# ANTIBACTERIAL EFFECT OF ENCAPSULATED NEW SYNTHETIC PEPTIDES AGAINST METHICILLIN RESISTANT *Staphylococcus aureus* (MRSA)


**Marlon Cáceres[1*], Brayann Martinez[3], Fanny Guzmán[2], Rodrigo Torres[3], and Claudia Ortiz[1]**

[1] *Escuela de Bacteriología y Laboratorio Clínico, Facultad de Salud, Universidad Industrial de Santander*
[2] *NBC Núcleo Biotecnología Curauma, Pontificia Universidad Católica de Valparaíso, Campus Curauma, Valparaíso, Chile*
[3] Escuela de Química, Facultad de Ciencias, Universidad Industrial de Santander
Bucaramanga, Colombia *E-mail:: marlon_caceres08@hotmail.com


## INTRODUCTION

Progressive Increase in antibiotic resistance of different bacteria has become a serious public health problem worldwide. Therefore, it has become necessary to increase research specifically aimed at finding different conventional antimicrobial agents {1]. In this context, antimicrobial peptides (AMPs) have emerged as a promising alternative for the treatment of antibiotic resistant bacterial infections [2]. However, AMPs have a low bioavailability and are also vulnerable to proteolytic action. Encapsulation of peptides into polymeric nanoparticles is proposed as a solution to improve biocompatibility and bioavailability, which is reflected in an increased antimicrobial action. In this work, we have designed synthetic AMPs with possible antimicrobial activity and their analogues using bioinformatics tools and algorithms for classification based on support vector machines (SVM). These AMPs and their respective analogues were synthesized by using F-moc methodology and encapsulated on polymeric nanopartides of poly(lactic-co-glycolic acid) by nanoprecipitation method. Encapsulated AMPs showed significant increases in antimicrobial activity against *MRSA*, obtaining minimum bactericide concentrations (MBC) of 25 µM

## MATERIAL AND METHODS

**Bioinformatics analysis, synthesis, characterization and encapsulation of the AMPs:** Design of new peptides with antimicrobial activity potential was performed through the support vector machines by means of the Quantitative Structure-Activity Relationship (QSAR). Then, AMPs and their analogs were synthesized through solid phase chemistry by using F-moc methodology and encapsulation was made by nanoprecipitation [3]. Nanostructurated peptides were characterized by dynamic light scattering (DLS) and scanning transmission electron microscopy (STEM) (**Figure 1A**).

**Determination of antimicrobial activity:** Studies on the antimicrobial activity of AMPs was carried out as described in the literature [4]. Bacterial growth kinetics was carried out in microwell cell culture quantifying bacterial growth by measurements of optical density at 595 (**Figure 1B**).

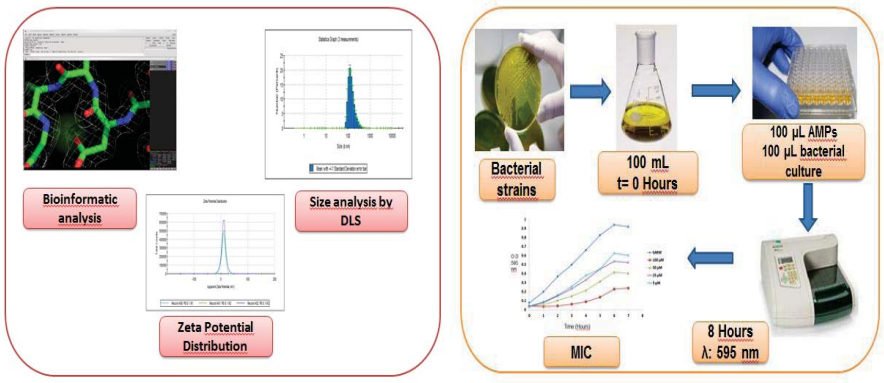


**Figure 1**. ***A) Design, synthesis and characterization of encapsulated AMPs B) Antimicrobial assay.***

## RESULTS AND DISCUSSION

**Design, synthesis and characterization of AMPs**: Structural modeling of peptides was performed using Pymol 3.0, as shown in **Figure 2A.** The secondary structure was determined by circular dichroism studies (**Figure 2B**).

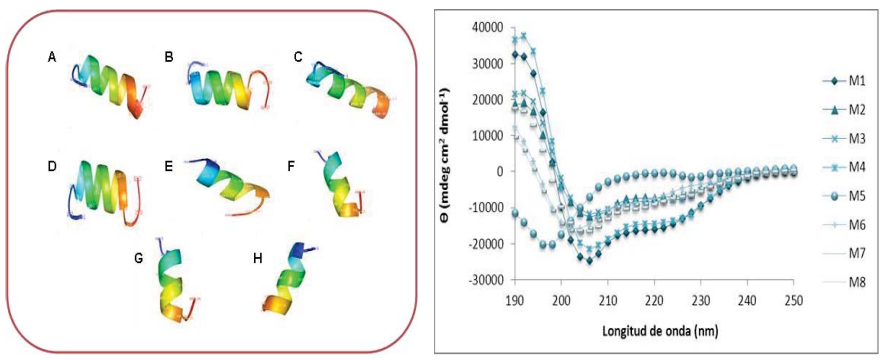


***Figure 2. Secondary structure of AMPs.*** *A)* Simulation of the secondary structure of different peptides, *B*) circular dichroism of synthesized peptides.

**Determination of the antimicrobial activity of AMPs:** **Figure 3** shows results of the antimicrobial activity of the encapsulated peptide LLKNIGLLSVFKKVLKG (M3), which is active against *MRSA* with minimum bactericide concentrations of 25µM, being three times more active compared to the free peptide (Table 2) (**Table 2**).

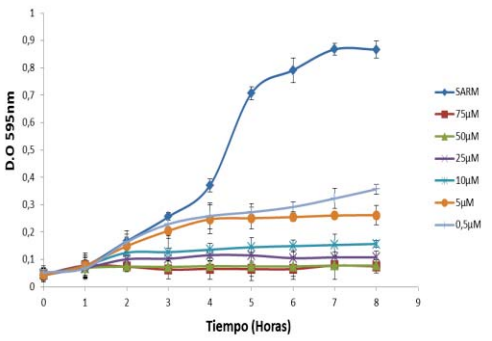


***Figure 3. Bactericidal activity of encapsulated LLKNIGLLSVFKKVLKG against MRSA strain.***

***Table 2: Bactericidal activity of AMPs against and MRSA***

| SYNTHETIC PEPTIDES | MBC |
|---|---|
| LLKNIGLLSVFKKVLKG Free | 75 µM |
| LLKNIGLLSVFKKVLKG Encapsulated | 25 µM |

## CONCLUSIONS

The analogs designed peptides, showed higher antibacterial activity against MRSA. M3 showed minimal bactericidal concentration of 75µM

The encapsulation of the peptide M3 by nanoprecipitation with biodegradable polymer PLGA, allowed to increase its antibacterial activity 3 times compared to the free peptide, reaching a CMB of 25µM against MRSA.

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## REFERENCES


1. J. Blazquez, A. Couce, J. Rodríguez-Beltrán, A. Rodríguez-Rojas, Curr. Opin. Microbiol 2012,15, 561-569.
2. R. Hicks, J. Bhonsle, D. Venugopal, B. Koser, A. Magill, Med. Chem2007, 50, 3026-3026 .
3. Bilati, U, Alleman E. European Journal of Pharmaceutical Sciencies 2005, 24, 67–75.
4. A. Espinel-Ingroff, F. Barchiesi, et al., J. Clin. Microbiol 2005,43, 3884-3889.