural agar plates; four Oxford cups were put on one of the agar plate surfaces with the test strain, and 100 µl CFS was pipetted into each Oxford cup. The whole cultural unit was incubated for 24 h at 30 or 37°C depending on the indicator organisms. Antimicrobial activity was defined by a clear zone of inhibition in the indicator lawn around the Oxford cup with CFS (Messi et al., 2001). The zones of diameters (mm) of inhibition were measured using sliding calipers, and each measurement was performed in triplicate.

Assay of sensitivity to enzyme and heat

In order to determine the type of compound causing bacterial growth inhibition, aliquots (500 µl) of the CFS were combined with equal volumes of either 0.1 mg/ml Proteinase K, 0.1 mg/ml Trypase, 0.1 mg/ml Benase, 0.1 mg/ml - amylase, 0.1 mg/ml catalase, or 1 mg/ml antalzyme (Sigma) and incubated overnight. The sterile CFS was used as a control for these incubations. The pH of the mixture and the incubation temperature were adjusted to those optimal for enzymatic activity. After 24 h the pH of all samples was readjusted to 6 to attain maximum antimicrobial activity and Oxford cup tests were conducted in triplicate against the indicator *Micrococcus flavus.*

Samples (500 µl) of the CFS were heated at 60, 70, 80, 90, and 100°C in water bath for 30 min before bacteriocin activity was measured using the Oxford cup test with *M. flavus* as the indicator strain. The experiments were carried out in triplicate using the untreated supernatant as control.

Bacteriocin synthesis in presence of complex carbon and nitrogen sources

E. faecium L17 was grown in 10 ml MRS broth for 24 h at 37°C, the

Table 1. Growth condition and subtilosin sensitivity of indicator organisms.

Organism	Growth agar medium	Growth temperature °C	Zone of inhibition (mm)
E. coli ATCC35218	LB	37	+ + +*
S. pullorum CMCC50115	LB	37	+ + +
M. flavus	LB	37	+ + +
S. aureus ATCC25923	Blood	37	+ +
S. agalactiae	Blood	37	+ +
C. mycoderma	Wort	30	+
S. cerevisiae	Wort	30	+
E. faecalis ATCC15724	MRS	37	-
Lactobacillus sakei	MRS	37	-
Lactobacillus curvatus	MRS	37	-
Lactobacillus plantarum	MRS	37	-
Lactobacillus salivarius	MRS	37	-

LB: Luria-Bertani, MRS: Mann rogosa sharpe, *-, no zone of inhibition, +, 5 mm< zone < 10 mm, + +: 10 mm < zone < 15 mm, + + +: zone > 15 mm.

culture was used to inoculate 200 ml of modified MRS broth with the following media, the carbon sources were: glucose, sucrose, maltose, amidulin, xylose, sorbitol, lactose and galactose and the nitrogen sources were: peptone, beef extract, soy peptone, tryptone, ammonium citrate, sodium nitrate, peptone plus beef extract, peptone plus soy peptone, peptone plus tryptone, peptone plus ammonium citrate, peptone plus sodium nitrate, beef extract plus soy peptone, beef extract plus tryptone, beef extract plus ammonium citrate, beef extract plus sodium nitrate in MRS respectively. Activity levels of bacteriocin L17 was determined as previously described using the Oxford cup tests and performed in triplicate. The final concentration of the total carbon or nitrogen source in the broth was 20 g/L, and each nitrogen source in the combination was 10 g/L.

Effect of stimulating factors in medium on antimicrobial activity

In order to study the effect of various stimulating factors on the antimicrobial activity of bacteriocin L17, an L8 ($4^2 \times 2^4$) orthogonal experiment was designed. Four concentration levels of Tween80 (0, 0.1, 0.6 and 1.5%), and two concentration levels of Tween20 and Macrogol 6000 (0 and 0.1%) were used as stimulating factors in MRS medium in this experiment. The activity level of bacteriocins was determined as described previously using the Oxford cup tests and all experiments were done in triplicate.

Partial purification and molecular size of bacteriocin

Strain L17 was inoculated (1% v/v) in 800 ml of MRS broth, and incubated without agitation at 37°C for 24 h. The cells were harvested (20 000 rpm, 20 min, 4°C) and the peptides precipitated from the cell -free supernatant by gradually adding various concentrations (60, 70 and 80%) saturated ammonium sulphate solution with gentle stirring at 4°C for 4 h, in independent experiment. Precipitated peptides in the pellets floating on the surface were collected and resuspended in one-tenth volume 25 mM ammonium acetate buffer (pH 6.5) . The samples were freeze dried and stored at -20°C. Antimicrobial activity test was performed according to the method previously described.

For determination of the molecular size of bacteriocin, frozen dried peptides were re-suspended in 25 mM ammonium acetate buffer (pH 6.5) and then separated by Tricine-SDS-PAGE. Low molecular weight markers with sizes ranging from 2.5 to 20 kDa (Amersham, Int. Germany) were used and standard Nisin (Sigma) was used as control. One gel was stained with Coomassie Brilliant Blue R250 (Invitrogen) after fixing, another gel was transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA) through semi-dry transfer apparatus (Bio-rad Laboratories, Inc.). The PVDF membranes were blocked at room temperature for 1 h with 5% non-fat dry milk powder dissolved in 1XTBS and then incubated at 4°C with a primary antibody. An anti-Nisin polyclonal antibody (goat anti-rabbit Nisin, 1:2000 dilute, Sc-7761, Santa Cruz Biotechnology, Inc) was used for detection of Nisin. Donkey anti-rabbit HRP-conjugated IgG (1:10000 dilute, Promega Corporation, Madison, WI) was the secondary antibody. Blots were visualized using the enhanced chemiluminescence detection system (Sigma, Oakville, ON, Canada). Photograhs of the film were scanned and densitometry was performed with Scion imaging software (Scion Corporation, Frederick, MD, USA).

Statistical analysis

Homogeneity of variances was examined and confirmed by the Levene's test for the inhibition zones using SAS (The SAS Institute, Cary, NC). Differences in these inhibition zones were compared by ANOVA Model of SAS. Where appropriate, data are presented as means $\pm$ SD. P values < 0.05 were considered significant.

RESULTS

Characterization of unknown isolate

In total 39 isolates were identified from the gut of poultry, 24 were with coccal-shaped cells and 15 of them were with rod-shaped cells. Among the 24 isolates with coccal-shapes, 20 matched *Enterococcus* and 4 matched *Lactococcus*; all of the 15 isolates with rod-shaped matched *Lactobacillus*. *E. faecium* L17 was the only organism among the coccal-shaped cells which showed antibacterial ability (Figure1) after eliminating the

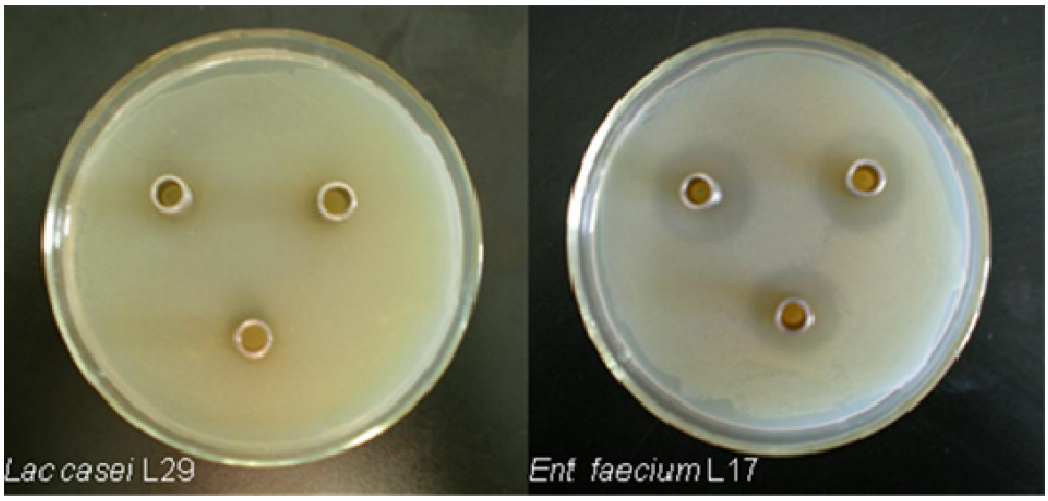

Figure 1. A comparison of isolates derived from gut of poultry, *E. faecium* L17 (on the right) and *Lactobacillus casei* L29 (on the left) in their antimicrobial activity against *Micrococcus flavus* on plates. The bacteriocin - like activity is shown by inhibition. CFS of lactic acid bacteria was spotted in triplicate; *M. flavus* was spread over the surface (plate 90 mm).

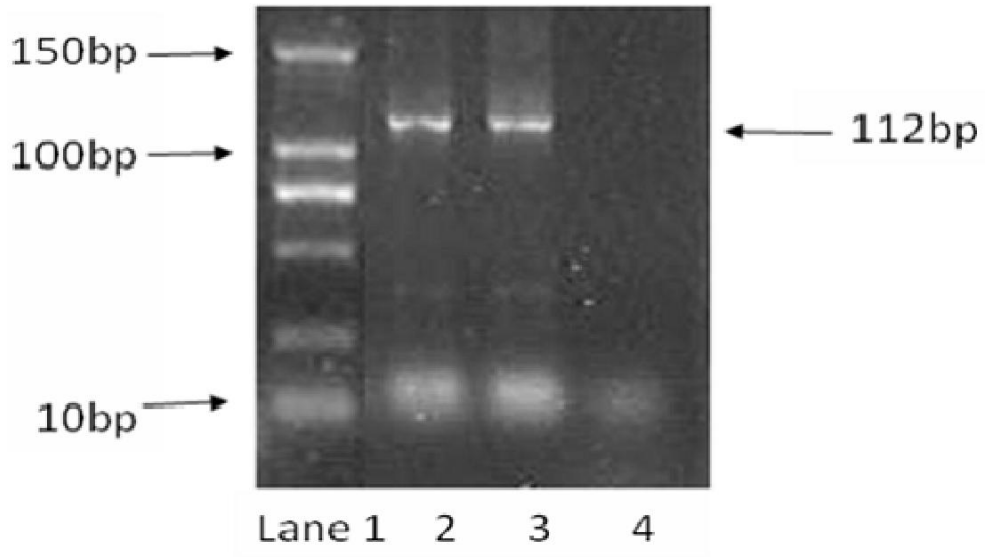

Figure 2. Agarose gel electrophoresis of PCR bands obtained after PCR amplification with genus- and species-specific primers. A DNA fragment was amplified at the size of 944bp. Lane 1, 100bp molecular marker; Lane 2, *E. faecalis* ATCC1571 as positive control; Lane 3 strain L17; Lane 4, sample without genomic DNA as negative control.

influence of lactic acid according to the Oxford cup test. *E. faecium* L17 was identified based on, gram positive reactions absence of catalase, and cell morphology (cocci in pairs). This was further confirmed by PCR with species-specific primers that yielded a 122bp fragment characteristic to the species (Figure 2). The sequence data of rDNA for L17 exhibited 99% similarities to the 16S rDNA sequences of *E. faecium* (accession no. FJ378677). Furthermore, the strain showed carbohydrate fermentation reactions characteristic for the species (data is not shown).

Screening for bacteriocin-producing lactic acid bacteria

The CFS of *E. faecium* L17 culture had antimicrobial activity against a wide range of bacterial species, including the pathogens *E. coli* and *S. pullorum*, *S. aureus* and *S. agalactiae*, as well as *C. mycoderma* and

Table 2. Effect of enzyme and heat on antimicrobial activity (expressed in zone of inhibition, mm).

Enzyme treatment (0.1 mg/ml) /zone diameter of inhibition (mm)	Heat treatment (°C) /zone diameter of inhibition (mm)
ProteinaseK/6.33* ± 0.18	60/16.55 ± 0.05
Trypase/6.15* ± 0.14	70/16.69 ± 0.30
Benase/6.09* ± 0.02	80 /14.66 ± 0.36
-amylase/13.44 ± 0.71	90/15.13 ± 0.18
Catalase/14.09 ± 0.20	100 /13.58 ± 0.29
Antalzyme/16.15 ± 0.92	Untreated CFS/16.82 ± 0.74

*Data are shown as means ± standard deviation, *P* values < 0.05 were considered significantly. The diameter of Oxford cup is about 6 mm.

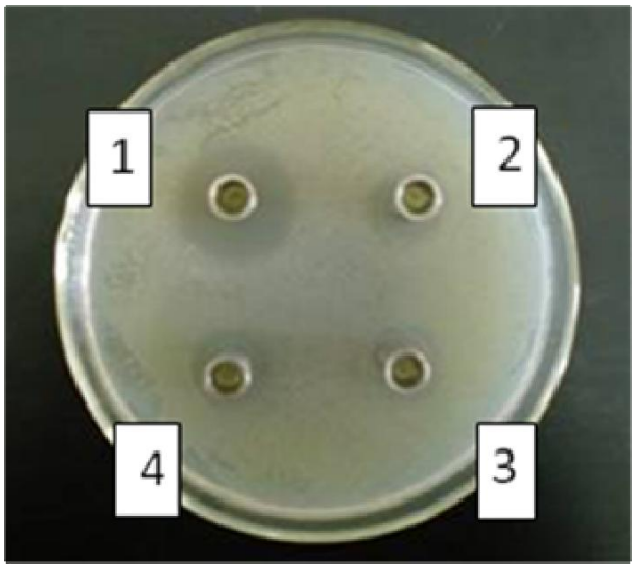

Figure 3. Antimicrobial activity of bacteriocin L17 treated with enzymes. 1, untreated CFS of *E. faecium* L17; 2, CFS treated with Proteinase K (0.1mg/ml); 3, CFS treated with Trypase (0.1 mg/ml); 4, CFS treated with Benase (0.1mg/ml). *M. flavus* was used as indicator strain.

S. cerevisiae. There was no activity against several strains of *Lactobacilli* also gathered from the clinical setting, shown in Table 1.

Effect of enzymes and heat on bacteriocin activity

Table 2 and Figure 3 showed that there was either complete inactivation or significant reduction in the antimicrobial activity after treatment with proteinase K, trypase, and benase respectively when compared with untreated CFS of *E. faecium* L17. Treatment with -amylase, catalase and antalzyme did not change the antimicrobial activity of bacteriocin L17. Similarly, there were no changes in antimicrobial activity when CFS was heated at 60, 70, 80, 90 and 100°C.

Effect of medium composition on antimicrobial activity

Figure 4 shows the results of the effect of medium composition on the antimicrobial potentials of bacteriocin L17. Results showed that addition of 0.1 and 0.6% Tween80 and 0.1% Macrogol to the MRS medium increased the antimicrobial activity of bacteriocin L17 with inhibition zones of 18.66, 17.9 and 17.6 mm respectively in comparison to the highest initial zone diameters of inhibition recorded as 16.8 mm in primary MRS broth. Conversely when 0.1% Tween20 or 1.5% Tween80 was used as the only stimulating factor, the antimicrobial activity was decreased with inhibition zones of 16.42 and 16.3 mm respectively. Furthermore, increasing the concentration of Tween80 reduced the inhibition zone. Thus the optimal production of enterotocin occurred with either 0.1 or 0.6%Tween80 or 0.1% Macrogol in MRS broth. The inhibition zone of bacteriocin L17 grown in the presence of glucose, lactose, sucrose or maltose was 18.84 mm, 18.72 mm, 16.89 mm and 17.64 mm respectively. Whereas, in the presence of galactose, xylose, sorbitol or amidulin, the inhibition zone was decreased to 15.32, 16, 15.48 and 13.46 mm. Results also showed that optimal stimulation of bacteriocin L17 production occurred with glucose, lactose, sucrose and maltose, hence their choice for the determination of effect of different nitrogen sources on bacteriocin L17 antimicrobial activity in this study.

Figure 5 shows the results of influence of lactose and glucose with different nitrogen sources separately on antimicrobial activity of bacteriocin L17. The peptone plus beef extract nitrogen source decreased the activity of bacteriocin L17 by 6.67% (15.68 mm) with glucose and by 13.45% (14.54 mm) with maltose. Figure 6 shows the results of influence of maltose and sucrose with different nitrogen sources separately on antimicrobial activity of bacteriocin L17. Despite the combinations of glucose, maltose or sucrose with different nitrogen sources, only one group of lactose (combined with peptone plus beef peptone) resulted in a reduction (inhibition zone less than 16.8 mm) of bacteriocin L17 production. The largest increase (36%) of bacteriocin production was obtained

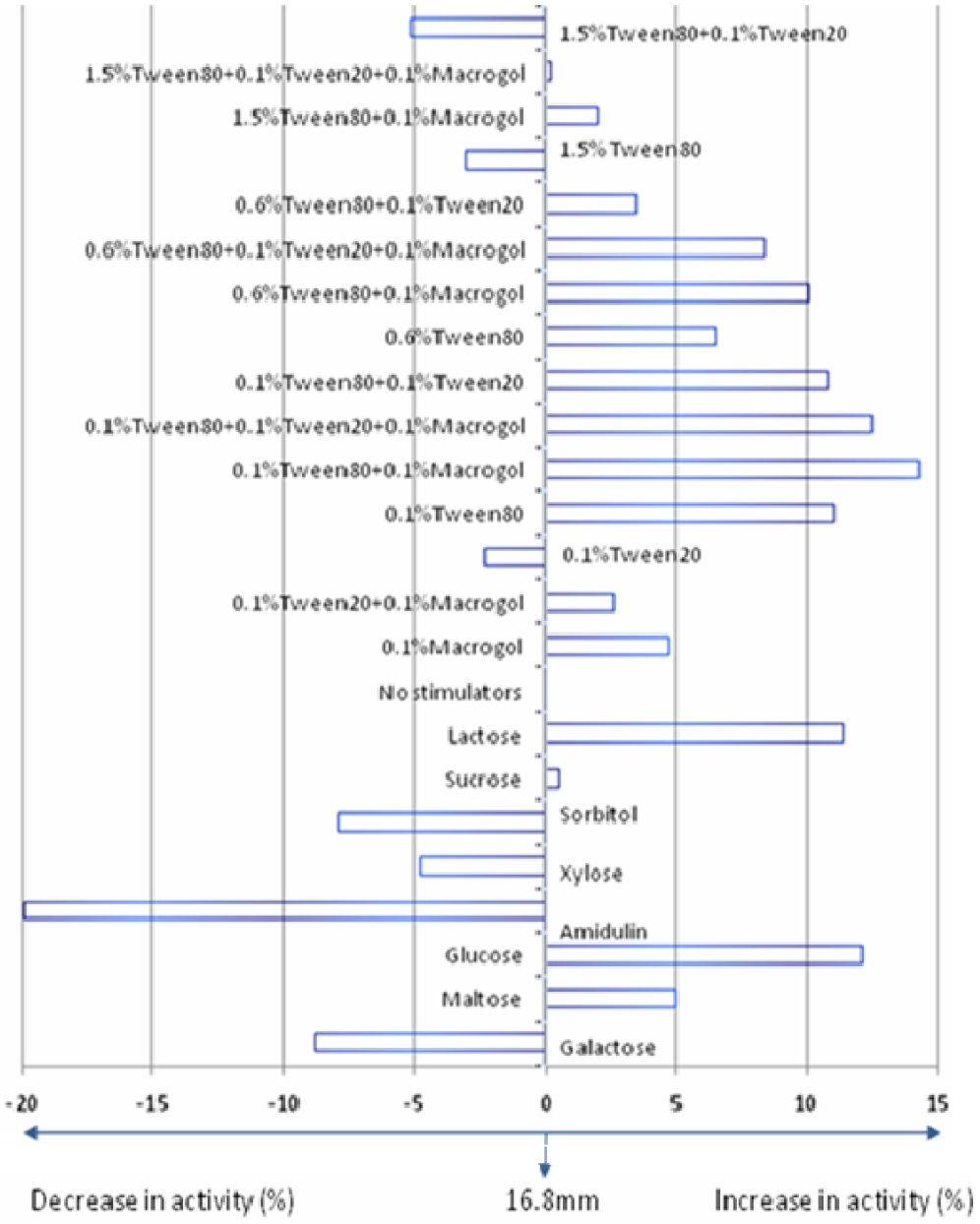

Figure 4. Influence of stimulating factors and carbon sources in MRS broth on antimicrobial activity of bacteriocin production by *E. faecium* L17. (Figure P software was used to make graphs).

with the lactose-beef extract-soy peptone combination compared to the combinations of glucose and maltose with various nitrogen sources.

Partial purification and molecular size of bacteriocins

The bacteriocin L17 was isolated from 24 h-old cultures in MRS medium and then was concentrated by precipitation and freeze-dried with 70% (w/v) ammonium sulphate. The analysis by Tricine-SDS-PAGE gel electrophoresis showed peptide bands of bacteriocin L17 with a molecular mass in the range of 3.5 kDa (Figure7; Supplement Figure 8).

DISCUSSION

Enterococci are natural inhabitants of the gastrointestinal tract (Kang and Lee, 2005). The bacteria are often used as additions to starter cultures for the preservation of various fermented foods. The preservative property of the

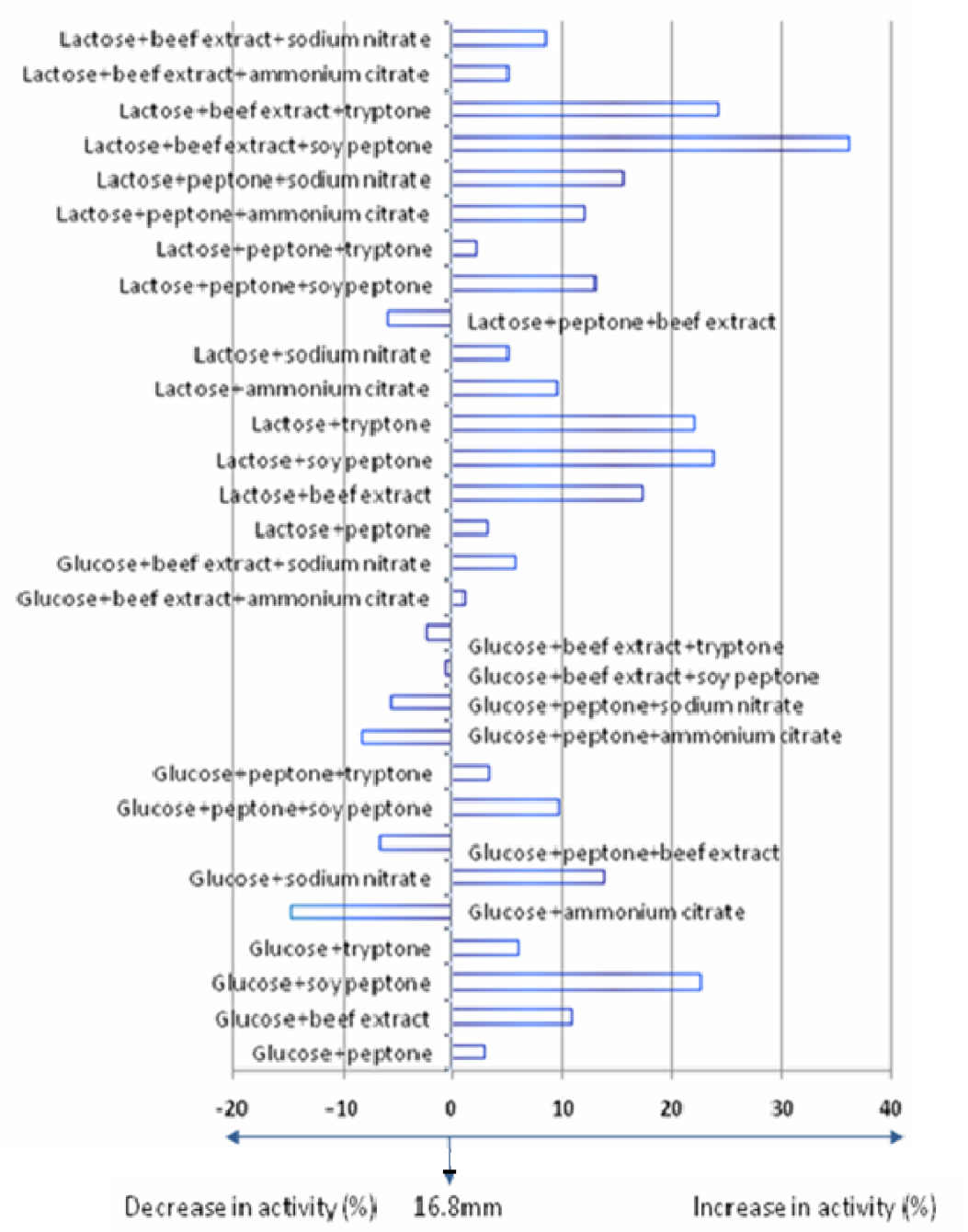

Figure 5. Influence of lactose and glucose with different nitrogen sources separately on antimicrobial activity of bacteriocin production by *E. faecium* L17. (Figure P software was used to make graphs). Wang et al.

bacteria is as a result of the inhibitory action of antibacterial peptides present in the bacteriocins (De Vuyst et al., 1996). Campo et al. (2001) reported a 28% frequency of bacteriocin producers among enterococcal isolates from chickens. Indeed, the antimicrobial activity of *E. faecium* has been widely investigated and discussed (Becquet, 2003; Alvarado et al., 2005; Strompfova and Laukova, 2007).

The present study reports the optimal conditions for the production of a bacteriocin from *E. faecium* strain L17 isolated from gut of poultry, which indicated that the production of bacteriocin L17 could be affected by composition in medium and improved by adjusting it growth condition to apply in practice. Result of the molecular size (3.5 kDa) of bacteriocin L17 obtained in this study is within the range of most bacteriocins

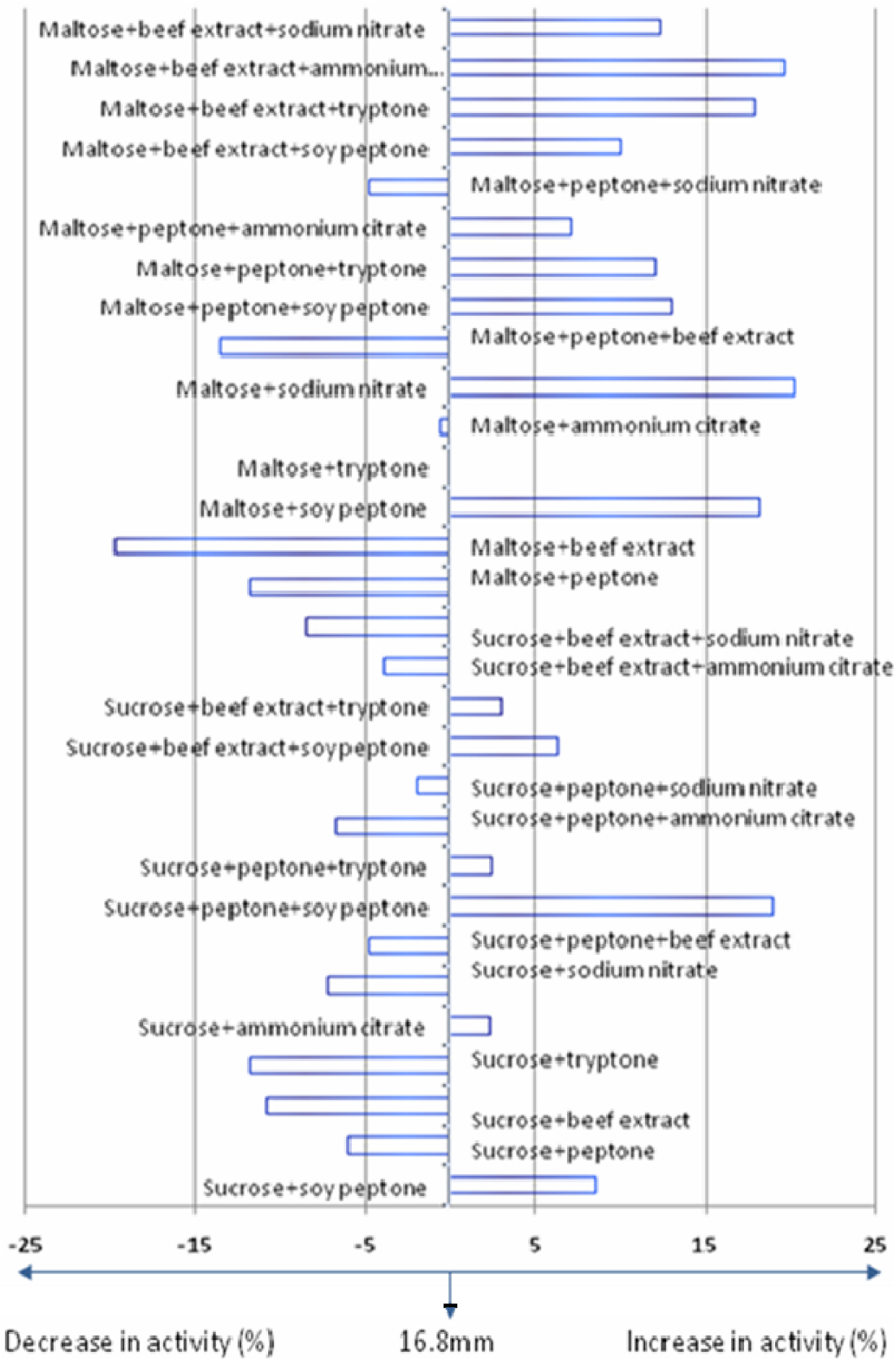

Figure 6. Influence of Maltose and Sucrose with different nitrogen sources separately on antimicrobial activity of bacteriocin production by *E. faecium* L17. (Figure P software was used to make graphs).

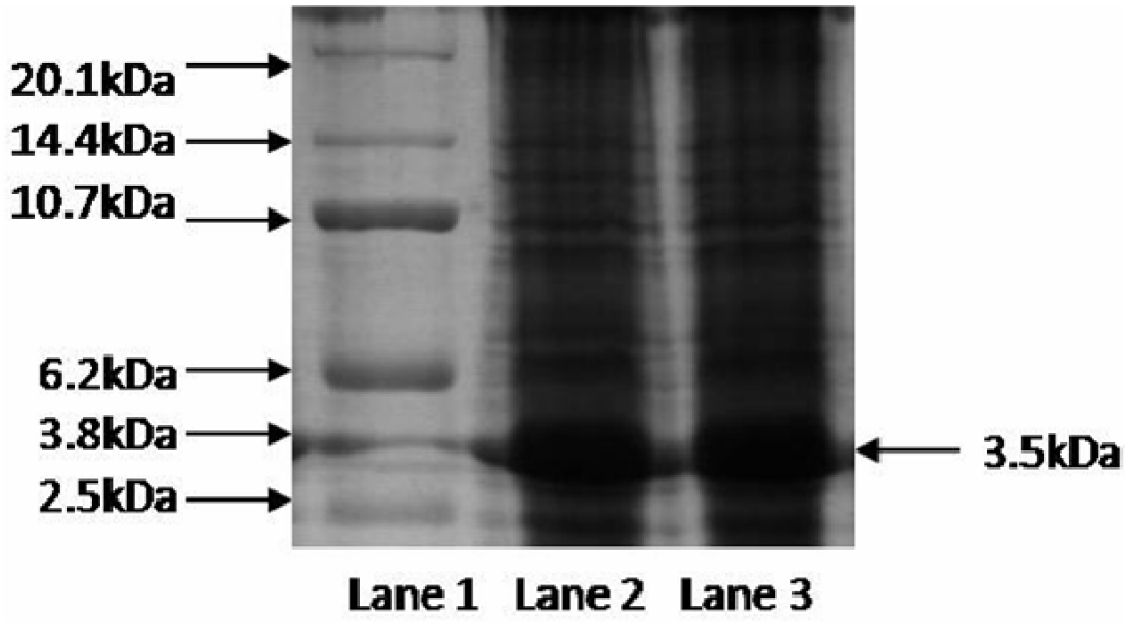

Figure 7. Tricine-SDS-PAGE gel of bacteriocin L17. Lane 1: molecular mass marker, Lane 2: band of standard NISIN, Lane 3: peptide band of bacteriocin L17 (concentrated by 70% saturated ammonium sulphate).

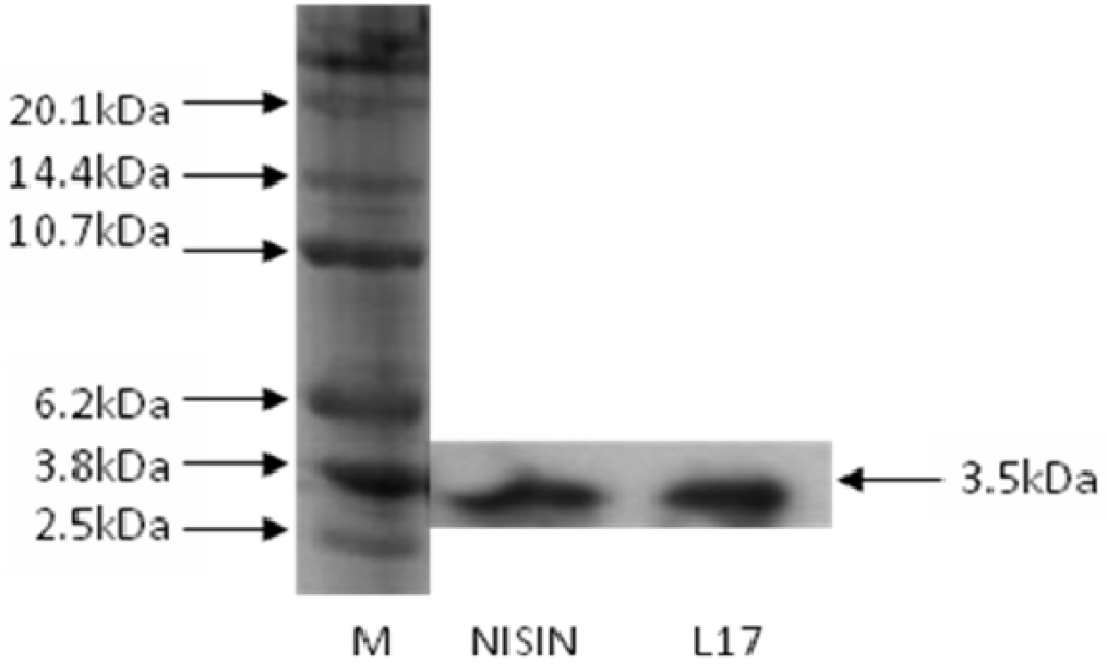

Figure 8. Western-blot of bacteriocin L17. Lane 1: molecular mass marker, Lane 2: band of standard NISIN, Lane 3: peptide band of bacteriocin L17 (concentrated by 70% saturated ammonium sulphate).

reported for the genus *Lactobacillus*. Many bacteriocins of lactic acid bacteria with activity against bacteria have been reported, viz. termophilin 81, produced by *S. thermophilus*; a bacteriocin produced by *L. lactis* KCA2386; and plantaricin 35 days, produced by *L. plantarum* (Caridi, 2002; Messi et al., 2001). The antimicrobial spectra for bacteriocin L17 include several genera with a broad spectrum of activity against gram-

positive organisms such as *M. flavus*. In addition bacteriocin L17 also demonstrated antimicrobial activity towards gram-negative indicators (Table 1), which is unusual, not in agreement with a number studies (De Vuyst et al., 2003; Marekova et al., 2003; Strompfova et al., 2007; Pinto et al., 2009), which showed antimicrobial activity to gram-positive indicators. It has been reported for few LAB bacteriocins (Messi et al., 2001; Todorov and Dicks, 2004; De Kwaadsteniet et al., 2005; Line et al., 2008) excluding *E. faecium* that showed antimicrobial activity towards gram-negative bacteria. Most bacteriocins investigated (Pinto et al. 2009; Tahiri et al., 2004; Tome et al., 2008), as in the current study, are secreted into the culture medium during the phase of growth. The ability of bacteriocin L17 to inhibit the growth of a number of bacteria, including pathogen like *Salmonellae* (Table 1), suggests that this bacteriocin may be a useful tool in feed production. However, little is known about the ability of the bacteriocin to reduce pathogen populations *in vivo*. In addition, more attention is being given to bacteriocins as possible antimicrobial agents for the reduction or elimination of pathogens like *E. coli* and *S. pullorum* (Strompfova et al., 2003; Lauková et al., 2004).

A decrease in bacteriocin concentration after the cessation of growth is a common observation that has been explained as a result of proteolytic activity, aggregation and/or adsorption to the cells (Lejeune et al., 1998; Aasen et al., 2000). In our study, enzymes treatment is indicative of the proteinaceous nature and confirms a general characteristic of bacteriocins. No change in activity was recorded when treated with -amylase, catalase and antalzyme (Table 2), indicating that H_2O_2 was not responsible for inhibition. Since the inhibitory substance produced by the strain was inactivated by proteolytic enzymes (Table 2), this confirms the proteinaceous nature and indicates the presence of bacteriocins (Todorov and Dicks, 2005). Moreover, since the activity was not affected by lipolytic or glycolytic enzymes, this suggests that the active moiety was not a lipid or a glucan, respectively as demonstrated by Tome et al. (2006) . The inhibitory effect of the bacteriocin in our study was stable when heated after 30 min at 60 -100°C, which is common with the bacteriocins, *Pendiococcus pentosaceus* ALP57 and *E. faecium* ALP7 (Pinto et al., 2009) as well as bacteriocin ST15 produced by *Enterococcus mundtii* (De Kwaadsteniet et al., 2005).

Glucose is considered the main carbon source by all microorganisms due to its size, rapid uptake, utilization and cellular energy conversion. However, some bacteria have a complete enzymatic machine that allows them to use complex carbohydrates; for example, *E. faecium* shows a variable sucrose fermentation pattern (Audisio et al., 2001), and in our study *E. faecium* L17 could utilize lactose and maltose to produce bacteriocin. The activity levels of bacteriocin L17 was strongly influenced by the carbon source added to MRS medium (Figure 4). In the presence of sorbitol, xylose, amidulin or galactose as a carbon source, low levels of the bacteriocin antimicrobial activity were produced. However, in the presence of lactose, glucose or maltose, more bacteriocin was produced as demonstrated with an inhibition zone greater than 16.8 mm.

Some studies of bacteriocin production in various lactic acid bacteria have shown that bacteriocin production can be influenced by pH, composition of growth media, temperature, inoculum size, and other environmental factors (Todorov and Dicks, 2004). Therefore, changing the components of the medium can alter the production of the bacteriocin. Despite relatively bad growth the high activity levels of bacteriocin L17 recorded in the presence of lactose, glucose, maltose, Tween80, beef extract, tryptone, soy peptone, and peptone, suggested that specific nutrients are required for bacteriocin production (Todorov and Dicks, 2004). Detectable levels of bacteriocin L17 were recorded after 3 h of growth in MRS broth (data not shown), indicating that the peptide is a primary metabolite. Similar results were reported for plantaricin Y (Chin et al., 2001) and bacteriocins produced by *Pendiococcus acidilactici* (Nieto-Lozano et al., 2002). The production of bacteriocin by *E. faecium* L17 was highly dependent on the cultural medium used and the combination of carbon source and nitrogen source yielded different levels of bacteriocin (Figures 5 and 6). This was demonstrated by the high bacteriocin production levels observed in the MRS containing lactose combined with either beef extract and soy peptone, or beef extract and tryptone, or soy peptone and tryptone. These results are supported by the observations of Verellen et al. (1998), in the case of plantaricin 423, where the highest production was obtained in MRS broth supplemented with bacteriological peptone. Similar results were also reported by Ogunbanwo et al. (2003) when the medium was supplemented with lactose, Tween20 and Tween80.

While many efforts are directed towards genetic methods to improve bacteriocin production, the metabolic regulation is still not well understood, further well-controlled studies to that continuously register base addition and calculate kinetic parameters are required.

Conclusion

In this study, bacteriocin L17 production was enhanced by the presence of 0.1 or 0.6% Tween80 and 0.1% Macrogol in MRS broth as shown in Figure 4. From this study therefore, the key carbon and nitrogen sources required for bacteriocin L17 production are lactose or lactose combined with beef extract and soy peptone respectively. Further studies on the behavior of bacteriocin in the intestinal environment are required to determine their suitability for potential biomedical applications.

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