BULGARIAN SOCIETY FOR MICROBIOLOGY
ACTA MICROBIOLOGICA BULGARICA Volume 40 / 1 (2024)

# Media Optimization for Bacteriocin Production by *Enterococcus faecium* Strains Isolated from Traditional Korean Soybean Paste

Ronaldo Rwubuzizi[1], Joanna Ivy Irorita Fugaban[1,#], Wilhelm Heinrich Holzapfel[2], Svetoslav Dimitrov Todorov[1,3-5,*]

[1]*ProBacLab, Department of Advanced Convergence, Handong Global University, Pohang, Republic of Korea*
[2]*Human Effective Microbes Laboratory, Department of Advanced Convergence, Handong Global University, Pohang, Republic of Korea*
[3]*ProBacLab, Laboratório de Microbiologia de Alimentos, Departamento de Alimentos e Nutrição Experimental, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, Brazil*
[4]*Food Research Center (FoRC), Departamento de Alimentos e Nutrição Experimental, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, Brazil*
[5]*CISAS- Center for Research and Development in Agrifood Systems and Sustainability, Instituto Politécnico de Viana do Castelo, Viana do Castelo, Portugal*
[#] *Current Address: National Food Institute, Technical University of Denmark, Kongens Lyngby, Denmark*

**Abstract**

Bacteriocinogenic *Enterococcus faecium* ST651ea, ST7119ea, and ST7319ea, were previously isolated from traditional Korean fermented soybean paste and identified by 16S rRNA sequencing and appropriate biochemical and physiological tests. Growth conditions for bacteriocin production by *E. faecium* ST651ea, ST7119ea, and ST7319ea were optimized regarding the inhibition of vancomycin-resistant *E. faecium* VRE19 of clinical origin, and *Listeria monocytogenes* ATCC15313 as a reference strain. When cultured in MRS broth for 24 h, *E. faecium* ST651ea, ST7119ea and ST7319ea produced 3200 AU/ml, 6400 AU/ml, and 6400 AU/ml, respectively, at 37°C, and 1600 AU/ml, 3200 AU/ml and 3200 AU/ml, respectively, at 30°C against *E. faecium* VRE19. MRS medium compositions were modified based on inclusion, exclusion, and/or replacement of components for optimizing the production of bacteriocins by these strains. Bacteriocins produced by *E. faecium* ST651ea, ST7119ea, and ST7319ea showed higher activity at 37°C after 15h with 20 g/l galactose replacing D-glucose and in setups containing organic nitrogen sources of only 10 g yeast extract/l and 15 g peptone/l in modified MRS. The bacteriocin produced by *E. faecium* ST651ea yielded 6400 AU/ml and 25600 AU/ml activity against *E. faecium* VRE19 and *L. monocytogenes* ATCC15313, respectively. For *E. faecium* ST7119ea results were 12800 AU/ml and 51200 AU/ml and for *E. faecium* ST7319ea were 12800 AU/ml and 51200 AU/ml against *E. faecium* VRE19 and *L. monocytogenes* ATCC15313, respectively. Based on the type and availability of different carbohydrates and organic nitrogen sources production of bacteriocins by *E. faecium* ST651ea, ST7119ea, and ST7319ea was reduced by up to 50%.

**Keywords**: *Enterococcus faecium*, *Listeria monocytogenes*, bacteriocin, production optimization

**Резюме**

Бактериоциногенните *Enterococcus faecium* ST651ea, ST7119ea и ST7319ea са изолирани преди това от традиционна корейска ферментирала соева паста и идентифицирани чрез секвениране на 16S рРНК и подходящи биохимични и физиологични тестове. Условията на растеж и получаване на бактериоцин от *E. faecium* ST651ea, ST7119ea и ST7319ea са оптимизирани по отношение инхибирането на ванкомицин-резистентен *E. faecium* VRE19 от клиничен произход и *Listeria monocytogenes* ATCC15313 като референтен щам. При култивиране в MRS бульон в продължение на 24 часа *E. faecium* ST651ea, ST7119ea и ST7319ea продуцират съответно 3200 AU/ml, 6400 AU/ml и 6400 AU/ml при 37°C и 1600 AU/ml, 3200 AU/ml и 3200 AU/ml при 30°C срещу *E. faecium* VRE19. Съставът на средата MRS е модифициран въз основа на включване, изключване и/или замяна на

*Corresponding author: slavi310570@abv.bg; todorov@usp.br
Acta Microbiol. Bulg. 2024; 40(01). https://doi.org/10.59393/amb24400109

компоненти за оптимизиране на продукцията на бактериоцини от тези щамове. Бактериоцините, продуцирани от *E. faecium* ST651ea, ST7119ea и ST7319ea, показват по-висока активност при 37°C след 15 часа с 20 g/l галактоза, заместваща D-глюкозата, и в състави, съдържащи органични източници на азот от само 10 g екстракт от дрожди/l и 15 g пептон/l в модифицираната MRS. Бактериоцинът, произведен от *E. faecium* ST651ea, проявява активност от 6400 AU/ml и 25600 AU/ml съответно срещу *E. faecium* VRE19 и *L. monocytogenes* ATCC15313. Резултатите за *E. faecium* ST7119ea са 12800 AU/ml и 51200 AU/ml, а за *E. faecium* ST7319ea са 12800 AU/ml и 51200 AU/ml срещу *E. faecium* VRE19 и *L. monocytogenes* ATCC15313, съответно. Въз основа на вида и наличието на различни въглехидрати и органични азотни източници производството на бактериоцини от *E. faecium* ST651ea, ST7119ea и ST7319ea е намалено с до 50%

## Introduction

The advancement of human society is reflected by the demand for a healthier and more sustainable environment. The nutritional industry adapted to these tendencies with the aim to provide high-quality and safe food products with health-promoting properties. The use of traditional conservation processes was questioned by consumers and the request for reduced chemical additives is part of the 21st-century perception of healthier food products. Demands for the use of natural conservation prompted scientific research for evaluation and use of different preservatives with natural origin. The application of essential oils of plant origin and different microbial metabolites with antimicrobial properties were suggested as potential substitutes for chemically defined preservatives (Damak *et al.*, 2019). Bacteriocins are antimicrobials produced by different microorganisms and belong to the largest family of AMP (antimicrobial peptides). They have a reasonably long history since their discovery back in 1928 when the first report on nisin was published (Rogers and Whittier, 1928). Authorized by EFSA and FDA, nisin as an antimicrobial peptide has been applied worldwide in different food products as a natural biopreservative for control of *Clostridium* spp., *Listeria monocytogenes* and other spoilage and food-borne pathogens (Mokoena *et al.*, 2021). One of the specific beneficial characteristics of bacteriocins is their narrow spectrum of activity, giving them an advantage in the reduction of food spoilage and pathogens without affecting the starter or beneficial cultures applied in the food preparation process (Holzapfel *et al.*, 1995).

However, the need for more bacteriocins with a specific spectrum of activity, produced at low cost and available for different food processing applications, is emerging. Production of bacteriocins is a complex process, associated with the expression of dedicated genes, related to the specific biosynthesis processes, influenced by the temperature, pH, and growth media components, ruled by induction or repression factors (Parente and Ricciardi, 1999).

Like all proteins, bacteriocins are produced via the ribosomal machinery of the bacterial cell (Altermann *et al.*, 2005; Chikindas *et al.*, 2018). However, different from some antibiotics with proteinaceous nature, bacteriocins do not enter the bacterial cytoplasm via post-production modification (Gan *et al.*, 2021). The bacteriocin operon normally consists of the genes encoding the mature antimicrobial peptide and leader peptide. In the next stage, the leader peptide is normally removed from the active bacteriocin in the process of secretion via the ABC transporter. Moreover, part of the operon includes the immunity protein involved in the protection of the producer to the effect of their own bacteriocin, accessory proteins, and the appropriate ABC transporter (Smits *et al.*, 2020). The presence of all components in the functional bacteriocin operon is essential for the effective production and expression of the mature bacteriocin (Drider *et al.*, 2006). Environmental conditions (temperature, pH, medium components, etc.) can influence different steps of the bacteriocin production and expression; and by this influence the bacteriocin activity detected.

The potential application of bacteriocins as bio-preservatives has been the subject of numerous studies (Choi *et al.*, 2022; Fugaban *et al.*, 2022). Further studies have also focused on their possible application as adjunct components in the treatment of infectious diseases of humans and animals (Lynn and Cohen, 1995) and, in addition, they have been suggested for the treatment of some types of cancers (Kaur and Kaur, 2015; Molujin *et al.*, 2022). However, a key issue for their application is how bacteriocins can be produced economically in sufficient quantity and semi-purified or purified to homogeneity. Bacteriocin production can be realized in rich laboratory media and conditions, however, a major problem would be their large-scale production in low-cost media (Kumar *et al.*, 2022). Thus, appropriate optimization of the production process and the use of low-cost media still constitute a great challenge for the industrial production of bacterio-

cins. The objective of this study was to explore possibilities for the optimization of bacteriocin production by previously characterized bacteriocinogenic *E. faecium* strains ST651ea, ST7119ea, and ST7319ea (Fugaban *et al.*, 2021). A specific focus has been on obtaining a favorable balance between production cost and the level of expressed bacteriocin/s.

## Material and Methods

*Bacterial strains*

Bacteriocinogenic *E. faecium* strains ST651ea, ST7119ea, and ST7319ea, isolated from traditional Korean fermented soybean paste, were previously identified based on partial 16S rRNA sequencing and appropriate biochemical and physiological tests (Fugaban *et al.*, 2021). Bacteriocionogenic strains were maintained and cultured in MRS broth (Difco, Franklin Lakes, NJ, USA) at 37°C for 24 h. Test organisms (indicator organism) applied in this study for determination of bacteriocin activity were vancomycin-resistant *E. faecium* VRE19, a strain of clinical origin, and *L. monocytogenes* ATCC15313, and were cultured in BHI (Difco) or BHI supplemented with 1% agar (LPS solution. Daejeon, Republic of Korea). All bacterial cultures were stored in their corresponding growth medium with 30% glycerol (Samchun Chemicals, Daejeon, Republic of Korea) at -20°C.

*Bacteriocin activity determination and principal parameters of bacterial behavior*

The bacterial cultures were grown in MRS broth at 37°C for 24 h. At selected intervals acidification was determined by changes in pH; bacterial growth was measured and monitored via changes in OD at 600 nm; and CFU/ml was calculated based on previously generated calibration curves providing the relation between $OD_{600}$ and CFU/ml for the studied *E. faecium* strains. Antimicrobial activity was expressed as AU/ml according to recommendations from Valledor *et al*. (2022), where serial 2-fold dilutions of the cell-free supernatant (CFS) collected after centrifugation (5000 ×g, 10 min, 20°C) were prepared and 10 µl from each dilution spotted on the surface of previously prepared plates with BHI soft agar (1% *w/v*) and seeded with the test organisms (*L. monocytogenes* ATCC 15313 or *E. faecium* VRE19) at $10^6$ CFU/ml final concentration. Plates were incubated at 37°C for 24 h and checked for inhibition zones, where zones of at least 2 mm were considered as positive evidence for the presence of a bacteriocin(s). Calculation of the AU/ml was based on the serial dilution, the volume of the spotted material, and the inhibition halo of the more diluted CFS presenting the inhibition zone of at least 2 mm in diameter, as follows:

$$\text{AU/ ml} = \frac{D^n \; x \; 1000}{p},$$

where *D* was the level of serial dilution, *n* was the highest dilution where at least 2 mm of inhibition was recorded, *p* was the volume of the spotted material containing bacteriocins in µl and 1000 was the conversion factor between µL and ml (Valledor *et al.*, 2022).

Bacteriocin activity was expressed as AU/CFU relative to the number of viable cells that were calculated based on the bacteriocin activity expressed as AU/ml and bacterial population, as CFU/ml. This, and further experiments were performed on at least 2 indepedent occasions.

*Effect of temperature on bacteriocin production*

For evaluation of the role of incubation temperature, the *E. faecium* strains ST651ea, ST7119ea, and ST7319ea were cultured in MRS broth (Difco) at 25, 30, and 37°C for 24 h with pH corrected to 6.5. Bacterial growth was recorded according to the changes in the OD monitored at 600 nm and the CFU/ml was estimated based on a previously prepared calibration curve. The pH evolution was monitored during the fermentation period. Samples were withdrawn for the determination of bacteriocin activity, expressed as AU/ml and AU/CFU calculated simultaneously.

*Effect of media components (organic nitrogen and carbon sources) on bacteriocin production*

Different modifications of the commercial MRS broth were prepared to evaluate the effect of the organic nitrogen sources and carbohydrates on growth, acidification, and bacteriocin production by *E. faecium* strains ST651ea, ST7119ea, and ST7319ea. For evaluating the role of carbohydrates in bacteriocin production, MRS broth was prepared according to the normal composition of the commercial product (Difco), but where glucose was replaced with the same amount (20 g/l) of lactose, sucrose, fructose, galactose, or maltose, while MRS with glucose served as control.

The effect of organic nitrogen sources (beef extract, yeast extract, and peptone) was determined in modified commercial MRS, prepared with variations in organic nitrogen components as follows [g/l]: 10.0 beef extract, 5.0 yeast extract, and 10 peptone; 25 beef extract; 25 yeast extract; 25 peptone; 10 yeast extract and 15 peptone; 12.5 beef ex-

**Table 1.** Effect of individual components (organic nitrogen and carbohydrates) on bacterial growth and production of bacteriocin/s by *Enterococcus faecium* ST651ea, ST7119ea, and ST7319ea recorded after 24 h incubation at 37°C

| Groups | Beef extract | Yeast extract | Peptone | Carbo-hydrate (20 g/l) | OD 600 nm | pH | Log CFU/ml | AU/log CFU | | AU/ml | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | vs. VRE19 | vs. ATCC 15313 | vs. VRE19 | vs. ATCC 15313 |
| *Enterococcus faecium* ST651ea | | | | | | | | | | | |
| Control (MRS) | 10 | 5 | 10 | glucose | 1.456 | 4.3 | 9.90 | 646.36 | 5170.86 | 6400 | 51200 |
| A | 25 | 0 | 0 | glucose | 1.046 | 4.46 | 9.62 | 332.53 | 2660.27 | 3200 | 25600 |
| B | 0 | 25 | 0 | glucose | 1.402 | 4.43 | 9.87 | 648.44 | 5187.53 | 6400 | 51200 |
| C | 0 | 0 | 25 | glucose | 1.478 | 4.42 | 9.91 | 322.77 | 2582.13 | 3200 | 25600 |
| D | 0 | 10 | 15 | glucose | 1.554 | 4.47 | 9.96 | 642.80 | 5142.36 | 6400 | 51200 |
| E | 12.5 | 0 | 12.5 | glucose | 1.440 | 4.45 | 9.89 | 323.48 | 5175.72 | 3200 | 51200 |
| F | 15 | 10 | 0 | glucose | 1.341 | 4.41 | 9.83 | 325.46 | 1301.83 | 3200 | 12800 |
| G | 10 | 5 | 10 | lactose | 1.423 | 4.69 | 9.88 | 323.81 | 5180.96 | 3200 | 51200 |
| H | 10 | 5 | 10 | sucrose | 1.483 | 4.46 | 9.92 | 645.35 | 5162.79 | 6400 | 51200 |
| K | 10 | 5 | 10 | fructose | 1.212 | 4.42 | 9.75 | 164.15 | 5252.82 | 1600 | 51200 |
| L | 10 | 5 | 10 | galactose | 1.495 | 4.69 | 9.74 | 657.40 | 2629.62 | 6400 | 25600 |
| M | 10 | 5 | 10 | maltose | 1.461 | 4.55 | 9.90 | 323.08 | 5169.35 | 3200 | 51200 |
| *Enterococcus faecium* ST7119ea | | | | | | | | | | | |
| Control (MRS) | 10 | 5 | 10 | glucose | 1.377 | 4.43 | 9.85 | 649.44 | 5195.51 | 6400 | 51200 |
| A | 25 | 0 | 0 | glucose | 1.007 | 4.48 | 9.59 | 667.29 | 2669.15 | 6400 | 25600 |
| B | 0 | 25 | 0 | glucose | 1.326 | 4.45 | 9.82 | 651.54 | 2606.16 | 6400 | 25600 |
| C | 0 | 0 | 25 | glucose | 1.504 | 4.46 | 9.93 | 322.29 | 2578.31 | 3200 | 25600 |
| D | 0 | 10 | 15 | glucose | 1.425 | 4.41 | 9.88 | 647.54 | 5180.34 | 6400 | 51200 |
| E | 12.5 | 0 | 12.5 | glucose | 1.348 | 4.49 | 9.84 | 325.31 | 1301.25 | 3200 | 12800 |
| F | 15 | 10 | 0 | glucose | 1.27 | 4.43 | 9.79 | 326.98 | 1307.92 | 3200 | 12800 |
| G | 10 | 5 | 10 | lactose | 1.398 | 4.66 | 9.87 | 324.30 | 2594.40 | 3200 | 25600 |
| H | 10 | 5 | 10 | sucrose | 1.384 | 4.47 | 9.86 | 324.58 | 2596.63 | 3200 | 25600 |
| K | 10 | 5 | 10 | fructose | 1.061 | 4.39 | 9.64 | 166.06 | 2656.96 | 1600 | 25600 |
| L | 10 | 5 | 10 | galactose | 1.568 | 4.7 | 9.72 | 658.71 | 5269.66 | 6400 | 51200 |
| M | 10 | 5 | 10 | maltose | 1.404 | 4.54 | 9.87 | 648.37 | 2593.45 | 6400 | 25600 |
| *Enterococcus faecium* ST7319ea | | | | | | | | | | | |
| Control (MRS) | 10 | 5 | 10 | glucose | 1.453 | 4.46 | 9.90 | 646.45 | 5171.59 | 6400 | 51200 |
| A | 25 | 0 | 0 | glucose | 1.019 | 4.44 | 9.60 | 333.30 | 2666.37 | 3200 | 25600 |
| B | 0 | 25 | 0 | glucose | 1.421 | 4.45 | 9.88 | 323.84 | 2590.71 | 3200 | 25600 |
| C | 0 | 0 | 25 | glucose | 1.582 | 4.43 | 9.97 | 320.90 | 2567.20 | 3200 | 25600 |
| D | 0 | 10 | 15 | glucose | 1.557 | 4.4 | 9.96 | 642.66 | 5141.32 | 6400 | 51200 |

| Groups | Beef extract | Yeast extract | Peptone | Carbo-hydrate (20 g/l) | OD 600 nm | pH | Log CFU/ml | AU/log CFU | | AU/ml | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | vs. VRE19 | vs. ATCC 15313 | vs. VRE19 | vs. ATCC 15313 |
| E | 12.5 | 0 | 12.5 | glucose | 1.405 | 4.41 | 9.87 | 162.08 | 2593.21 | 1600 | 25600 |
| F | 15 | 10 | 0 | glucose | 1.324 | 4.46 | 9.82 | 325.80 | 1303.22 | 3200 | 12800 |
| G | 10 | 5 | 10 | lactose | 1.377 | 4.71 | 9.85 | 324.72 | 2597.76 | 3200 | 25600 |
| H | 10 | 5 | 10 | sucrose | 1.436 | 4.46 | 9.89 | 323.56 | 5176.95 | 3200 | 51200 |
| K | 10 | 5 | 10 | fructose | 1.209 | 4.4 | 9.75 | 164.19 | 5253.94 | 1600 | 51200 |
| L | 10 | 5 | 10 | galactose | 1.572 | 4.7 | 9.72 | 329.26 | 5268.10 | 6400 | 51200 |
| M | 10 | 5 | 10 | maltose | 1.442 | 4.55 | 9.89 | 323.44 | 2587.56 | 3200 | 25600 |

VRE19: *E. faecium* VRE19; ATCC 15313: *L. monocytogenes* ATCC 15313. description of the media composition: Control: 10 g/l BE (beef extract), 5 g/l YE (yeast extract), 10 g/l PE (peptone), 20 g/l glucose; A: 25 g/l BE, 0 g/l YE, 10 g/l PE, 20 g/l glucose; B: 0 g/l BE, 25 g/l YE, 0 g/l PE, 20 g/l glucose; C: 0 g/l BE, 0 g/l YE, 25 g/l PE, 20 g/l glucose; D: 0 g/l BE, 10 g/l YE, 15 g/l PE, 20 g/l glucose; E: 12.5 g/l BE, 0 g/l YE, 12.5 g/l PE, 20 g/l glucose; F: 15 g/l BE, 10 g/l YE, 0 g/l PE, 20 g/l glucose; G: 10 g/l BE, 5 g/l YE, 10 g/l PE, 20 g/l lactose; H: 10 g/l BE, 5 g/l YE, 10 g/l PE, 20 g/l sucrose; K: 10 g/l BE, 5 g/l YE, 10 g/l PE, 20 g/l fructose; L: 10 g/l BE, 5 g/l YE, 10 g/l PE, 20 g/l galactose; M: 10 g/l BE, 5 g/l YE, 10 g/l PE, 20 g/l maltose

tract and 12.5 peptone; 15.0 beef extract and 10.0 yeast extract (Table 1). The strains were grown in commercial 20 ml MRS (Difco) for 24 h at 37°C, harvested by centrifugation (5000 ×g, 10 min, 20°C), washed 2 times with sterile saline (0.85% NaCl, *w/v*) and resuspended in the original volume (20 ml) in sterile saline. Twenty milliliters of the different variations of MRS broth (listed in Table 1) were inoculated with 2% of prepared cell suspensions and incubated for 24 h at 37°C. At specific intervals aliquots were withdrawn and evaluated for changes in pH, OD at 600 nm, and bacteriocin activity (AU/ml) against *L. monocytogenes* ATCC 15313 and *E. faecium* VRE19. Based on previously the generated calibration curve, CFU/ml was calculated and the AU/CFU was determined.

*Comparison of bacterial growth in commercial MRS and modified MRS composed according to the individual parameters for optimization*

The modified MRS broth was designed based on previous results on the effect of carbohydrate and organic nitrogen sources on growth and bacteriocin production by *E. faecium* ST651ea, ST7119ea, and ST7319ea strains. The strains were grown in 20 ml commercial (Difco) MRS broth for 24 h at 37°C, washed 2 times in sterile saline, and resuspended in the original volume (20 ml) in physiological saline solution. Aliquots of 2% were used for the inoculation of standard MRS broth (MRS, Difco) and modified MRS according to the results from previously optimized individual parameters.

Cultures were grown at 37°C for 24 h and aliquots were withdrawn at set intervals for determination of pH, $OD_{600}$, and bacteriocin activity, expressed as AU/ml against *L. monocytogenes* ATCC 15313 and *E. faecium* VRE19. In addition, based on the previously generated calibration curve, CFU/ml for each strain was calculated and AU/CFU was estimated.

**Results and Discussion**

Industrial production of antimicrobial metabolites is a complex process where cost-effective practices have always been considered a priority. In research protocols of time-consuming and expensive processes, the objective is to produce the metabolite in high purity and by constant expression. However, for industrial production, low-cost growth media have been suggested for bacterial growth and the production of particular metabolites (Manzoor *et al.*, 2017). Temperature control of the fermentation processes was selected aiming to save energy and only partial purifications and even the use of the crude bacterial extracts were applied for the applications of the antimicrobial metabolites (Al-Saraireh *et al.*, 2015). Moreover, even in proposed applications of bacteriocins for pharmaceutical purposes, semi-purified preparations were often suggested to reduce the cost of purification steps, however, without compromising the safety issues (Fugaban *et al.*, 2022).

In general, MRS growth medium is considered as expensive for the commercial production of bacteriocins, and therefore different side streams from the food processing industry were suggested as substrates for the culturing bacteriocinogenic strains with the aim of low-cost production of

antimicrobial peptides. Previously, molasses was suggested as a substrate for bacteriocin production (Todorov and Dicks, 2005a; Metsoviti *et al.*, 2011) by *Lactiplantibacillus plantarum* strains, while waste residues from the sugar industry (Kuniyoshi *et al.*, 2021) can be applied in the production of bacteriocin by *Pediococcus pentosaceus* ET34. These further highlighted the effect of different additives and media component levels on bacteriocin activity expressed by *P. pentosaceus* ET34 (Kuniyoshi *et al.*, 2021). Cheese whey was also suggested as a growth media for bacteriocin production by bacteriocinogenic *Pediococcus pentosaceus* 147 in co-culture with an inducer strain *L. plantarum* LE27 (Gutiérrez-Cortés *et al.*, 2018). In addition, waste products from the fish processing industry can be applied as substrates for the production of bacteriocins (Vázquez-Rowe *et al*., 2010).

The principal challenges in using different waste products from the food industry are associated with the fastidious growth requirement of most LAB that often do not grow very well in these "natural" substrates which, in addition, cannot be considered as media with standardized chemical composition. Levels of carbohydrates, organic nitrogen sources, and their mineral composition vary, and this can influence bacterial growth, which, consequently affects the predictability of bacteriocin expression. It has been shown that some changes in the composition of the growth media can result in a significant reduction or even complete inhibition of bacteriocin production (Vimont *et al.*, 2017). Thus, the influence of the food waste components in a culturing medium on bacterial growth and bacteriocin production are critical points that need to be clarified when industrial food waste products are considered for the biotechnical production of antimicrobial peptides.

For the production of bacteriocins by *E. faecium* ST651ea, ST7119ea, and ST7319ea, incubation temperatures (25°C, 30°C, 37°C) were not associated with significant variation in bacteriocin activity recorded versus *L. monocytogenes* ATCC 15313 or *E. faecium* VRE19 (Table 2). Moreover, neither was the incubation temperature shown to be a principal factor affecting bacteriocin production for the studied enterococci. In principle, the incubation temperature of the MRS broth was affecting bacterial growth, as expected; however, when calculating bacteriocin activity relative to bacterial growth (AU/CFU), similar levels of bacteriocin expression were recorded (Table 2). Thus, to conclude the effect of the specific environmental factor on bacteriocin production, the growth of the producer strains needs to be monitored, and parameters such as AU/CFU are calculated for comparison of different conditions.

*Enterococcus* spp. are known for their relative tolerance to different variations in temperature and pH. According to Bergey's Manual (de Vos *et al.*, 2011), *E. faecium* strains can tolerate growth temperatures between 10°C and 45°C with an optimal for growth at 35°C. Moreover, *E. faecium* strains can multiply at pH between 5.0 and 9.0 (de Vos *et al.*, 2011). Regarding the effect of incubation temperature and initial pH of the growth media, optimal conditions for bacteriocin production of *L. plantarum* ST194BZ was previously reported to be 30°C and 37°C, and an initial pH of 4.5, 5.0, 5.5, 6.0, and 6.5 (Todorov and Dicks, 2005b). Moreover, Malheiros *et al*. (2015) pointed out the influence of the incubation temperature and initial pH of the growth media on bacteriocin production, however, linking the data with bacterial growth is still unsatisfactory.

The principal role of organic nitrogen in the growth media is to provide structural material for the production and synthesis of proteins by the microorganisms. Whereas, the addition of yeast extract can be considered a source of vitamins, co-factors, and specific growth-promoting factors (Chauhan and Chundawat, 2019). For the growth and expression of bacteriocins by bacteria, not only proteinaceous components should be part of the cultivation media, but also the compatibility and right proportions of amino acids are considered to be crucial factors, particularly in the productivity of bacteriocin formation.

By definition, most of the bacteriocins are cationic peptides, several of them containing disulfide bridges, this being related to the predominant presence of positively charged and sulfur-containing amino acids (methionine, cysteine, homocysteine, and taurine). Methionine and cysteine are classified as proteinogenic, canonic amino acids incorporated in protein structure (Colovic *et al.*, 2018). Limited availability of these specific amino acids can result in reduced bacteriocin expression. Moreover, several LABs require the presence of some vitamins as important co-factors in the enzymatic anabolic processes, and yeast extract can play an important role in providing these micronutrients for the functionality of the bacterial cells.

In this study, we have observed that the organic nitrogen source plays a limited role in both the growth of the studied enterococci and in the pro-

**Table 2.** Effect of incubation temperature of the modified MRS broth and cultivation temperature on bacterial growth and production of bacteriocin/s by *E. faecium* ST651ea, ST7119ea, and ST7319ea recorded after 24 h

| Groups | pH | Temperature (°C) | OD 600 nm | pH | Log CFU/ml | AU/log CFU | | AU/ml | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | vs. VRE19 | vs. ATCC 15313 | vs. VRE19 | vs. ATCC 15313 |
| *Enterococcus faecium* ST651ea | | | | | | | | | |
| A | 6.5 | 37 | 1.046 | 4.46 | 9.62 | 332.64 | 2661.12 | 3200 | 25600 |
| B | 6.5 | 37 | 1.402 | 4.43 | 9.87 | 648.43 | 5187.44 | 6400 | 51200 |
| C | 6.5 | 37 | 1.478 | 4.42 | 9.91 | 322.91 | 2583.25 | 3200 | 25600 |
| D | 6.5 | 37 | 1.554 | 4.47 | 9.96 | 642.57 | 5140.56 | 6400 | 51200 |
| E | 6.5 | 37 | 1.44 | 4.45 | 9.89 | 323.56 | 5176.95 | 3200 | 51200 |
| F | 6.5 | 37 | 1.341 | 4.41 | 9.83 | 325.53 | 1302.14 | 3200 | 12800 |
| A | 6.5 | 30 | 1.072 | 4.62 | 9.64 | 331.95 | 1327.80 | 3200 | 12800 |
| B | 6.5 | 30 | 1.035 | 4.64 | 9.61 | 166.49 | 5327.78 | 1600 | 51200 |
| C | 6.5 | 30 | 1.024 | 4.59 | 9.61 | 332.99 | 2663.89 | 3200 | 25600 |
| D | 6.5 | 30 | 1.057 | 4.63 | 9.63 | 332.29 | 1329.18 | 3200 | 12800 |
| E | 6.5 | 30 | 1.103 | 4.62 | 9.67 | 165.46 | 2647.36 | 1600 | 25600 |
| F | 6.5 | 30 | 1.053 | 4.61 | 9.63 | 332.29 | 1329.18 | 3200 | 12800 |
| A | 6.5 | 25 | 1.037 | 5.14 | 9.62 | 166.32 | 1330.56 | 1600 | 12800 |
| B | 6.5 | 25 | 1.046 | 5.08 | 9.62 | 166.32 | 2661.12 | 1600 | 25600 |
| C | 6.5 | 25 | 1.022 | 5.15 | 9.6 | 333.33 | 1333.33 | 3200 | 12800 |
| D | 6.5 | 25 | 1.053 | 5.15 | 9.63 | 332.29 | 1329.18 | 3200 | 12800 |
| E | 6.5 | 25 | 1.104 | 5.26 | 9.67 | 330.92 | 1323.68 | 3200 | 12800 |
| F | 6.5 | 25 | 1.075 | 5.02 | 9.65 | 165.80 | 1326.42 | 1600 | 12800 |
| *Enterococcus faecium* ST7119ea | | | | | | | | | |
| A | 6.5 | 37 | 1.007 | 4.48 | 9.59 | 667.36 | 2669.45 | 6400 | 25600 |
| B | 6.5 | 37 | 1.326 | 4.45 | 9.82 | 651.73 | 2606.92 | 6400 | 25600 |
| C | 6.5 | 37 | 1.504 | 4.46 | 9.92 | 322.58 | 2580.65 | 3200 | 25600 |
| D | 6.5 | 37 | 1.425 | 4.41 | 9.88 | 647.77 | 5182.19 | 6400 | 51200 |
| E | 6.5 | 37 | 1.348 | 4.49 | 9.84 | 325.20 | 1300.81 | 3200 | 12800 |
| F | 6.5 | 37 | 1.27 | 4.43 | 9.79 | 326.86 | 1307.46 | 3200 | 12800 |
| A | 6.5 | 30 | 1.069 | 4.61 | 9.64 | 663.90 | 1327.80 | 6400 | 12800 |
| B | 6.5 | 30 | 1.052 | 4.67 | 9.63 | 332.29 | 1329.18 | 3200 | 12800 |
| C | 6.5 | 30 | 1.056 | 4.63 | 9.63 | 332.29 | 1329.18 | 3200 | 12800 |
| D | 6.5 | 30 | 1.062 | 4.66 | 9.64 | 165.98 | 5311.20 | 1600 | 51200 |
| E | 6.5 | 30 | 1.085 | 4.7 | 9.65 | 331.61 | 1326.42 | 3200 | 12800 |
| F | 6.5 | 30 | 1.083 | 4.63 | 9.65 | 165.80 | 2652.85 | 1600 | 25600 |

| Groups | pH | Temperature (°C) | OD 600 nm | pH | Log CFU/ml | AU/log CFU | | AU/ml | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | vs. VRE19 | vs. ATCC 15313 | vs. VRE19 | vs. ATCC 15313 |
| A | 6.5 | 25 | 1.079 | 5.04 | 9.65 | 331.61 | 1326.42 | 3200 | 12800 |
| B | 6.5 | 25 | 1.043 | 5.06 | 9.62 | 332.64 | 2661.12 | 3200 | 25600 |
| C | 6.5 | 25 | 1.086 | 5.01 | 9.65 | 165.80 | 1326.42 | 1600 | 12800 |
| D | 6.5 | 25 | 1.029 | 5.00 | 9.61 | 166.49 | 2663.89 | 1600 | 25600 |
| E | 6.5 | 25 | 1.096 | 5.03 | 9.66 | 165.63 | 1325.05 | 1600 | 12800 |
| F | 6.5 | 25 | 1.039 | 5.12 | 9.62 | 166.32 | 1330.56 | 1600 | 12800 |
| *Enterococcus faecium* ST7319ea | | | | | | | | | |
| A | 6.5 | 37 | 1.019 | 4.44 | 9.61 | 332.99 | 2663.89 | 3200 | 25600 |
| B | 6.5 | 37 | 1.421 | 4.45 | 9.88 | 323.89 | 2591.09 | 3200 | 25600 |
| C | 6.5 | 37 | 1.582 | 4.43 | 9.97 | 320.96 | 2567.70 | 3200 | 25600 |
| D | 6.5 | 37 | 1.557 | 4.4 | 9.96 | 642.57 | 5140.56 | 6400 | 51200 |
| E | 6.5 | 37 | 1.405 | 4.41 | 9.87 | 162.11 | 2593.72 | 1600 | 25600 |
| F | 6.5 | 37 | 1.324 | 4.46 | 9.82 | 325.87 | 1303.46 | 3200 | 12800 |
| A | 6.5 | 30 | 1.14 | 4.65 | 9.71 | 329.56 | 2636.46 | 3200 | 25600 |
| B | 6.5 | 30 | 1.142 | 4.63 | 9.71 | 329.56 | 1318.23 | 3200 | 12800 |
| C | 6.5 | 30 | 1.085 | 4.63 | 9.65 | 331.61 | 1326.42 | 3200 | 12800 |
| D | 6.5 | 30 | 1.147 | 4.67 | 9.71 | 659.11 | 1318.23 | 6400 | 12800 |
| E | 6.5 | 30 | 1.126 | 4.68 | 9.69 | 165.12 | 2641.90 | 1600 | 25600 |
| F | 6.5 | 30 | 1.094 | 4.62 | 9.66 | 331.26 | 1325.05 | 3200 | 12800 |
| A | 6.5 | 25 | 1.083 | 5.17 | 9.65 | 331.61 | 1326.42 | 3200 | 12800 |
| B | 6.5 | 25 | 1.074 | 5.03 | 9.65 | 165.80 | 1326.42 | 1600 | 12800 |
| C | 6.5 | 25 | 1.062 | 5.03 | 9.64 | 331.95 | 2655.60 | 3200 | 25600 |
| D | 6.5 | 25 | 1.033 | 5.08 | 9.61 | 166.49 | 1331.95 | 1600 | 12800 |
| E | 6.5 | 25 | 1.148 | 5.02 | 9.71 | 329.56 | 2636.46 | 3200 | 25600 |
| F | 6.5 | 25 | 1.049 | 5.10 | 9.63 | 332.29 | 2658.36 | 3200 | 25600 |

duction of related bacteriocins (Table 1). Moreover, the calculation of the AU/CFU showed a small effect on growth and bacteriocin production proportions. These results agree with the known facts that representatives of the genus *Enterococcus* are less dependent on organic nitrogen sources as compared to the genus *Lactiplantibacillus*.

Enterococci are known for their ability to grow in different environmental conditions, including a wide range of temperatures, environmental pH, and bioavailability of nutrients (de Vos *et al.*, 2011). Comparing the results on bacterial growth of *E. faecium* ST651ea, ST7119ea, and ST7319ea and bacteriocin production as determined versus *L. monocytogenes* ATCC15313 and *E. faecium* VRE19 showed a similar pattern, not only in OD, CFU/ml, and AU/ml but also in the respective AU/CFU. This may support the hypothesis that bacteriocin expression (by these strains) is a constant process not associated with fastidious requirements for specific co-factors. Moreover, for some other bacteriocins produced by different *Enterococcus* strains, it was reported that organic nitrogen sources can be characterized as growth regulators and are associated with bacteriocin production abilities (Furlaneto-Maia *et al.*, 2020; Foudjing *et al.*, 2023).

Moreover, each bacteriocin is characterized by its own specific amino acid sequence and composition, and in the case of some exclusive bacteriocins with primary structures, the bioavailability of specific amino acids can be a limiting biosynthesis factor (Choi *et al.*, 2022).

The presence of carbohydrates as a source of energy is essential for bacterial growth. Moreover, the use of different carbohydrates can serve as phenotypic characteristics in the identification of a bacterial species. Enterococci are known for their ability to grow in different ecological environments and to use numerous carbohydrates as energy sources (de Vos *et al.*, 2011). However, as reported previously, bacterial growth in the presence of a specific carbohydrate source does not always correlate with bacteriocin production (Todorov and Dicks, 2005a; Todorov and Dicks, 2005b). Thus, it was important to evaluate the role of different sugars in the microbial growth of *E. faecium* ST651ea, ST7119ea, and ST7319ea and the production of their bacteriocins.

*E. faecium* ST651ea, ST7119ea, and ST7319ea showed similar growth patterns when cultured in MRS modified by the replacement of glucose with lactose, sucrose, fructose, galactose, or maltose (Table 1; Figs. 1-3).

However, better growth parameters were recorded in galactose and the lowest in the presence of fructose. Moreover, monitoring of the bacteriocin activity, expressed in AU/ml with *L. monocytogenes* ATCC15313 or *E. faecium* VRE19, showed some variation depending on the type of sugar (glucose or galactose) used in the basic MRS medium (Table 1; Figs. 1-3). However, when the AU/CFU ratio was calculated, media optimal for bacteriocin production could be selected based on the utilization of galactose as the carbon source with fructose supporting the lowest bacteriocin production ratio per viable cell (Table 1; Figs. 1-3). This suggests that bacteriocin production needs to be associated with bacterial multiplication as a way to compare different growth conditions. Previous results also showed that carbohydrates can play a key role not only in the growth of bacteriocinogenic strains but can also comprise a limiting factor in the expression of these antimicrobial peptides (Abbasiliasi *et al.*, 2017).

An older hypothesis suggests that bacterial strains carrying more than one gene for bacteriocin production may express specific genes at different growth conditions (Poeta *et al.*, 2007). Appropriate expression experiments on RNA level need to be performed to confirm this hypothesis for the stud-

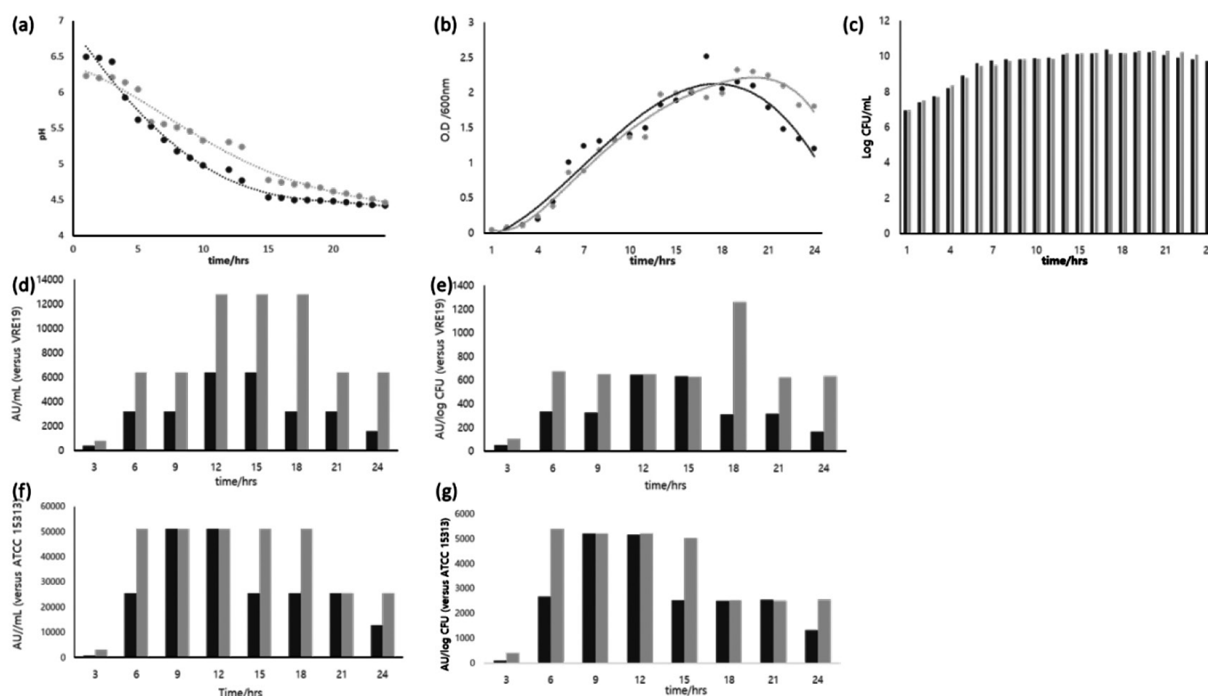


**Fig. 1**. *Enterococcus faecium* strain ST651ea: Comparison of changes in culture environmental pH (a), bacterial growth (OD at 600nm (b) and CFU/ml calculated based on previously built standard curve (c) and production of bacteriocin ST651ea (expressed as AU/ml (d, f) and AU/CFU (e, g)) evaluated against *Enterococcus faecium* VRE19 (d, e) and *Listeria monocytogenes* ATCC 15313 (f, g) on commercial MRS (dark grey) and modified media (proteose peptone 10 g/l, yeast extract 5 g/l, galactose 20 g/l, polysorbate_80 1 g/l, ammonium citrate 2 g/l, sodium acetate 5 g/l, magnesium sulfate 0.1 g/l, manganese sulfate 0.05 g/l, dipotassium phosphate 2 g/l) (light grey)

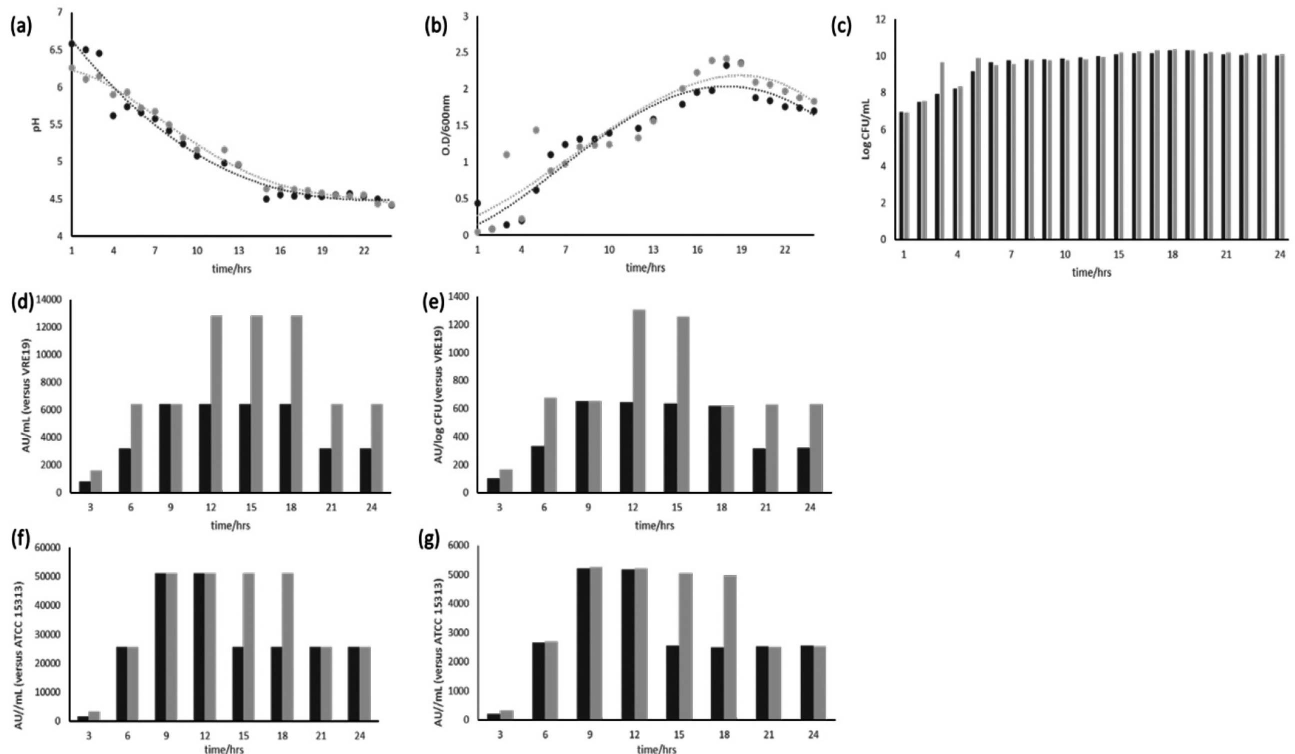


**Fig. 2**. *Enterococcus faecium* strain ST7119ea: Comparison of changes in culture environmental pH (a), bacterial growth (OD at 600nm (b) and CFU/ml calculated based on previously build standard curve (c)) and production of bacteriocin ST7119ea (expressed as AU/ml (d, f) and AU/CFU (e, g)) evaluated against *Enterococcus faecium* VRE19 (d, e) and *Listeria monocytogenes* ATCC 15313 (f, g) on commercial MRS (dark grey) and modified media (proteose peptone 10 g/l, yeast extract 5 g/l, galactose 20 g/l, polysorbate_80 1 g/l, ammonium citrate 2 g/l, sodium acetate 5 g/l, magnesium sulfate 0.1 g/l, manganese sulfate 0.05 g/l, dipotassium phosphate 2 g/l) (light grey)

ied *E. faecium* strains ST651ea, ST7119ea, and ST7319ea and their bacteriocins. A different approach would be to perform appropriate purification and amino acid sequencing and/or composition experiments and to confirm which single or combination of bacteriocins is produced. Fugaban *et al*. (2021) showed that these strains carry at least 2 sets of genes associated with the expression of bacteriocins with appropriate amino acid sequence, *ent*B: "ENDHRMPNEL NRPNNLSKG**G A**KCGAAIAGG LFGIPKGPLA WAAGLANVYS KCN" and *ent*P*: "*MRKKLFSLTL IGKFGLVVTN FGTKVDAATS YDNGIYCNNS KCWVNWGEAK ENIAGIVISG WASGLAGMGH" in *E. faecium* ST651ea, *ent*A: "SKDPKYSDIL EVLQKVYLKL EKQKYELDPG PLINRLVN_T AYTNKIRFTE YQEELIRNLS EIGRTAGING LYRADYG" and *ent*B: "ENDHRMPNEL NRPNNLSKG**G A**KCGAAIAGG LFGIPKGPLA WAAGLANVYSK C**N"** in *E. faecium* ST7119ea, and *ent*A: "GSAKMKKNAK QIVHELYNDI SISKDPKYSD ILEVLQKVYL KLEKQKYELD PGPLINRLVN YLYFTYTNKI RFTEYQEELI RNLSEIGRTA GINGLYRADY GE" and *ent*B*: "*ENDHRMPNEL NRPNNLSKGG AKCGAAIAGG LFGIPKGPLA LVAGLANVYS KCN" in *E. faecium* ST7319ea.

Modifications in growth conditions (cultivation temperature, initial pH of the culture media, specificity in the composition of the growth media regarding organic nitrogen and carbohydrate sources) can play a modulatory role in the expression of specific bacteriocin genes or in both of the previously reported enterocins in the studied strains (*E. faecium* strains ST651ea, ST7119ea, and ST7319ea). Such an issue merits additional research and more precise characterization of the expression of the specific bacteriocin/s by the studied *E. faecium* ST651ea, ST7119ea, and ST7319ea strains.

Based on the data obtained for the effect of temperature, initial pH, the role of organic nitrogen and carbohydrate source, modification of the commercial MRS media was investigated, and bacterial growth, acidification, and bacteriocin production by *E. faecium* ST651ea, ST7119ea, and ST7319ea were monitored for 24 h (Figs. 1-3).

Compared to the commercial medium, the growth of the *E. faecium* strains ST651ea, ST7119ea, and ST7319ea were not significantly influenced when cultured in modified MRS, and the

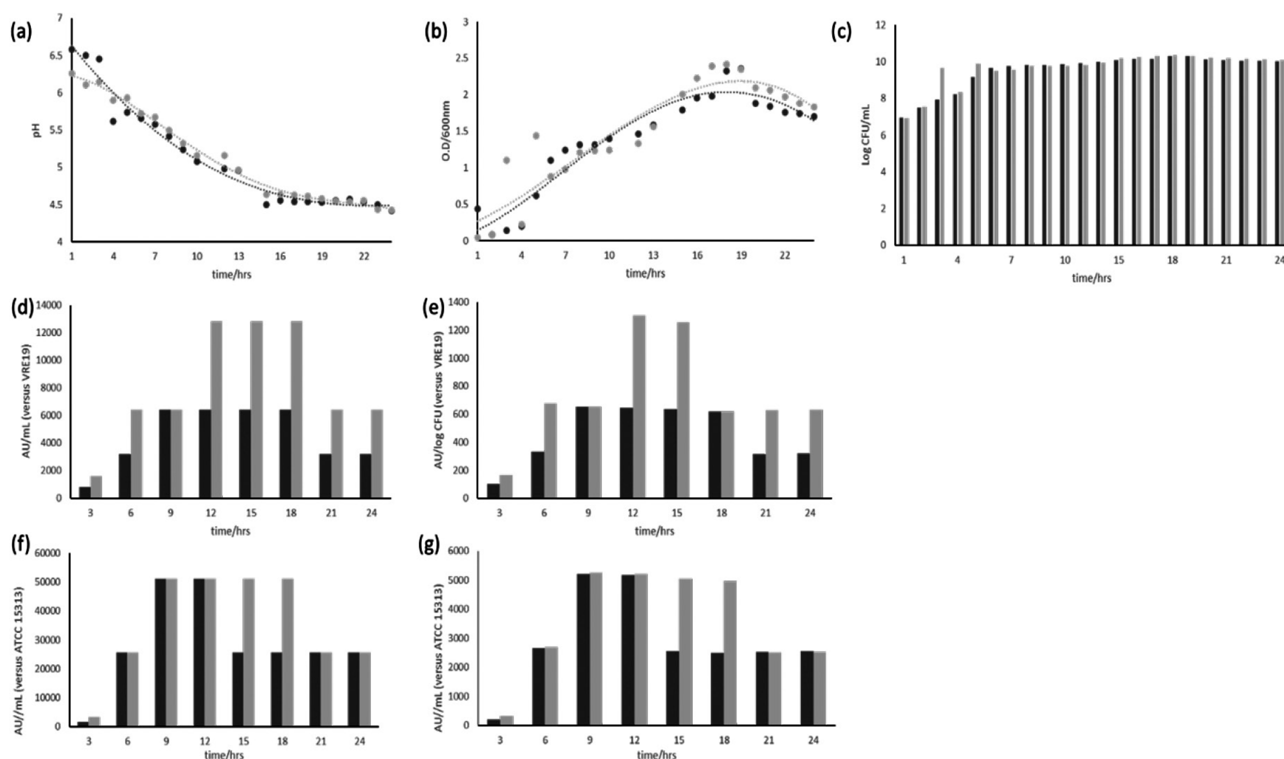


**Fig. 3**. *Enterococcus faecium* strain ST7319ea: Comparison of changes in culture environmental pH (a), bacterial growth (OD at 600nm (b) and CFU/ml calculated based on previously build standard curve (c)) and production of bacteriocin ST7319ea (expressed as AU/ml (d, f) and AU/CFU (e, g)) evaluated against *Enterococcus faecium* VRE19 (d, e) and *Listeria monocytogenes* ATCC 15313 (f, g) on commercial MRS (dark grey) and modified media (proteose peptone 10 g/l, yeast extract 5 g/l, galactose 20 g/l, polysorbate_80 1 g/l, ammonium citrate 2 g/l, sodium acetate 5 g/l, magnesium sulfate 0.1 g/l, manganese sulfate 0.05 g/l, dipotassium phosphate 2 g/l) (light grey)

cultures entered the exponential phase at approximately the same time point (Figs. 1-3).

This suggests that differences between commercial and modified MRS did not significantly affect the growth of *E. faecium* ST651ea, ST7119ea, and ST7319ea. Similar observations were recorded regarding acidification, as monitored by changes in the pH (Figs. 1-3). However, the objective of this study is to focus on the production of bacteriocins by *E. faecium* ST651ea, ST7119ea, and ST7319ea strains, hence, the focus on the monitoring of levels of antimicrobial activity (AU/mL) as presented in Figures 1-3. Consequently, the calculated AU/CFU ratio (Figs. 1-3) showed that increased production occurred when strains ST651ea, ST7119ea, and ST7319ea were cultured in modified media when their peak growth time was reached between 15-18 hours and where optimal levels of studied bacteriocins were also recorded.

Challenges for the application of modified media in the production of bacteriocins are not only related to the point of higher recovery of antimicrobial peptides but also to the potential reduction of the cost of the industrial process. In academic research, the objective is to obtain desired metabolite(s) with high purity, appropriate for the performance of analytical experiments. However, when transferred to the industrial scale, the production economy is a decisive factor, and the price-benefit ratio needs to be considered.

## Conclusions

Environmental and culturing conditions can play a significant role in bacteriocin production and thus appropriate optimization is essential for reaching the objective of optimal recovery of the expressed bacteriocins. It is essential to cut the cost of bacteriocin production, e.g., by the use of fewer resources (growth media, energy) and reducing the fermentation time. In our investigation we have evaluated the effect of media components and incubation temperatures by comparing not only absolute levels of expressed bacteriocins, recorded as AU/ml, but also relative antimicrobial levels, taking into consideration the relation between bacterial growth (CFU/ml) and the calculated appropriate AU/CFU. The obtained data confirmed the influence of the culturing conditions on the expression of the studied bacteriocins.

## Acknowledgements

To Mia Miau for her unconditional support.

## Fundings

This research was funded by FAPESP (Grant 2013/07914-8), University of Sao Paulo, Sao Paulo, SP, Brazil; to Centre for Research and Development in Agrifood Systems and Sustainability, funded by FCT (UIDB/05937/2020 and UIDP/05937/2020), Fundação para a Ciência e a Tecnologia, Portugal; to National Research Foundation (NRF) funded by the Ministry of Science and ICT (NRF-2018M3A9F3021964), Seoul, Republic of Korea; to Handong Global University, Pohang, Republic of Korea for providing the infrastructure and facilities.

## References

Abbasiliasi, S., J. S. Tan, T. A. T. Ibrahim, F. Bashokouh, N. R. Ramakrishnan, S. Mustafa, A. B. Ariff (2017). Fermentation factors influencing the production of bacteriocins by lactic acid bacteria: A review. *Res. Advances* **7**: 29395-29420. https://doi.org/10.1039/C6RA24579J.

Al-Saraireh, H., W. A. Al-Zereini, K. A. Tarawneh (2015). Antimicrobial activity of secondary metabolites from a soil *Bacillus* sp. 7B1 isolated from south Al-Karak, Jordan. *Jordan. J. Biol. Sci.* **147**: 127-132. https://doi.org/10.12816/0027558.

Altermann, E., W. M. Russell, M. A. Azcarate-Peril, R. Barrangou, B. L. Buck, O. McAuliffe, N. Souther, A. Dobson, T. Duong, M. Callanan, S. Lick, A. Hamrick, R. Cano, T. R. Klaenhammer (2005). Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. *Proc. Nat. Acad. Sci.* **102**: 3906-3912. https://doi.org/ 10.1073/pnas.0409188102.

Chauhan, N. P. S., N. S. Chundawat (2019). Inorganic and organometallic polymers. Walter de Gruyter GmbH & Co KG, Berlin, Germany. ISBN 9781501518669.

Chikindas, M. L., R. Weeks, D. Drider, V. A. Chistyakov, L. M. Dicks (2018). Functions and emerging applications of bacteriocins. *Curr. Opin. Biotechnol*. **49**: 23-28. htpps://doi.org/ 10.1016/j.copbio.2017.07.011.

Choi, G. H., W. H. Holzapfel, S. D. Todorov (2022). Diversity of the bacteriocins, their classification and potential applications in combat of antibiotic resistant and clinically relevant pathogens. *Crit. Rev. Microbiol.* **49**: 578-597. https://doi.org/10.1080/1040841X.2022.2090227.

Colovic, M. B., V. M. Vasic, D. M. Djuric, D. Z. Krstic (2018). Sulphur-containing amino acids: protective role against free radicals and heavy metals. *Curr. Med. Chem.* **25**: 324-335. https://doi.org/10.2174/0929867324666170609075434

Damak, F., M. Asano, K. Baba, A. Suda, D. Araoke, A. Wali, H. Isado, M. Nakajima, M. Ksibi, K. Tamura (2019). Interreginal traceability of Tunisian olive oils to the provenance soil by multielental fingerprinting and chemometrics. *Food Chem*. **283**: 656-664. https://doi.org/ 10.1016/j.foodchem.2019.01.082

De Vos, P., G. Garrity, D. Jones, N. R. Krieg, W. Ludwig, F. A. Rainey, K. H. Schlelfer, W. B. Whitman (Eds.) (2011). Bergey's manual of systematic bacteriology: Volume 3: The Firmicutes. Springer Science & Business Media. https://doi.org/10.1007/b92997.

Drider, D., G. Fimland, Y. Héchard, L. M. McMullen, H. Prévost (2006). The continuing story of class IIa bacteriocins. *Microbiol. Mol. Biol. Rev.* **70**: 564-582. https://doi.org/10.1128/MMBR.00016-05.

Foudjing, G. G. D., E. Sarmast, Z. Allahdad, S. Salmieri, M. Lacroix (2023). Influence of growth parameters on bacteriocin-like inhibitory substances (BLIS) production by lactic acid bacteria. *Lett. Appl. Microbiol.* **76**: ovac013. https://doi.org/10.1093/lambio/ovac013.

Fugaban, J. I. I., W. H. Holzapfel, S. D. Todorov (2022). The overview of natural by-products of beneficial lactic acid bacteria as promising antimicrobial agents. *Appl. Food Biotechnol.* **9**: 127-143. https://doi.org/10.22037/afb.v9i2.37544.

Fugaban, J. I. I. , J. E. Vazquez Bucheli, W. H. Holzapfel, S. D. Todorov (2021). Characterization of partially purified bacteriocins produced by *Enterococcus faecium* strains isolated from soybean paste active against *Listeria* spp. and vancomycin-resistant enterococci. *Microorganisms* **9**: 1085. https://doi.org/10.3390/microorganisms9051085

Furlaneto-Maia, L., R. Ramalho, K. R. Rocha, M. C. Furlaneto (2020). Antimicrobial activity of enterocins against *Listeria* sp. and other food spoilage bacteria. *Biotechnol. Lett.* **42**: 797-806. https://doi.org/10.1007/s10529-020-02810-7.

Gan, B. H., J. Gaynord, S. M. Rowe, T. Deingruber, D. R. Spring (2021). The multifaceted nature of antimicrobial peptides: Current syntetic chemistry approaches and future directions. *Chem. Sco. Rev.* **50**: 7820-7880. https://doi.org/ 10.1039/d0cs00729c. Erratum: Gan, B. H., J. Gaynord, S. M. Rowe, T. Deingruber, D. R. Spring (2022). The multifaceted nature of antimicrobial peptides: Current synthetic chemistry approaches and future directions. *Chem. Soc. Rev.* **50**: 7820–7880. https://doi.org/10.1039/D0CS00729C.

Gutiérrez-Cortés, C., H. Suarez, G. Buitrago, L. A. Nero, S. D. Todorov (2018). Enhanced bacteriocin production by *Pediococcus pentosaceus* 147 in co-culture with *Lactobacillus plantarum* LE27 on cheese whey broth. *Front. Microbiol.* **9**: 2952. https://doi.org/10.3389/fmicb.2018.02952.

Holzapfel, W. H., R. Geisen, U. Schillinger (1995). Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *Int. J. Food Microbiol.* **24**: 343-362. https://doi.org/ 10.1016/0168-1605(94)00036-6.

Kaur, S., S. Kaur (2015). Bacteriocins as potential anticancer agents. *Front. Pharmacol.* **6:** 272. https://doi.org/10.3389/fphar.2015.00272

Kumar, P., A. A. Shaikh, P. Kumar, V. K. Gupta, R. Dhyani, T. K. Sharma, A. Hussain, K. Gandele, K. M. Poluri, K. N. Rao, R. K. Malik, R. Pathania, N. K. Navani (2022). Double-edged nanobiotic platform with protean functionality: Leveraging the synergistic antibacterial activity of a food-grade peptide to mitigate multidrug-resistant bacterial pathogens. *ACS Appl. Mater. Interf.* **14:** 20652-20668. https://doi.org/10.1021/acsami.2c01385.

Kuniyoshi, T. M., C. M. N. Mendonça, V. B. Vieira, D. Robl, B. D. G. M. Franco, S. D. Todorov, E. Tome, P. M. O'Connor, A. Converti, W. L. Araujo, L. P. S. P. Vaconcellos, A. de Mello Varani, P. D. Cotter, S. C. Rabelo, R. P. de Souza Oliveira (2021). Pediocin PA-1 production by *Pediococcus pentosaceus* ET34 using non-detoxified hemicellulose hydrolysate obtained from hydrothermal pretreatment of

sugarcane bagasse. *Biores. Technol.* **338**: 125565. https://doi.org/10.1016/j.biortech.2021.125565.

Lynn, W. A., J. Cohen (1995). Menagment of spetic shock. *J. Infect.* **30**: 207-212. https://doi.org/ 10.1016/S0163-4453(95)90670-3.

Malheiros, P. S., V. Sant'Anna, S. D. Todorov, B. D. Franco (2015). Optimization of growth and bacteriocin production by *Lactobacillus sakei* subsp. *sakei* 2a. *Braz. J. Microbiol.* **46**: 825-834. https://doi.org/10.1590/S1517-838246320140279.

Manzoor, A., J. I. Qazi, I. ul Haq, H. Mukhtar, A. Rasool (2017). Significantly enhanced biomass production of a novel bio-therapeutic strain *Lactobacillus plantarum* (AS-14) by developing low-cost media cultivation strategy. *J. Biol. Engin.* **11**: 1-10. https://doi.org/10.1186/s13036-017-0059-2.

Metsoviti, M., S. Paramithiotis, E. H. Drosinos, P. N. Skandamis, M. Galiotou-Panayotou, S. Papanikolaou (2011). Biotechnological valorization of low-cost sugar-based media for bacteriocin production by *Leuconostoc mesenteroides* E131. *New Biotechnol.* **28**: 600-609. https://doi.org/ 10.1016/j.nbt.2011.03.004.

Mokoena, M. P., C. A. Omatola, A. O. Olaniran (2021). Applications of lactic acid bacteria and their bacteriocins against food spoilage microorganisms and foodborne pathogens. *Molecules* **26**: 7055. https://doi.org/ 10.3390/molecules26227055.

Molujin, A. M., S. Abbasiliasi, A. Nurdin, P. C. Lee, J. A. Gansau, R. Jawan (2022). Bacteriocins as potential therapeutic approaches in the treatment of various cancers: A review of *in vitro* studies. *Cancers* **14**: 4758. https://doi.org/10.3390/cancers14194758.

Parente, E., A. Ricciardi (1999). Production, recovery and purification of bacteriocins from lactic acid bacteria. *Appl. Microbiol. Biotechnol.* **52**: 628-638. htpps://doi.org/10.1007/s002530051570.

Poeta, P., D. Costa, B. Rojo-Bezares, M. Zarazaga, N. Klibi, J. Rodrigues, C. Torres (2007). Detection of antimicrobial activities and bacteriocin structural genes in faecal enterococci of wild animals. *Microbiol. Res.* **162**: 257-263. https://doi.org/10.1016/j.micres.2006.06.003.

Rogers, L. A., E. O. Whittier (1928). Limiting factors in the lactic fermentation. *J. Bacteriol.* **16**: 211-229. https://doi.org/ 10.1128/jb.16.4.211-229.1928.

Smits, S. H. J., L. Schmitt, K. Beis (2020). Self-immunity to antibacterial peptides by ABC transporters. *FEBS Lett.* **594**: 3920-3942. https://doi.org? 10.1002/1873-3468.13953.

Todorov, S. D., L. M. T. Dicks (2005a). *Lactobacillus plantarum* isolated from molasses produces bacteriocins active against Gram-negative bacteria. *Enzyme Microb. Technol.* **36**: 318-326. https://doi.org/10.1002/1873-3468.13953.

Todorov, S. D., L. M. T. Dicks (2005b). Effect of growth medium on bacteriocin production by *Lactobacillus plantarum* ST194BZ, a strain isolated from boza. *Food Technol. Biotechnol.* **43**: 165-173. UDC 57.083.332:579.864.1.

Valledor, S. J. D., C. M. Dioso, J. E. V. Bucheli, Y. J. Park, D. H. Suh, E. S. Jung, B. Kim, W. H. Holzapfel, S. D. Todorov (2022). Characterization and safety evaluation of two beneficial, enterocin-producing *Enterococcus faecium* strains isolated from kimchi, a Korean fermented cabbage. *Food Microbiol.* **102**: 103886. https://doi.org/10.1016/j.fm.2021.103886.

Vázquez-Rowe, I., D. Iribarren, M. T. Moreira, G. Feijoo (2010). Combined application of life cycle assessment and data envelopment analysis as a methodological approach for the assessment of fisheries. *Int. J. Life Cycle Assess.* **15**: 272-283. https://doi.org/10.1007/s11367-010-0154-9.

Vimont, A., B. Fernandez, R. Hammami, A. Ababsa, H. Daba, I. Fliss (2017). Bacteriocin-producing *Enterococcus faecium* LCW 44: a high potential probiotic candidate from raw camel milk. *Front. Microbiol.* **8**: 865. https://doi.org/10.3389/fmicb.2017.00865.