OPEN ACCESS

GAVIN PUBLISHERS

Food & Nutrition Journal

Research Article

Costa GA, et al. Food Nutr J 4: 209.
DOI: 10.29011/2575-7091.100109

Antibiofilm Activity of the Antimicrobial Peptide P34 against Multispecies Biofilms

Géssica Aracéli Costa[1], Adriano Brandelli[2], Ana Paula Guedes Frazzon[1], Amanda de Souza da Motta[1]*

[1]Instituto de Ciências Básicas da Saúde, Programa de Pós-Graduação em Microbiologia Agrícola e do Ambiente, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

[2]Laboratório de Bioquímica e Microbiologia Aplicada, Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul (ICTA-UFRGS), Porto Alegre, Brazil

***Corresponding author:** Amanda de Souza da Motta, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Sarmento Leite, n. 500, sala 216, CEP: 90050- 170, Porto Alegre, RS, Brazil. Tel: +555133084111; Fax: +555133083455; Email: amanda.motta@ufrgs.br

Citation: Costa GA, Brandelli A, Frazzon APG, Motta AS (2019) Antibiofilm Activity of the Antimicrobial Peptide P34 against Multispecies Biofilms. Food Nutr J 4: 209. DOI: 10.29011/2575-7091.100109

Received Date: 23 October, 2019; **Accepted Date:** 01 November, 2019; **Published Date:** 07 November, 2019

Introduction

Microbial biofilms are present in different habitats and are considered highly relevant in clinical environments, where they cause many types of chronic infections in patients. Biofilms are also a concern within the industrial environment, where they are associated with the contamination of products and foodborne illnesses [1-3]. These structures are constituted by sessile cells embedded in a polymeric extracellular matrix composed of polysaccharides, proteins, DNA and lipids. When compared to planktonic cells, sessile cells generally have higher resistance to adverse environmental conditions, as well as to antimicrobials and sanitizers [4,5]. Multispecies biofilms predominate in many environments. These biofilms are composed of a heterogeneous population of bacteria and yeasts, forming a more complex structure that is favored by synergistic interactions and commensalism [6,7].

Because they are resistant structures, strategies for cleaning and disinfecting surfaces by conventional methods are often ineffective in combating biofilms. Consequently, new control approaches, including the use of bioactive peptides, has been the subject of recent research [8-10]. Antimicrobial peptides produced by *Bacillus* sp. can present a low rate of toxicity, high biodegradability and anti-adhesive properties [11,12], providing its use as an alternative in the control of biofilms. The antimicrobial peptide P34 was initially characterized as a bacteriocin-like substance by Motta, Cannavan, Tsai, and Brandelli (2007a) [13] and has a molecular weight of 1,456 Da. It is a proteolytic enzyme which is relatively heat stable and exhibits activity in a pH range of 3.0 to 10 at a temperature between 25 and 37 ˚C. Furthermore, this substance demonstrates inhibitory activity against Gram-positive bacteria such as *Listeria monocytogenes*, *Bacillus cereus* and *Rhodococcus* sp. [14,15]. In addition, this peptide shows antiviral capacity against some animal pathogenic viruses [16-18], potential to prevent and eradicate the biofilm formation by *Staphylococcus aureus* and *Enterococcus faecalis* [19] and low cytotoxicity on eukaryotic cells [20]. However, until now, little has been known about the antibiofilm potential of this substance.

In this context, the aim of this study was to evaluate the behavior of planktonic and sessile cultures of *S. aureus* F4-1, *S.* Enteritidis ATCC 13076 and *C. tropicalis* 72-A, under different nutritional conditions. Moreover, the responses of individual cultures and mixed cultures and multispecies biofilms formed on stainless steel, within the presence of peptide P34, were observed.

Materials and Methods

Bacterial strains

The isolate *Staphylococcus aureus* F4-1 was obtained from frozen chicken meat and characterized as a strong biofilm-producing strain [21]. The clinical isolate *Candida tropicalis* 72-A, which is resistant to fluconazole, amphotericin B and voriconazole, has been characterized as a weak biofilm producer (Bergamo et al., 2014) [22] whereas the strain *Salmonella* Enteritidis ATCC 13076 has been characterized as a non-former of biofilms. Both of these were also evaluated. The isolates *S. aureus* F4-1 and *C. tropicalis* 72-A have been previously identified by [22,23], respectively. The bacterial isolates were maintained at -20 ˚C in cryotubes containing 10% (v/v) skim milk and 20% (v/v) glycerol. The yeast *C. tropicalis* 72-A was maintained on Sabouraud Dextrose Agar (SDA, Acumedia), covered with mineral oil and stored at 25

°C. The cultures were grown in Brain Heart Infusion agar (BHI, Himedia) at 37 °C for 24 h to obtain pure colonies before use.

Production and partial purification of peptide P34

For the production of peptide P34, *Bacillus* sp. P34 was grown in 100 ml BHI medium at 30 °C in a rotary shaker at 180 rpm. After cultivation for 24 h, the cells were harvested by at 10,000 *g* for 15 min at 4 °C. The filtrate was precipitated with ammonium sulfate at 20% (w/v centrifugation) saturation, and then dissolved in 10 mM phosphate buffer pH 7.0. This solution was purified by gel filtration chromatography using a Sephadex G-100 column [13]. The antimicrobial activity titre was determined by the serial twofold dilution method previously described by Mayr-Harting, Hedjes, and Berkeley (1972) [24]. Activity was defined as the reciprocal of the last serial dilution giving an inhibition zone and expressed as Activity Units (AU) per milliliter. The AU/ml was determined against *Listeria monocytogenes* ATCC 7644 as the indicator strain. The fractions showing antimicrobial activity were pooled, sterilized through a 0.22 µm filter (Millipore, Bedford, USA) and frozen stored.

Antimicrobial activity of peptide P34 against planktonic cells

Antimicrobial activity was determined essentially as described by Motta and Brandelli (2002) [25]. An aliquot of 20 µl of peptide P34 (1600 AU) was applied onto BHI agar plates previously inoculated with a swab submerged in suspensions of *S. aureus* F4-1, *C. tropicalis* 72-A or *S*. Enteritidis ATCC 13076, which corresponded to a 0.5 McFarland turbidity standard solution. The strain *L. monocytogenes* ATCC 7644 was used as a control. Plates were incubated at the optimal growth temperature of the test organisms and inhibitory zones were measured after 24 h.

Evaluation of antimicrobial activity of peptide P34 on the growth of individual cultures and mixed culture

For the evaluation of peptide P34 against individual cultures of *S. aureus* F4-1, *C. tropicalis* 72-A and *S.* Enteritidis ATCC 13076, a standard concentration of 10^3 CFU/ml (Colony-Forming Units) of each isolate was inoculated in 50 ml of BHI broth. After 3 h incubation at 37 °C and 120 rpm, 5 ml of either 10 mM phosphate buffer pH 7.0 or 1600 AU/ml-1 of peptide P34 were added to the control and treated samples respectively. Aliquots of 100 µl were collected at 0 h, 8 h and 24 h of treatment to perform an evaluation of viable cell counts.

The mixed culture assay was performed by the inoculation of a standardized concentration of 10^3 CFU/ml of each isolate in BHI broth (BHI, Himedia). Equal amounts of each strain were added to the assay tube, which was incubated for 3 h at 37 °C and 120 rpm. After incubation, the procedures were performed as described above. For the counting of individual microbial species, aliquots from each treatment were inoculated into selective media: Mannitol Salt Agar for *S. aureus* F4-1, MacConkey Agar for *S.* Enteritidis ATCC 13076, and Sabouraud Dextrose Agar containing 1% chloramphenicol for *C. tropicalis* 72-A. The incubation was carried out at 37 °C for 24 h, for subsequent determination of the CFU/ml, according to the protocol established by Milles, Misra, and Irwin (1938) [26]. The assays were performed in duplicate and the results were expressed as log CFU/ml.

Evaluation of antibiofilm activity of the peptide P34 on the multispecies biofilm formed on stainless steel

The ability of the peptide P34 to prevent biofilm formation was evaluated on AISI 304 stainless steel probes (2 x 2 cm). The probes were previously prepared by soaking in neutral detergent at 0.3% for 60 min and subsequently immersing them in acetone, flushing with distilled water and spraying them with 70% (v/v) ethanol for disinfection. After this sanitization process, the probes were subjected to drying for 2 h at 60 °C and autoclaved at 121°C for 15 min [27]. The control probe was submerged in sterile water for 4 h while the treated probe was submerged in the solution containing 1600 AU/ml of the peptide P34 for the same period. After this pretreatment, the probes were immersed in 20 ml of TSB (Himedia) containing 1% glucose which was previously inoculated with *S. aureus* F4-1, *S.* Enteritidis ATCC 13076 and *C. tropicalis* 72-A, at a concentration of 10^8 CFU/ml each. The samples were incubated at 37 °C for 24 h at 180 rpm in a rotary shaker.

Scanning Electron Microscopy (SEM)

The stainless steel AISI 304 surfaces, which were used for the development of multispecies biofilms, were fixed with 3% (v/v) glutaraldehyde in 0.2 M phosphate buffer pH 7.2 for 8 days. After this process, the probes were washed three times with the same buffer and dehydrated using an acetone gradient from a 30% (v/v) to 100% concentration. The probes were processed in a critical point dryer (Balzers CPD030; Bal-Tec, Balzers, Liechtenstein) and then gold and platinum particles were deposited on the samples. The visualization was performed using a Scanning Electron Microscope JSM6060 (JEOL, Tokyo, Japan).

Statistical analysis

All experiments were performed at least in duplicates and standard deviations from the mean values were calculated. The effect of peptide P34 on biofilm formation was analyzed using Parametric Analysis by Statistica 10.0 software (Statsoft, Tulsa, OK, USA). Values were considered significantly different from each other at $P<0.05$.

Results and Discussion

Antimicrobial activity against planktonic cells

In the present study, cultures of *S. aureus* F4-1, *S.* Enteritidis ATCC 13076 and *C. tropicalis* 72-A were evaluated in planktonic and sessile conditions, as multispecies biofilms, in the absence and in the presence of the peptide P34. Only the *S. aureus* F4-1 strain showed an inhibition zone of 13.0 mm, while other strains were not sensitive to peptide P34 (data not shown). The indicator strain *L. monocytogenes* ATCC 7644, which was used as a positive control, presented an inhibition zone of 13.6 mm.

The behavior of microbial cells in planktonic form is distinct from their behavior in sessile form, whether in monospecies or multispecies biofilms. In recent years, many studies have been performed with a focus on the complexity of microbial interactions in heterogeneous biofilms, in order to understand the consequences of the associations between different microorganisms [7,28]. Although the mechanism of action of this substance is not fully understood, its potential has been tested against a variety of microbial species [13]. The authors reported no antimicrobial activity against isolates of *S. aureus*, *S.* Enteritidis ATCC 13076 and *Candida* sp. This discrepancy of results for *S. aureus* may be due to peptide concentration, composition of culture media, concentration of indicator culture, or the peculiar phenotypic and genotypic characteristics of each microorganism [25,29]. According to Otto (2012) [30] the proportion of teichoic acids present in the cell wall of *Staphylococcus* sp. seems to be involved in the bacterial adhesion process and the susceptibility to different antimicrobial peptides. The planktonic cells are important for the dissemination of pathogenic microorganisms, which can adhere to and colonize new environments [5,31].

The effect of peptide P34 on the growth of individual and mixed cultures

The analysis of individual cultures, with the addition of the peptide P34, demonstrated that only *S. aureus* F4-1 suffered a bacteriostatic effect at 8 h of culture, the moment at which the control sample count was 7.81 log CFU/ml and the treated sample count was 4.20 log CFU/ml (Table 1). The statistical analysis indicated a growth difference between the two conditions tested ($P<0.05$). After 8 h of incubation, an increase in cell growth was verified, and at the end of 24 h no differences in the cell population were verified among the samples. The peptide P34 caused no effect on the cultures of *S.* Enteritidis ATCC 13076 and *C. tropicalis* 72-A (Table 1). This behavior was similar to that observed in agar diffusion tests. Antimicrobial peptides, such as those evaluated in this study, can have bactericidal or bacteriostatic effects. The mentioned effects have been attributed to the concentration and the degree of purity of the antimicrobial peptide, as well as the concentration of the target cell. In many cases, these molecules present such effects on a narrow spectrum of bacteria [32-34]. Lisboa, Bonatto, Bizani, Henriques, and Brandelli (2006) [29] evaluated an antimicrobial peptide produced

Strain	Time (h)	Control (log CFU/ml)	Sample with peptide P34 (log CFU/ml)
C. tropicalis 72-A	0	3.60 + 0.06a	3.59 + 0.01a
	8	5.74 + 0.03a	5.55 + 0.04a
	24	7.36 + 0.03a	6.62 + 0.05a
S. aureus F4-1	0	3.11 + 0.02a	3.15 + 0.02a
	8	7.81 + 0.04a	4.20 + 0.04b
	24	9.07 + 0.02a	8.36 + 0.12a
S. Enteritidis ATCC 13076	0	3.10 + 0.08a	3.00 + 0.01a
	8	7.76 + 0.06a	6.70 + 0.04a
	24	9.07 + 0.11a	8.75 + 0.08a
*Averages followed by the same letter in the same row do not differ by the Parametric Analysis at 5% significance (P>0.05).			

Table 1: Growth of *S. aureus* F4-1, *S.* Enteritidis ATCC 13076 and *C. tropicalis* 72-A, individually evaluated with peptide P34 during 24 h.

by *B. amyloliquefaciens* and observed a bacteriostatic effect on planktonic cells of *L. monocytogenes*. Deng, Su, and Zhang (2011) [35], while using the peptide subticin 112 against *S. aureus* CVCC 1885, also reported a bacteriostatic effect. The peptide P34 did not influence the development of planktonic cells of *S.* Enteritidis ATCC 13076 and *C. tropicalis* 72-A. Gram-positive bacteria are the main targets of this peptide, which acts against Gram-negative bacteria in the presence of EDTA [15]. Generally, the resistance to antimicrobial peptides presented by most *Salmonella* spp. is primarily due to the presence of an external membrane [36,37]. The cell reduces the external membrane fluidity, which in turn causes an increase in hydrophobic interactions [38]. Therefore, the hydrophobicity generated decreases the possibility of the inclusion of the antimicrobial peptide, and consequently the reduction of pore formation in the membrane, resulting in resistance to the antimicrobial substance. In agreement with our study, other authors have reported that *Candida* spp. are not susceptible to these kinds of antimicrobial peptides [13,29,39]. It is suggested that the differences in the lipid composition of the yeast membrane may be related to the ineffectiveness of the peptides against eukaryotic microorganisms [40].

In the evaluation of mixed culture, with or without the addition of the peptide P34, it was observed that in a period of 24 h, the development of *S.* Enteritidis ATCC 13076 was superior when compared to *S. aureus* F4-1 and *C. tropicalis* 72-A (Table 2). No significant differences in cell counts were observed when the strains were cultivated simultaneously in the presence of peptide P34. The coexistence of different microbial species in the same environment favors synergistic interactions that influence their distribution in such milieu. These multispecies interactions may affect the cell physiology, functionality and virulence [7,10,41]. The ineffectiveness of different antimicrobial compounds in controlling multispecies cultures has been reported [10,42,43]. However, there is a lack of reports about the effects of antimicrobial peptides on mixed cultures. In the present study, the peptide P34 did not influence the development of the mixed culture. Nevertheless, a decreased growth rate in *S. aureus* F4-1 was recorded, resulting in the smallest amount of viable cells. Similar to our study, when Waili, Al- Ghamdi, Ansari, Al-Attal, and Salon (2012) [42] evaluated synergism in multispecies culture of *S. aureus*, *E. coli* and *C. albicans*, they also noted a small reduction in the growth of the isolates. This reduction was speculated to be the result of competition among the isolates for a limited amount of nutrients.

Scanning electron microscopy

The use of antimicrobial peptides has been proved to be an interesting approach to prevent microbial adhesion to surfaces [8,44,45]. The scanning electron microscopy evidenced a change in the arrangement of microbial cells which adhered to the surface when compared to the control sample and the sample treated with the peptide P34 (Figure 1). Isolated cells of *S.* Enteritidis were observed in both samples, suggesting that bacterial adhesion failure was not influenced by other microbial species.

Strain	Time (h)	Control (log CFU/ml)	Sample with peptide P34 (log CFU/ml)
C. tropicalis 72-A	0	3.08 + 0.12a	3.05 + 0.07a
	8	4.75 + 0.02a	4.77 + 0.02a
	24	6.61 + 0.06a	6.59 + 0.04a
S. aureus F4-1	0	3.49 + 0.07a	3.44 + 0.07a
	8	4.64 + 0.04a	5.31 + 0.06a
	24	6.71 + 0.04a	6.70 + 0.12a
S. Enteritidis ATCC 13076	0	3.23 + 0.04a	3.26 + 0.01a
	8	7.51 + 0.06a	6.80 + 0.03a
	24	9.14 + 0.04a	8.75 + 0.05a
*Averages followed by the same letter in the same row do not differ by the Parametric Analysis at 5% significance (P>0.05).			

Table 2: Effect of the peptide P34 on mixed culture with *S. aureus* F4-1, *S.* Enteritidis ATCC 13076 e *C. tropicalis* 72-A, in BHI broth at 37 °C during 24 h.

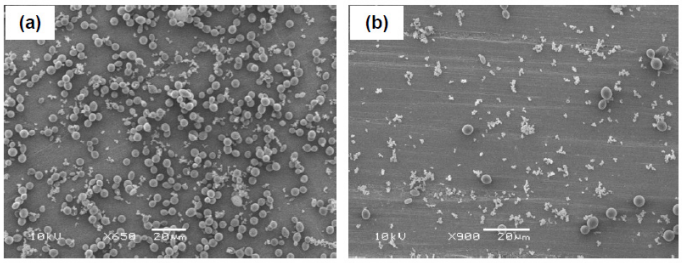

Figure 1: Images of Scanning Electron Microscopy (SEM) of multispecies biofilm with o *S. aureus* F4-1*, S.* Enteritidis ATCC 13076 e *C. tropicalis* 72-A formed on stainless steel surface - positive control (a); surface treated with BLS P34 showing smaller *C. tropicalis* 72-A population (b).

An irregular biofilm was observed in the control sample, without large cell agglomerations and with a prevalence of yeast cells (Figure 1 (a)). However, in the treated sample, the use of peptide P34 reduced the population of yeast cells when compared to the control sample (Figure 1 (b)). Furthermore, there was a change in the arrangement of *S. aureus* cells which adhered to the surface: larger cell agglomerations appeared in the treated sample when compared to the control sample (Figure 1 (a) and (b)).

The SEM images confirm the ability of *S. aureus* F4-1 and *C. tropicalis* 72-A to adhere to and from biofilm on stainless steel. Although there are several studies about the interaction between *C. albicans* with *S. aureus* [46-49], *C. tropicalis* species have not been studied in multispecies biofilms. *S. aureus* and *C. albicans* are commonly found together in different types of infections, showing increased resistance when they infect the host [46,47]. It has been suggested that this behavior can be mediated by protein filaments of *Candida* sp., as described by Peters et al. (2010) and Peters et al. (2012) [6,48]. The presence of peptide P34 on the stainless steel surface decreased the adhesion of *C. tropicalis* 72-A cells. It is possible that the peptide formed a film that somehow interacted with the yeast cell surface and decreased its adhesion. Some antimicrobial molecules can reduce the surface hydrophobicity, and therefore, reduce cellular adhesion. This characteristic suggests a potential application in the destabilisation of the biofilm [10].

The adherence of *S. aureus* F4-1 was not influenced. According to [50], *S. aureus* is able to produce proteins and secrete enzymes that perform modifications on the cell surface and ensure the resistance against action of antimicrobial peptides. It was also possible to note surface irregularities in the stainless steel (Figure 1 (b)) that probably hamper the efficiency of the cleaning process, serving as a source of substrate accumulation and therefore favoring the adhesion of cells and biofilm formation.

Conclusion

In summary, the results of this study demonstrate that the peptide P34 presents bacteriostatic potential on planktonic cells of *S. aureus* F4-1. However, it does not interfere in the development of *S.* Enteritidis and *C. tropicalis* in mixed culture. Besides this, it was observed that the stainless steel surface treated with peptide P34 showed a change in the distribution and architecture of C. *tropicalis* 72-A cells, suggesting a potential anti-adhesive action.

Acknowledgements

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil).

References

1. Janssens JCA, Steenackers H, Robijns S, Gellens E, Levin J, et al. (2008) Brominated furanones inhibit biofilm formation by *Salmonella enterica* serovar Typhimurium. Applied and Environmental Microbiology 74: 6639-6648.
2. Bjarnsholt T (2013) The role of bacterial biofilms in chronic infections. *APMIS* Supplementoum 121: 1-5.
3. Srey S, Jahid IK, Ha SD (2013) Biofilm formation in food industries: A food safety concern. Food Control 31: 572-585.
4. Simões M, Simões LC, Machado I, Pereira MO, Vieira MJ (2006) Control of flow-generated biofilms using surfactants - evidence of resistance and recovery. Food Bioproducts Processing 84: 338-345.
5. Simões M, Simões LC, Vieira MJ (2010) Review of current and emergent biofilm control strategies. LWT - Food Science Technology 43: 573-583.
6. Peters BM, Ovchinnikova ES, Krom BP, Schlecht LM, Zhou H, et al. (2012) *Staphylococcus aureus* adherence to *Candida albicans* hyphae is mediated by the hyphal adhesin Als3p. Microbiology 158: 2975-2986.
7. Burmølle M, Ren D, Bjarnsholt T, Sørensen SJ (2014) Interactions in multispecies biofilms: do they actually matter?. Trends Microbiology 22: 84-91.
8. Héquet A, Humblot V, Berjeaud JM, Pradier CM (2011) Optimized grafting of antimicrobial peptides on stainless steel surface and biofilm resistance tests. Colloids Surfaces B: Biointerface 84: 301-309.
9. Espitia PJP, Soares NFF, Coimbra JSR, Andrade NJ, Cruz RS, et al. (2012) Bioactive Peptides: Synthesis, properties, and applications in the packaging and preservation of food. Comprehensive Reviews in Food Science and Food Safety 11: 187-204.
10. Gomes MZV, Nitschke M (2012) Evaluation of rhamnolipid and surfactin to reduce the adhesion and remove biofilms of individual and mixed cultures of food pathogenic bacteria. Food Control 25: 441-447.
11. Van Hamme JD, Singh A, Ward OP (2006) Physiological aspects Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. Biotechnology Advances 24: 604-620.
12. Nitschke M, Costa SGVAO (2007) Biosurfactants in food industry. Trends in Food Science Technology 18: 252-259.
13. Motta AS, Cannavan FS, Tsai SM, Brandelli A (2007a) Characterization of a broad range antibacterial substance from a new *Bacillus* species

isolated from Amazon basin. Archives Microbiology 188: 367-375.

14. Motta AS, Lorenzini DM, Brandelli A (2007b) Purification and partial characterization of an antimicrobial peptide produced by a novel *Bacillus* sp. isolated from the Amazon Basin. Current Microbiology 54: 282-286.

15. Motta AS1, Flores FS, Souto AA, Brandelli A (2008) Antibacterial activity of a bacteriocin-like substance produced by *Bacillus* sp. P34 that targets the bacterial cell envelope. Antonie Van Leeuwenhoek 93: 275-284.

16. Silva DS, Castro CC, Silva FS, Sant'anna V, Vargas GD, et al. (2014) Antiviral activity of a *Bacillus* sp. P34 peptide against pathogenic viroses of domestic animals. Brazilian Journal of Microbiology 45: 1089-1094.

17. Castro CC, Silva DS, Costa GA, Fischer G, Vargas GD, et al. (2017) Activity of the antimicrobial peptide P34 against bovine alphaherpesvirus type 1. Ciência Rural 47: 01-06.

18. Silva DS, Castro CC, Silva FS, Soares MP, Vargas GD, et al. (2017) Inhibition of equine arteritis virus by an antimicrobial peptide produced by Bacillus sp. P34. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 69: 535-542.

19. Costa GA, Rossato FCP, Medeiros AW, Correa APF, Brandelli A, et al. (2018) Evaluation antibacterial and antibiofilm activity of the antimicrobial peptide P34 against *Staphylococcus aureus* and *Enterococcus faecalis*. Anais da Academia Brasileira de Ciências 90: 73-84.

20. Vaucher RA, Motta AS, Brandelli A (2010) Evaluation or the *in vitro* cytotoxicity of the antimicrobial peptide P34. Cell Biology International 34: 317-323.

21. Pinto JB, Rossatto FCP, Martins PD, Frazzon APG (2014) Genetic relationships and virulence factors in *Staphylococcus aureus* isolated from raw poultry in South Brazil. Annals Microbiology 65: 1933-1940.

22. Bergamo VZ, Donato RK, Dalla Lana DF, Donato KJ, Ortega GG, et al. (2014) Imidazolium salts as antifungal agents: strong antibiofilm activity against multidrug-resistant *Candida tropicalis* isolates. Letters in Applied Microbiology 60: 66-71.

23. Martins PD, De Almeida TT, Basso AP, De Moura TM, Frazzon J, et al. (2013) Coagulase-positive *staphylococci* isolated from chicken meat: pathogenic potential and vancomycin resistance. Foodborne Pathogens and Disease 10: 771-776.

24. Mayr-Harting A, Hedjes AJ, Berkeley CW (1972) Methods for studying bacteriocins. In: Norris JB, Ribbons D. Methods Microbiology 7: 315-412.

25. Motta AS, Brandelli A (2002) Characterization of an antibacterial peptide produced by *Brevibacterium linens*. Journal of Applied Microbiology 92: 63-71.

26. Milles AAL, Misra SS, Irwin JO (1938) The estimation of bacterial power of blood. Journal of Hygine (Lond) 38: 732-749.

27. Rossoni EM, Gaylarde CC (2000) Comparison of sodium hypochlorite and peracetic acid as sanitizing agents for stainless steel food processing surfaces using epifluorescence microscopy. Internetional Journal of Food Microbiol 61: 81-85.

28. Zhang W, Sileika T, Packman AI (2013) Effects of fluid flow conditions on interactions between species in biofilms. FEMS Microbiology Ecology 84: 344-354.

29. Lisboa MP, Bonatto D, Bizani D, Henriques JA, Brandelli A (2006) Characterization of a bacteriocin-like substance produced by *Bacillus amyloliquefaciens* isolated from the Brazilian Atlantic forest. International Microbiology 9: 111-118.

30. OTTO M (2012) Molecular basis of *Staphylococcus epidermidis* infections. Seminars in Immunopathology 34: 201-214.

31. Van Houdt R, Michiels CW (2010) Biofilm formation and the food industry, a focus on the bacterial outer surfasse. Journal of Applied Microbiology 109: 1117-1131.

32. Nes IF, Yoon SS, Diep DB (2007) Ribosomally synthesiszed antimicrobial peptides (bacteriocins) in lactic acid bacteria: A review. Food Science Biotechnology 16: 675-690.

33. Balciunas EM, Martinez FAC, Todorov SD, Franco BDGM, Convertia A, et al. (2013) Novel biotechnological applications of bacteriocins: A review. Food Control 32: 134-142.

34. Güllüce M, Karaday M, Bariş Ö (2013) Bacteriocins: promising natural antimicrobials. In: Méndez-Vilas A (ed,). Microbial Pathogens and Strategies for Combat-ing them: Science, Technology and Education. (2nd edition), Formatex Research Center Pg No: 1016-1027.

35. Deng K, Su LF, Zhang RJ (2011) Bacteriostatic activity of bacteriocin Subtilin112 against *Staphylococcus aureus* CVCC 1885 *in Vitro* and *in Vivo*. Scientia Agricultura Sinica 44: 2830-2837.

36. Chalón MC, Acuña L, Morero RD, Minahk CJ, Bellomio A (2012) Membrane-active bacteriocins to control *Salmonella* in foods Are they the definite hurdle?. Food Research International 45: 735-744.

37. Band VI, Weiss DS (2015) Mechanisms of antimicrobial peptide resistance in Gram-negative bacteria. Antibiotics 4: 18-41.

38. Brogden KA (2015) Antimicrobial Peptides: Pore formers or metabolic inhibitors in bacteria?. Nature Reviews Microbiology 3: 208-250.

39. Khochamit N, Siripornadulsil S, Sukonb P, Siripornadulsil W (2015) Antibacterial activity and genotypic-phenotypic characteristics of bacteriocin-producing *Bacillus subtilis* KKU213: Potential as a probiotic strain. Microbiological Research 170: 36-50.

40. Pereira HA, Tsyshevskaya-Hoover I, Hinsley H, Logan S, Nguyen M, et al. (2010) Candidacidal activity of synthetic peptides based on the antimicrobial domain of the neutrophil-derived protein, CAP37. Medical Mycology 48: 263-272.

41. da Silva Fernandes M, Kabuki DY, Kuaye AY (2015) Behavior of *Listeria monocytogenes* in a multi-species biofilm with *Enterococcus faecalis* and *Enterococcus faecium* and control through sanitation procedures. International Journal of Food Microbiology 200: 05-12.

42. Waili NAL, Al- Ghamdi A, Ansari MJ, Al-Attal Y, Salom K (2012) Synergistic effects of honey and propolis toward drug multi-resistant *Staphylococcus aureus, Escherichia coli* and *Candida albicans* isolates in single and polymicrobial cultures. International Journal of Medical Sciences 9: 793-800.

43. Giaouris E, Chorianopoulos N, Doulgeraki A, Nychas GJ (2013) Co-culture with *Listeria monocytogenes* within a dual-species biofilm community strongly increases resistance of *Pseudomonas putida* to benzalkonium chloride. PLoS ONE 8: 77276.

44. Blanco Massani M, Fernandez MR, Ariosti A, Eisenberg P, Vignolo G

(2008) Development and characterization of an active polyethylene film containing *Lactobacillus curvatus* CRL705 bacteriocins. Food Additives & Contaminants 25: 1424-1430.

45. Batoni G, Maisetta G, Brancatisano FL, Esin S, Campa M (2011) Use of Antimicrobial Peptides Against Microbial Biofilms: Advantages and Limits. Current Medicinal Chemistry 18: 256-279.

46. Harriott MM, Noverr MC (2009) *Candida albicans* and *Staphylococcus aureus* form polymicrobial biofilms: effects on antimicrobial resistance. Antimicrobial Agents and Chemotherapy 53: 3914-3922.

47. Shirtliff ME, Peters BM, Jabra-Rizk MA (2009) Cross-kingdom interactions: *Candida albicans* and bacteria. FEMS Microbiology Letters 299: 1-8.

48. Peters BM, Jabra-Rizk MA, Scheper MA, Leid JG, Costerton JW, et al. (2010) Microbial interactions and differential protein expression in *Staphylococcus aureus* - *Candida albicans* dual-species biofilms. FEMS Immunology and Medical Microbiology 59: 493-503.

49. Lindsay AK, Hogan DA (2014) *Candida albicans*: Molecular interactions with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Fungal Biology Reviews 28: 85-96.

50. Nawrocki KL, Crispell EK, McBride SM (2014) Antimicrobial peptide resistance mechanisms of Gram-positive bacteria. Antibiotics 3: 461-492.