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Par

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Obtention et évaluation de l'activité antibiofilm de peptides et peptidomimétiques issus de *Capsicum baccatum* var. *pendulum* (Solanaceae)

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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

Peptides and peptidomimetics obtention from *Capsicum baccatum* var. *pendulum* (Solanaceae) aiming to the antibiofilm activity

Thesis presented by **Rafael Gomes Von Borowski** for the obtention of (**PhD.**) **DOCTOR** degree:
Mention: Docteur en Microbiologie et Biochimie by Ecole doctorale Biologie Santé.
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Résumé

Le biofilm est une matrice complexe, composée de substances polymères extracellulaires enveloppant des communautés de micro-organismes, adhérant de manière irréversible à une surface biotique (tissus et organes) ou abiotique (cathéters et prothèses). Les biofilms permettent à de nombreuses bactéries pathogènes d'adhérer à des dispositifs implantés tels que sondes, prothèses, cathéters ou tissus endommagés, avec un impact majeur sur la santé des patients infectés. La formation de biofilms représente donc une menace clinique croissante, d'autant qu'aucun médicament anti-biofilm n'est actuellement disponible (Bjarnsholt et al., 2013).

Les bactéries du genre *Staphylococcus* comprennent un groupe diversifié de commensaux et d'agents pathogènes qui colonisent la peau et les muqueuses des mammifères (Méric et al., 2018). Certains des membres les plus connus de ce genre, tels que *S. aureus* et *S. epidermidis*, sont des agents pathogènes responsables de la formation de biofilms (Paharik e Horswill, 2016). *S. epidermidis* est notamment à l'origine d'infections associées aux dispositifs médicaux (Rogers et al., 2009; Uçkay et al., 2011; Nishizaki et al., 2013). De plus, *S. epidermidis* développe une multi-résistance aux antibiotiques et, bien que cette bactérie exprime de nombreux facteurs de virulence, la formation de biofilm est le mécanisme le plus important contribuant à l'infection (Otto, 2008; Fey e Olson, 2010; Laverty et al., 2013; Otto , 2013; Otto, 2014).

Cette émergence rapide de la résistance aux antibiotiques est due à diverses conditions associées aux biofilms, telles que la facilitation de l'échange de matériel génétique (Madeo e Frieri, 2013; Scopel et al., 2013; Travier et al., 2013). En ce sens, la matrice de biofilm de *S. epidermidis* joue un rôle fondamental en raison de sa constitution complexe en exopolysaccharides, protéines, ADN et acides teichoïques (Otto, 2008; Paharik et Horswill, 2016). Cette matrice, en plus de conférer une stabilité mécanique au biofilm, rend l'adhésion des bactéries irréversible et difficile à traiter (Haussler e Fuqua, 2013). Ainsi, ces composants sont des cibles importantes dans la recherche d'agents anti-biofilm.

Dans la recherche de nouvelles molécules antibiofilm nous nous sommes particulièrement intéressés à diverses espèces de piments appartenant au genre

Capsicum, originaires des zones tropicales et humides d'Amérique et actuellement cultivées dans le monde entier (Govindarajan, 1986; Menichini et al., 2009; Kim et al., 2014). Certains piments *Capsicum* présentent une production mondiale d'environ 19 millions de tonnes de fruits frais sur 1,5 million d'hectares (FAOSTAT 2001: bases de données statistiques de la FAO, 2003). Ils sont communément appelés tili, piments forts, paprika et poivrons rouges. Les espèces de *C. baccatum* sont communément connues au Brésil sous les noms de poivron «dedo-de moça» et de « cambuci », avec des colorations et des formes différentes. Leur culture est pratiquée dans presque toutes les régions du Brésil et constitue un excellent exemple d'agriculture familiale (Embrapa, 2002; Ecocrop, 2013). *Capsicum* baccatum var. *pendulum* (NCBI: txid40320) est caractérisé par des fleurs crème dotées de corolles dorées / vertes. En règle générale, les fruits sont allongés avec des graines de couleur crème (Betemps e Eloi Pinto, 2015).

Notre groupe de recherche a développé un important travail de caractérisation chimique et de bioactivité à partir de *Capsicum* baccatum var. *pendulum* (Zimmer, Aline Rigon et al., 2012; Zimmer, AR et al., 2012; Gomes Von Borowski, 2015; Molon, 2016, Leonardi, 2017. ; Von Borowski et al., 2017). Précisément, nous avons identifié et normalisé différents extraits de graines de *C. baccatum* présentant une importante activité antibiofilm sur *S. epidermidis* et *Pseudomonas aeruginosa*. Nous avons également évalué leur profil toxicologique *in vitro* et *in vivo*. L'extrait le plus actif de *C. baccatum* a été incorporé aux lames de Permanox ™ par une technique de « spin-coating » afin de produire une surface anti-infectieuse. De plus, une évaluation toxicologique *in vivo* a été réalisée à l'aide du modèle de larves de *Galleria (G. mellonella)*. L'extrait aqueux résiduel des graines de *C. baccatum* (RAqS) est l'extrait le plus actif, capable d'inhiber respectivement jusqu'à 80% et 60% du biofilm de *S. epidermidis* et de *P. aeruginosa*, sans inhiber la croissance des bactéries planctoniques et sans favoriser la toxicité aiguë chez *G. mellonella*. Le traitement de revêtement de surface par le RAqS a modifié la caractéristique hautement hydrophobe des lames Permanox ™ et a empêché l'adhésion bactérienne et le développement du biofilm. Une analyse par microscopie électronique à balayage a montré la présence de petits groupes ou de cellules individuelles sans la présence de matrice sur les échantillons traités au RAqS, même lorsque le RAqS était en

solution ou en tant que revêtement. L'évaluation phytochimique du RAqS a révélé la présence principale d'acides aminés / protéines et de tanins (Gomes Von Borowski et al., 2019). Nous avons ensuite procédé au fractionnement du RAqS, qui a finalement abouti à une fraction active semi-purifiée de peptides (AF). Nous avons procédé à la caractérisation de l'AF et déterminé sa composition. Après cette purification, il a été démontré que les peptides d'AF étaient plus actifs contre la formation de biofilm de *S. epidermidis,* tout en maintenant l'absence d'activité antibactérienne, de revêtement de biomatériau et l'absence d'effet cytotoxique (Gomes Von Borowski, 2015; Gomes Von Borowski et al., 2019).

Dans le contexte des bactéries résistantes/tolérantes aux antibiotiques, les peptides naturels jouent un rôle de plus en plus important en tant qu'agents antimicrobiens et anti-biofilm (Feuillie et al., 2017; Grassi et al., 2017; Von Borowski et al., 2017). Les plantes sont constamment soumises à la présence d'une variété d'agents pathogènes et, pour se défendre, synthétisent des facteurs de protection tels que peptides et protéines de défense (Castro e Fontes, 2005). Ces peptides sont des composants chimiques courants dans les graines du genre *Capsicum* (Lee et al., 2004; Ribeiro et al., 2007; Ribeiro et al., 2012; Dias et al., 2013; Ribeiro et al., 2013). Certaines études suggèrent que ces protéines d'origine végétale pourraient présenter des activités anti-adhésives (Lengsfeld et al., 2004; Wittschier et al., 2007; Bensch et al., 2011).

Dans cette étude, nous avons identifié et caractérisé un peptide antibiofilm particulièrement actif (brevet/Europe nº 19305205.7), nommé capsicumicine. La capsicumicine est capable d'empêcher l'établissement et le maintien de l'architecture du biofilm de *S. epidermidis*. Il diminue notamment l'adhésion et l'agrégation cellulaire de *S. epidermidis* résistant à la méthicilline. Nous avons démontré que le mécanisme d'action de la capsicumicine est lié à une interaction de ce dernier avec la matrice de biofilm en phase initiale, entraînant une modification de l'autoassemblage de la matrice (*Matrix anti-assembly, MAA*) et par là-même une perte de fonctionnalité. Ce mécanisme est indépendant de la régulation cellulaire.

La réduction de l'adhérence bactérienne et de la formation de biofilms par une voie n'impliquant pas la mort cellulaire constitue une nouvelle approche de traitement par antivirulence. Un tel traitement vise en effet à rendre les

microorganismes plus sensibles aux agents antimicrobiens et au système immunitaire (Brancatisano et al., 2014).

Ainsi, cette étude rapporte la découverte de la capsicumicine, un nouveau peptide antibiofilm qui empêche de manière significative l'établissement et le maintien des biofilms bactériens. Il diminue l'adhésion et l'agrégation cellulaire de *S. epidermidis* résistant à la méthicilline, sans pour autant avoir une activité antibiotique directe. Ce mécanisme d'action est nouveau et très prometteur. La prochaine étape consistera à élaborer des stratégies peptidomimétiques afin d'en améliorer la stabilité et l'activité de la capsicumicine *in vivo*.

Resumo

O biofilme apresenta vários benefícios às bactérias devido à existência de uma matriz que confere resistência e tolerância aos antibioticos. O *Staphylococcus epidermidis* é uma das bactérias com maior relevância clínica devido à sua capacidade de formar biofilmes em dispositivos médicos, tais como, marca-passos, cateteres urinários e próteses. Neste contexto, os peptídeos têm sido propostos como uma alternativa importante, tanto como tratamento, quanto como agentes anti-infecciosos de superfície. Este estudo consiste na identificação de novos peptídeos naturais e sintéticos, derivados da pimenta *Capsicum baccatum* var. *pendulum*, com atividade antibiofilme. Por conseguinte, foi selecionado e estudado extensivamente um peptídeo de referência que apresentou a melhor atividade antibiofilme contra *S. epidermidis*. Este peptídeo atua através de um novo mecanismo de ação que descrevemos e chamamos de "anti-montagem de matriz" (AMM). No primeiro capítulo deste trabalho foi abordado a ligação entre peptídeos, biofilmes patogênicos e a atividade antibiofilme. O Capítulo 2 consiste nos principais resultados experimentais desta tese como a caracterização da atividade antibiofilme do peptídeo de referência, que age através do novo mecanismo de ação AMM independente da regulação celular e os testes de citotoxicidade. Esses resultados nos permitiram patentear o peptídeo em questão, referenciado no Capítulo 3. Finalmente, o último capítulo descreve o possível uso de peptidomiméticos antibiofilme como uma perspectiva. A estratégia é criar pequenas moléculas semelhantes ao peptídeo de referência. Estes peptidomiméticos mantêm as capacidades inerentes ao peptídeo principal, porém são mais resistentes a proteases e / ou mais ativos.

Abstract

Biofilm confers to bacteria many benefits due to the production of a matrix that improves their resistance and tolerance to antibiotics. *Staphylococcus epidermidis* is one of the most important clinical bacteria, able to form biofilm on medical devices such as pacemakers, urinary catheters and prostheses. In this context, peptides have been proposed as an important alternative as a treatment or as anti-infective surface agents. This study focuses on the identification of new antibiofilm natural and synthetic peptides from the *Capsicum baccatum* var. *pendulum* pepper. As a result, a lead peptide responsible for the antibiofilm activity against *S. epidermidis* was selected and extensively studied. It acts by a new mechanism of action that we call "matrix anti-assembly" (MAA). In the first chapter, we explore the link between peptides, pathogenic biofilms and the antibiofilm activity. Chapter 2 consists of the main experimental results of this thesis. It describes the antibiofilm characterization of the lead peptide acting by the AAM new mechanism of action, independent of cell regulation. Cytotoxicity tests are also presented. These results allowed us to patent this peptide, referenced in Chapter 3. The last chapter presents the possible use of antibiofilm peptidomimetics as a perspective. The strategy is to create small peptide-like molecules. These peptidomimetics retain the inherent capabilities of the lead peptide, but are more resistant to proteases and / or more active.

SUMMARY

1. General introduction

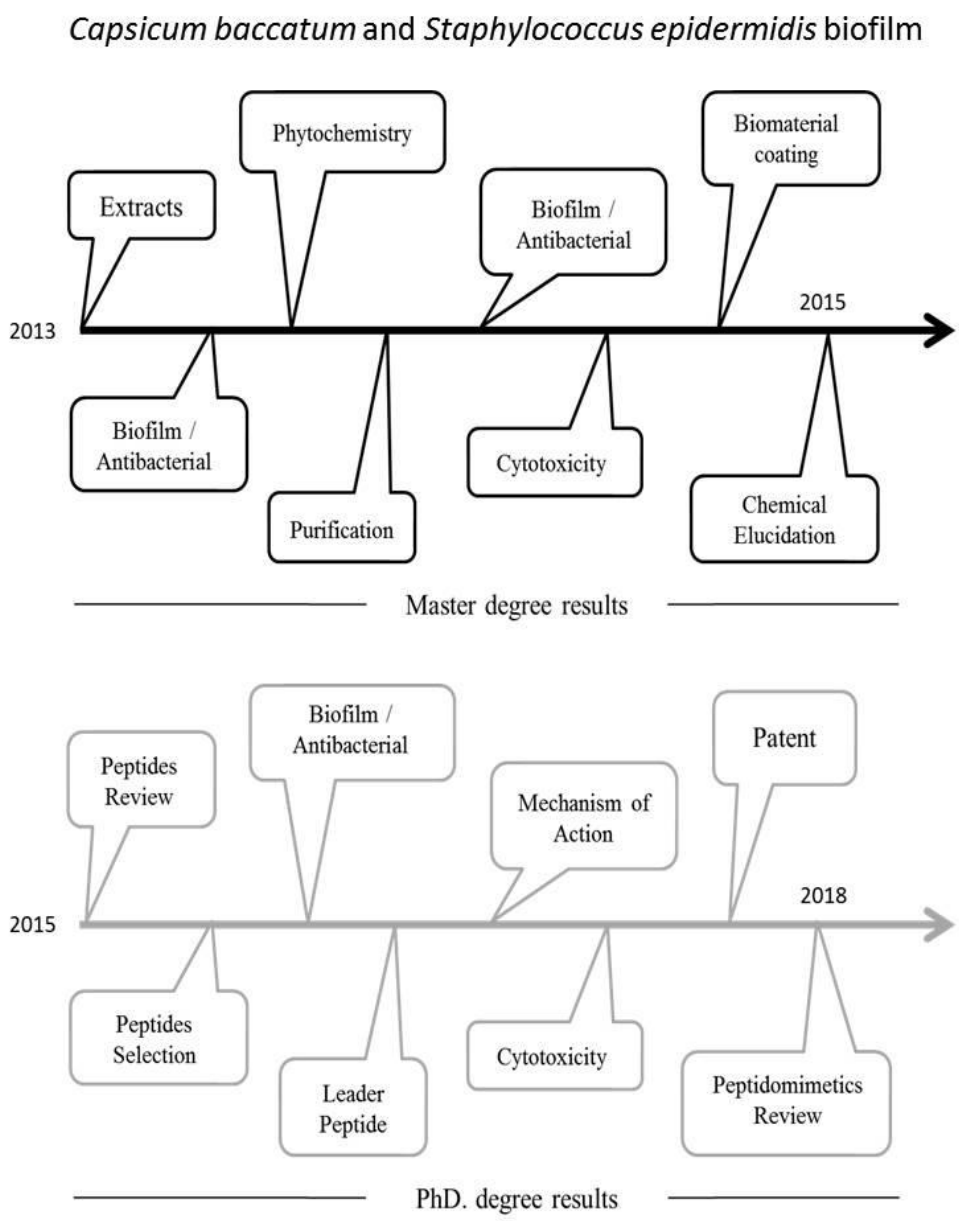
This thesis reports the discovery and antibiofilm evaluation of peptides from *Capsicum baccatum* seeds. We evidence the selection and identification of a lead peptide responsible for *Staphylococcus epidermidis* antibiofilm activity and the elucidation of its mechanism of action.

Biofilm formation may cause or worsen infections, since the microorganisms are resilient to most of the treatments available. Besides the recognized and increasing problematic issue of bacterial resistance, biofilm formation represents a rising clinical threat without available drugs (Bjarnsholt *et al.*, 2013).

The first chapter of this study is a review of the scientific literature (2005-2015) and introduces the universe of peptides, pathogenic biofilms concerns and antibiofilm activity. It presents a vast review article (Von Borowski *et al.*, 2017) contemplating a perspective of peptides and antibiofilm structure activity relationship (SAR). This review was also used as an instrument to select the lead peptide used in the experimental part of this work.

To better guide the readers through this thesis, the history of the studies with *Capsicum baccatum* pepper as well as my scientific course up to now are briefly described (Flow chart 1). Our research group has been developing an important work of chemical and bioactivity characterizations with this species (Zimmer, Aline Rigon *et al.*, 2012; Zimmer, A. R. *et al.*, 2012; Gomes Von Borowski, 2015; Molon, 2016; Leonardi, 2017; Von Borowski *et al.*, 2017). Precisely, we identified and standardized different extracts of *C. baccatum* seeds displaying *S. epidermidis* and *Pseudomonas aeruginosa* antibiofilm activity and evaluated its toxicological profiles *in vitro* and *in vivo*. The most active extract of *C. baccatum* was incorporated in Permanox™ slides by spin-coated technique in order to produce an anti-infective surface. In addition, an *in vivo* toxicological evaluation was performed using the alternative host model of *Galleria mellonella larvae*. The residual aqueous extract from *C. baccatum* seeds (RAqS) was the most active extract, able to inhibit up to 80% and 60% of the *S. epidermidis* and *P. aeruginosa* biofilm, respectively, without inhibiting the planktonic bacterial growth, neither promoting acute toxicity in *G. mellonella*. The surface coating with the RAqS modified the highly hydrophobic feature of Permanox™ slides and prevented the bacterial adhesion and biofilm development. Scanning electron microscopy analysis showed the presence of only small clusters or individual cells without the presence of matrix on the RAqS treated

samples, even when RAqS was in solution or as coating. The phytochemical evaluation of RAqS indicated the main presence of amino acids/proteins and tannins (Gomes Von Borowski *et al.*, 2019). Then, we operated the bioguided fractionation of RAqS, finally leading to a semi-purified active fraction (AF) of peptides. We proceeded with the “AF peptides” characterization and determined its sequence composition and protein origin. After this purification, the “AF peptides” was shown to be more active against *S. epidermidis* biofilm formation keeping the absence of antibacterial activity, biomaterial coating and non-cytotoxic effect (Gomes Von Borowski, 2015; Gomes Von Borowski *et al.*, 2019). This original article is in progress.



Flow chart 1. *Capsicum baccatum* and *Staphylococcus epidermidis* biofilm studies. Time line in black displaying a summary of master degree results by Gomes Von Borowski, 2015. Brefly extracts obtention from *C. baccatum* seeds, antibiofilm/antibacterial screenings, phytochemical caracterization, bio-guided purification of the most active extract, biofilm/antibacterial activity of the purified fraction (PF) and cytotoxicity, biomaterial coating, chemical elucidation essays of the PF. Time line in grey displaying a summary of PhD degree results by Gomes Von Borowski, 2018. Brefly, a peptide and antibiofilm activity review, the selection of three natural peptides from PF to be synthesize and the identification of the lead antibiofilm peptide (LAP). The elucidation of LAP mechanism of action and cytotoxicity essays. These results leaded to the register of a patent deposit. Finally, we used a scientific review to base ourselves on the best peptidomimetic models for antibiofilm activity.

Chapter 2 describes the main experimental results of this thesis. It contains the antibiofilm characterization of the lead peptide, evidences about its mechanism of action and cytotoxicity assays as experimental work, discussion and conclusions.

In addition, the consistence of this study drove us to an international patent registration. The patent description is also available in chapter 3 and strengthens the relevance and applicability of our research.

Finally, in chapter 4 we propose a molecular simplification and peptidomimetic derivatives synthesis to improve the lead peptide efficiency. Peptidomimetics are a strategy of molecular improvements to advance some aspects like resistance to proteases or to boost its bioactivity. Therefore, we used a scientific review to base ourselves on the best peptidomimetic models for antibiofilm activity. Consequently we published a review article (Gomes Von Borowski *et al.*, 2018) whose manuscript is presented in this chapter.

1.1 *Staphylococcus epidermidis*

Bacteria from the genus *Staphylococcus* include a diverse group of commensals and pathogenic that colonize mammals on the skin or mucous membranes (Méric *et al.*, 2018). Some of the best-known members of this genus, such as *S. aureus* and *S. epidermidis*, are also opportunistic biofilm forming pathogens (Paharik e Horswill, 2016).

Concerning pathogenic biofilms, *S. epidermidis* is the most frequently coagulase negative *Staphylococcus* (CoNS) infection, highly associated to health care bloodstream and medical devices infections (Rogers *et al.*, 2009; Uçkay *et al.*, 2011; Nishizaki *et al.*, 2013).

Furthermore, *S. epidermidis* is developing antibiotic multi-resistance and while this bacterium expresses many virulence factors, the biofilm formation is the most important mechanism contributing to infection (Otto, 2008; Fey e Olson, 2010; Laverty *et al.*, 2013; Otto, 2013; Otto, 2014). This rapid emergence of antibiotic resistance occurs due to various conditions associated to biofilm lifestyle such as facilitating the exchange of genetic material (Madeo e Frieri, 2013; Scopel *et al.*, 2013; Travier *et al.*, 2013).

In this sense, *S. epidermidis* biofilm matrix has a fundamental role due to its complex constitution such as exopolysacharides, proteins, extra-DNA (eDNA) and

teichoic acids (Figure 1) (Otto, 2008; Paharik e Horswill, 2016). This matrix besides conferring mechanical stability to the biofilm, makes bacteria adhesion irreversible and difficult to treat (Haussler e Fuqua, 2013). Thus, these components are important targets in the search for antibiofilm agents.

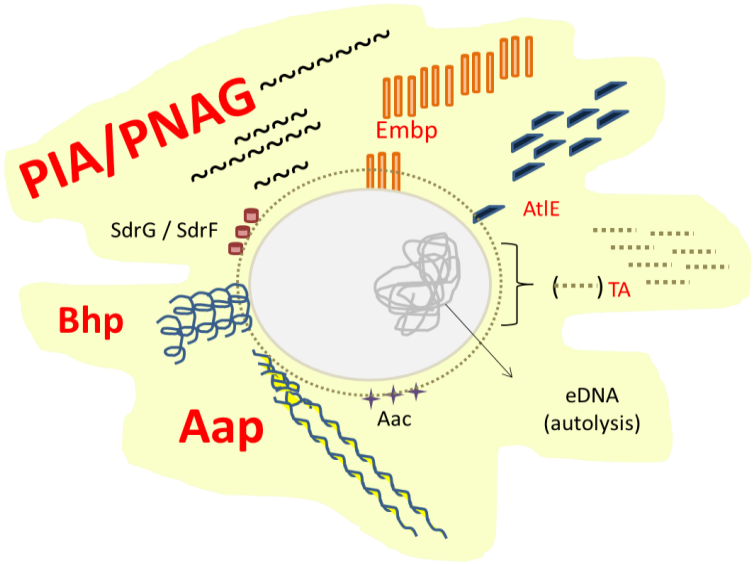


Figure 1. Summary of *Staphylococcus epidermidis* matrix composition. Bacterial cell is illustrated in the center and matrix around in light yellow. The main components of the matrix are named in red and police size is proportional to relevance. PIA /PNAG are polysaccharides, eDNA are extra-cellular DNA, TA are teichoic acids and others are proteins (Gomes Von Borowski, R., 2018).

1.2 *Capsicum baccatum* var. *pendulum*

Many species of red peppers come from *Capsicum* genus, native from the tropical and humid zones of America and currently cultivated worldwide (Govindarajan, 1986; Menichini *et al.*, 2009; Kim *et al.*, 2014). Some *Capsicum* peppers present a world production of about 19 million tons of fresh fruit from 1.5 million ha (FAOSTAT 2001: FAO Statistical Databases, 2003).

These peppers are popularly known as tili, hot peppers, paprika and red pepper. In particular, *Capsicum baccatum* species are popularly known as “dedo-de moça” pepper (finger pepper) and cambuci, with different colorations and forms. Its cultivation occurs practically in all regions of the country (Brazil) and it is a great example of familiar agriculture (Embrapa, 2002; Ecocrop, 2013).

The *Capsicum baccatum* var. *pendulum* (NCBI: txid40320) plant is characterized by cream flowers with gold/green corolla markings. Typically, fruits are elongated with cream colored seeds (Figure 2), (Betemps e Eloi Pinto, 2015).

The specimen used in this study was obtained from a controlled cultivated area in Turuçu, Rio Grande do Sul (RS), Brazil (latitude: 31º 25 '18 "S, longitude: 52 ° 10 '42 "W, altitude: 30m and area: 286.1 Km^2). A voucher specimen (number P278) was identified and deposited at the Herbarium of Brazilian Government Research Institute EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária, Pelotas, RS, Brazil).

Figure 2. Pictures of dedo-de-moça pepper (*C. baccatum var. pendulum*), author archive; A) fruits with 7 x 1,5cm (length x width), red colored (mature); B) seeds, light yellow colored and C) Flower with white corolla, a pair of yellowish or greenish spots at the base of each wolf of the petals and anthers usually yellowish (one to two flowers per node).

2. Objectives

The main objectives of this study were to select and obtain natural and synthetic peptides from *Capsicum baccatum* with significant bacterial antibiofilm activity and to establish their mechanism of action.

2.1 Aims of the study

- To identify the amino acid sequence that forms the composition of natural peptides of interest by mass analysis and bioinformatics;
- To determine the minor peptides based on the most promising natural peptide by molecular simplification;
- To assay *in vitro* the antibiofilm and the antibacterial activity of peptides, by crystal violet method and optical density and CFU/mL, using *Staphylococcus epidermidis* ATCC 35984 model;
- To evaluate the cytotoxicity of peptides with multiparameter high-throughput image analysis (HCS: High Content Screening and HCA: High Content Analysis), using 7 different mammalian lines: HuH7, CaCo-2, MDA, HCT116, PC3, NCI-H727 and MCF7;
- To establish the target of the lead bioactive peptide in treated bacteria (Intra or extra-cells) by fluorescence labeling and visualization in Microscopy of Fluorescence;
- To analyze the ultrastructural interactions of exposed bacteria by Transmission Electron Microscopy;
- To analyze topographic structural interactions of biomaterials exposed to bacteria in the presence/absence of lead peptide by Scanning Electron Microscopy;
- To evaluate gene expression variation by qRT-PCR in the presence/absence of lead peptide;
- To design peptidomimetics based on the lead bioactive peptide.

3. Chapter 1. Literature review: article 1

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Peptides as a strategy against biofilm-forming microorganisms: Structure-activity relationship perspectives

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ABSTRACT

Biofilm forming microorganisms substantially enhance their virulence and drug resistance causing and alternatives are need to combat this health problem. In this context, peptides are an exceptional strategy in drug design and pharmaceutical innovation due to their diverse chemical features, biological activity and biotechnological relevance. Therefore, this study proposes a comprehensive assessment of a wide range of peptides, targeting biofilms. It provides chemical and molecular information and a Structural Activity Relationship perspective in order to delineate minimal requirements for antibiofilm activity and contributing to the development of new antibiofilm agents. In light of this, it was possible to propose a peptide design model (X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}-X_{16}-X_{17}-X_{18}-X_{19}-X_{20}) to be tested in the war against resistant microorganisms.

1. Introduction

Besides the recognized and increasing problematic issue of bacterial resistance, biofilm formation represents a rising clinical threat. In a hospital scenario, biofilm formation may cause or worsen infections, since the microorganisms are resilient to most of the treatments available (Bjarnsholt et al., 2013). Biofilm is defined as a microbial lifestyle in which microbial adhere to a surface and produce a matrix that enables them to overcome a series of environmental stresses due to their interaction abilities. Several mechanisms have been proposed to explain the drug resistance within biofilms, including the delayed/suppressed penetration of the antimicrobial agent into the extracellular matrix, the presence of metabolically inactive 'persister' cells, and the increased ability to exchange mobile genetic elements encoding resistance (Hoiby et al., 2010; Mah, 2012a; Mah, 2012b).

It is increasingly evident that alternatives are need to combat drug-resistant organisms and biofilms. In this context, peptides are an outstanding strategy because they able to establish diverse biomolecules (Yoshikawa, 2015) due to chemical features like malleability and multifunctionality. Peptides are the source of many bioactive compounds with distinct activities, such as immunomodulation (Faruqi, 2013; Sanchez-Margalet et al., 2003; Pennington et al., 2015; Pasikowski et al., 2011), antitumor (Bajou et al., 2014; Chernysh et al., 2002; Martinez-Hoyer et al., 2015), anticancer (Leuschner and Hansel, 2004; Berge et al., 2010; Ciocca et al., 2012), antimicrobial (Hancock and Sahl, 2006; Dinh et al., 2015; Ganz, 2003), and, currently, antibiofilm (Lum et al., 2015; Wu et al., 2015; de la Fuente-Nunez et al., 2015). Also, several physico-chemical parameters including charge, hydrophobicity, and secondary structure are possible targets to modify and to influence the peptide activity and differential selectivity.

In addition, it is well established that peptides can play an interesting role in development of new biomaterials having anti-infective activity. They act in the earliest step in the pathogenesis of foreign-body-related infections in bacterial adhesion; the colonization will hardly occur if bacteria can not adhere to a surface (Campoccia et al., 2013; Glinel et al., 2012).

A few publications have addressed Antimicrobial Peptides (AMPs) as peptide models against biofilm-forming microorganisms (Jorge et al., 2012; Strempel et al., 2015; Di Luca et al., 2014; Schillaci et al., 2013) then, it will not be the focus of this work.

Even more, applicability of AMPs as antibiofilm agents is hindered by some problems, like the absence of a defined mechanism of action, cytotoxicity, stability, bioavailability and adaptive bacterial resistance (Jorge et al., 2012; Di Luca et al., 2014; Schillaci et al., 2013; Wang et al., 2014).

Despite these peptide advantages, the structure-activity relation-

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Table 1
Peptide information in alphabetical order: peptides with both Gram positive (G +) and negative (G −) antibiofilm relative activity tested.

ID	Peptide	General information/range of concentration tested	N° AA	Sequence	*Theoretical	
					Molecular weight	Total net charge
1	AS10	It is a shortened variant of the known cathelicidin-related antimicrobial peptide (CRAMP) discovered in the	26	KIGEKLKKIAQKIKNFFQKL-VPQPEQ	3.069.717	5
2	P318	islets of Langerhans of the murine pancreas, in which each amino acid of the native sequence was individually (10th aa) replaced by an alanine residue; 6.25–12.25 μM	26	KIGEKLKKIAQKIKNFFAKL-VAQPEQ	2.992.627	5
3	Battacin analogue	A cyclic lipopeptide produced by the soil isolate *Paenibacillus tianmunesis* that belongs to the octapeptin group of peptide antibiotics characterized by a high percentage of the nonprotein amino acid α,γ-diaminobutyric acid (Dab) and a branched β-hydroxy fatty acid tail linked to a cyclic heptapeptide moiety; 1–100 μM	17	$_{D}$.Dab-Dab-Dab-L-$_{D}$-F-Dab-Dab-L	x	x
4	BL-DZ1	Identified (14 kDa) as BL00275, accession number gi52082584, from *B. licheniformis* ATCC 14580. The purified protein was stable at 75 °C for 30 min and in the pH range between 3.0 and 11.0; however, it was sensitive to the enzymes trypsin and proteinase K; 1.60 mg/mL	ns	Ns	x	x
5	BMAP27-Melittin	Both are antimicrobial peptides (AMPs), the bovine derived BMAP-27 and the bee venom derived melittin. This hybrid peptide consists of an N-terminal fragment obtained from residues 9–20 of BMAP-27 and a C-terminal fragment from residues 2–9 of melittin. It displays a total helicity of 76.2%, net charge of + 6, hydrophobicity of 0.531, and hydrophobic moment of 0.64	21	KFKKLFKKLSPVIGAVLKVLT	2.352.019	6
6	Coprisin	It is a defensin-like peptide (AMP) from the dung beetle *Copris tripartitus*. Combinations of coprisin and other conventional antibiotics (ampicillin, vancomycin, and chloramphenicol) also showed antibiofilm properties against preformed biofilms; 8–16 μg/mL	43	VTCDVLSFEAKGIAVNHSA-CALHCIALRKKGGSCQNGV-CVCRN	4.477.251	3
7	DispersinB™ and KSL combination	DispersinB™ is a naturally occurring enzyme (40 kDa, glycoside hydrolase) produced by an oral bacterium *Aggregatibacter actinomycetemcomitans*, an antibiofilm	10	KKVVFKVKFK	1.250.618	5
8	DispersinB™ and KSL-W combination	enzyme based wound gel in combination with a synthetic broad-spectrum cationic antimicrobial decapeptide, 1.31 kDa; 0.9–8 μg/mL	10	KKVVFWVKFK	1.308.661	4
9	Gramicidin A	They are linear pentadecapeptides with a molecular mass of approximately 1900 Da and consist of alternating L- and D-amino acids. The natural mixture of gramicidins contains predominantly (85%) gramicidin A, which is hydrophobic, forming a β-bonded helix; 20 mg/L	15	XGALAVVVWLWLWLW	1.712.116	0
10	Holothuroidin 1	Natural peptide fraction (AMP) from the coelomocyte cytosol from *Holothuria tubulosa* (sea-cucumber).	12	HLGHHALDHLLK	1.389.5	0
11	Holothuroidin 2	1389.5 Da, 7.56 of pl, and 1547.6 Da, + 0.9 total net charge, 42.86% total hydrophobic ratio, 7.56 of pl, both with an α-helical secondary structure; 3.1–6.2 mg/mL	14	ASHLGHHALDHLLK	1.547.6	0
12	IDR-HH2	Synthetic analogs of host defense peptide (HDP) termed	12	VQLRIRVAVIRA	1.393.747	3
13	IDR-1002	innate defense regulator (IDR) peptides; 5–80 μg/mL	12	VQRWLIVWRIRK	1.6530.52	4
14	IDR-2009		12	KWRLLIRWRIQK	1.696.116	5
15	K4K20S4	They are dermaseptins, linear polycationic peptides arranged in amphipathic α-helices in nonpolar solvents.	28	ALWKTLLKKVLKAAAKAAL-KAVLVGANA	2.861.579	6
16	K4S4	They all have a conserved Trp residue at position 3, an AG(A)KAAL(V/G)G(N/K)AV(A) consensus motif in the middle region and a positive charge attributable to the presence of Lys residues that punctuate an alternating hydrophobic-hydrophilic sequence. These derivatives combine two substitutions: methionine with lysine at position 4 and asparagine with lysine at position 20; 0.4–25 μg/mL	28	ALWKTLLKKVLKAAAKAAL-NAVLVGANA	2.847.513	5
17	NapFFKK	They are ultrashort aromatic peptides that self-assemble	4	NapFFKK	568.699	x
18	NapFFFKK	into inherently antimicrobial hydrogel nanostructures. They consist of two phenylalanine building blocks conjugated to a molecule of high aromaticity, such as naphthalene (Nap) or 9-fluorenylmethoxycarbonyl (Fmoc); 0.5–2 (*w/v* %)	5	NapFFFKK	715.875	x
19	Paracentrin 1	Based on a peptide fraction from the coelomocyte cytosol (5-kDa), it is composed of fragments of a β-thymosin from *Paracentrotus lividus*, mainly enriched by residues such as lysine, with a pI of 10.72 and a net charge of + 1	11	EVASFDKSKLK	12.514.417	1

(continued on next page)

Table 1 *(continued)*

ID	Peptide	General information/range of concentration tested	N° AA	Sequence	*Theoretical	
					Molecular weight	Total net charge
		at pH 7.0. It has properties similar to those of AMPs with a hydrophobic core; 25 to 0.07 mg/mL				
20	PAβN	It is the Phe-Arg β-naphthylamide dipeptide and is an efflux pump inhibitor (EPI); 20–50 μg/mL		FR-FR	624.726	x
21	Pleuricidin	It is an AMP derived from the skin mucous secretions of the winter flounder *Pleuronectes americanus*. It showed synergistic combinations with several other antibiotics and its mechanisms; 1.9–3.8 μg/mL	25	GWGSFFKKAAHVGKHVGK-AALTHYL	2.711.144	4
22	1037	It is a small cationic peptide chosen based on a consensus identified amphipathic sequence (FRIRVRV), with 3 cationic residues (i.e., R) and 4 hydrophobic amino acids; analogous to host defense peptides (predecessor LL-37); 5–25 μg/mL	9	KRFRIRVRV	1.229.54	5

ID	*Theoretical		Antibiofilm activity		Antimicrobial activity		References
	Hydrophobicity ⟨H⟩	Protein-binding potential (Boman index)	G −	G +	G −	G +	
1	0.235	1.68 kcal/mol	*E. coli* (TG1); *P. aeruginosa* (PA14); *P. gingivalis* (ATCC33277)	*S. epidermidis* (ns)	ns	ns	(De Brucker et al., 2014)
2	0.240	1.33 kcal/mol					
3	x	x	*P. aeruginosa* (16207)	*S. auereus* (ns)	*P. aeruginosa* (16207)	*S. auereus* (ns)	(De et al., 2015)
4	x	x	*P. aeruginosa* (PAO1)	*B. pumilus* (TiO1)	*P. aeruginosa* (PAO1)	*B. pumilus* (TiO1)	(Dusane et al., 2014)
5	0.544	− 0.29 kcal/mol	*P. aeruginosa* (2114)	*S. aureus* (43330)	*P. aeruginosa* (2114)	*S. aureus* (43330)	(Almaaytah et al., 2015)
6	0.450	0.96 kcal/mol	*E. coli* (O-157, ATCC43895, ATCC25922); *P. aeruginosa* (ATCC27853)	*S. mutans* (KCTC3065)	*E. coli* (O-157, ATCC43895, ATCC25922); *P. aeruginosa* (ATCC27853)	*S. mutans* (KCTC3065)	(Hwang et al., 2013)
7	0.229	0.96 kcal/mol	(X)/(X); A. baumannii (63270)	*S. epidermidis* (1457); MRSA (Gav16a); Coagulase-negative *Staphylococci* (42)	*K. pneumoniae* (P30); *P. aeruginosa* (232); *A. baumannii* (63270)	*S. epidermidis* (1457); MRSA (Gav16a); Coagulase-negative *Staphylococci* (42)	(Gawande et al., 2014)
8	0.553	0.17 kcal/mol					
9	1.434	− 3.26 kcal/mol	*E. faecalis* (III); *E. coli* (ATCC25922, XL1Blue)	*S. aureus* (ATCC29213, RN 4220); *Listeria ivanovii* (Li4pVS2); *Listeria innocua* (8811)	*E. faecalis* (III); *E. coli* (ATCC25922, XL1Blue)	*S. aureus* (ATCC29213, RN 4220); *Listeria ivanovii* (Li4pVS2); *Listeria innocua* (8811)	(Yala et al., 2011)
10	0.489	0.87 kcal/mol	*P. aeruginosa* (ATCC15442)	*S. aureus* (ATCC25923); *S. epidermidis* (ATCC35984)	P. aeruginosa (ATCC15442)	S. aureus (ATCC25923); S. epidermidis (ATCC35984)	(Schillaci et al., 2013)
11	0.439	0.86 kcal/mol					
12	0.527	1.65 kcal/mol	*P. aeruginosa* (PAO1 LPS)	*S. aureus* (MRSA SAP0017, clinical isolate)	(X)	(X)	(Haney et al., 2015)
13	0.667	2.36 kcal/mol					
14	0.523	3.08 kcal/mol					
15	0.451	− 0.66 kcal/mol	*E. coli* (MG1655); *P. aeruginosa* (PA01)	*S. aureus* (15981)	*E. coli* (MG1655); *P. aeruginosa* (PA01)	*S. aureus* (15981)	(Zaïri et al., 2014)
16	0.465	− 0.62 kcal/mol					
17	x	x	*P. aeruginosa* (PAO1); *E. coli* (NCTC11303)	*S. epidermidis* (ATCC35984); *S. aureus* (ATCC29213)	(X)	(X)	(Laverty et al., 2014)
18	x	x					
19	0.051	2.29 kcal/mol	*P. aeruginosa* (ATCC15442)	*S. aureus* (ATCC29213, 25,923, 6538); *S. epidermidis* (RP62A); *staphylococcal* isolates from sheep (strains 657, 688, 700, 702)	*P. aeruginosa* (ATCC15442)	*S. aureus* (ATCC29213, 25,923, 6538); *S. epidermidis* (RP62A); *staphylococcal* isolates from sheep (strains 657, 688, 700, 702)	(Schillaci et al., 2014)
20	x	x	*E. coli* (clinical isolate, 83,972); *E. coli*	*S. aureus* (8324)	*E. coli* (clinical isolate, 83,972); *E. coli* (wild-type strain	*S. aureus* (8324)	(Kvist et al., 2008)

(continued on next page)

Table 1 *(continued)*

ID	*Theoretical		Antibiofilm activity		Antimicrobial activity		References
	Hydrophobicity ⟨H⟩	Protein-binding potential (Boman index)	G −	G +	G −	G +	
			(wild-type strain F18); *K. pneumoniae* (i222–86) and *P. putida* (KT2442)		F18); *K. pneumoniae* (i222–86) and *P. putida* (KT2442)		
21	0.421	0.2 kcal/mol	*E. coli* (ATCC25922, O-157, ATCC43895); *P. aeruginosa* (ATCC27853)	*S. aureus* (ATCC25923); *E. faecium* (ATCC19434); *P. acnes* (ATCC6919)	*E. coli* (ATCC25922, O-157, ATCC43895); *P. aeruginosa* (ATCC27853)	*S. aureus* (ATCC25923); *E. faecium* (ATCC19434); *P. acnes* (ATCC6919)	(Choi and Lee, 2012)
22	0.111	5.47 kcal/mol	*P. aeruginosa* (PA14 and PAO1); *B. cenocepacia* (4813)	*L. monocytogenes* (568)	*P. aeruginosa* (PA14 and PAO1); *B. cenocepacia* (4813)	*L. monocytogenes* (568)	(de la Fuente-Nunez et al., 2012)

"X", not tested; "ns", not shown. *Theoretical: analyzed by computational model.

ships (SAR) contribution to dispersion or inhibition of biofilms and their limitations are still lacking.

Accordingly, the aim of this review is to offer a comprehensive assessment of the wide range of peptides (in total 85 peptides, of which 46% are from natural (animal, bacterial and plant) sources, 27% from synthetic, and 27% from mixed) having antibiofilm activity, not restricted to AMPs, in order to delineate minimal requirements for this activity (in the period of 2005–2015). Also we provide chemical and molecular information (general information/range of concentration tested, number of amino acids, amino acids sequences, molecular weight, total net charge, hydrophobicity, protein-binding potential and antibiofilm and antimicrobial activities) as basis for a SAR study (Tables 1–4) contributing to the development of new antibiofilm agents. More than 200 papers from the main databases (SciFinder, PubMed and Web of Science) were analyzed, and 54 were reviewed for this study in accordance with inclusion/exclusion criteria.

2. AMINO acids (AA) composition and frequencies

The amino acid residues that constitute the peptides are fundamentally important for their characterization, molecular interactions and activity (Nefedov and Sadygov, 2011). These residues are chemically responsible for all the molecular characteristics including the secondary conformation and hydrophobicity and biotic or abiotic interactions (biomaterials) (Lim et al., 2013; Burton et al., 2006).

Accordingly, all the peptides that appeared in the sequence in the references were analyzed in order to identify the most relevant AA and its SAR. First, they were categorized as *Antibiofilm Peptides against Bacteria* (**APB**) (see Tables 1–3) and *Antibiofilm Peptides against Fungus* (**APF**) (see Table 4) due to their biocide and non-biocide actions. Then, APB was subdivided into Gram-negative, Gram-positive, and both activities together in accordance with the original article.

The tables included in this article show the different peptides in alphabetic order and with individual identification numbers (ID). Repeated peptides in these tables are shown because they were tested for distinct microorganisms. The theoretical analyses were performed only with the peptides from the original articles in which AA sequences appeared, considering the software limitations (atypical AA and minimal number of AA, for example) (Gautier et al., 2008; Wang et al., 2009; Wang et al., 2016). All these results were submitted to cluster and descriptive analysis using the software SPSS (Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.) at the Statistical Assistance Core of the Federal University of Rio Grande do Sul.

2.1. Antibiofilm peptides against bacteria (APB)

2.1.1. APB with both Gram-negative and -positive activities (G ±)

In order to study the AA composition we have analyzed the following aspects: Range of presence of each AA into each single peptide (*individual range)*; Range of presence of each AA for each group of microorganism (APB (G −), APB (G +) or APB (G ±)) (*group range)*; Overall presence of AA in both groups of microorganisms (*overall range)*. Then, the frequencies (in percentage) of these ranges were calculated.

The APB (G ±) included 21 different peptides that could be analyzed. Among them, lysine (K) was the most prevalent AA (APB (G ±) *group range*), present in 19% of them (with a maximum value of 50% of this AA on the constitution of a single peptide (*individual range*)). Also, it was observed high amounts of phenylalanine (F) and leucine (L) (*group range*), with 12% of each one (with maximum values of 60% and 33%, respectively in a single peptide (*individual range*)), followed by valine (V) with 10% (with a maximum value of 30% of this AA in a single peptide) and alanine (A) and arginine (R) with 8% each one (with maximum values of 32% and 50% respectively in a single peptide) (Fig. 1).

Table 2
Peptide information in alphabetical order: peptides with Gram-positive (G +) antibiofilm relative activity tested.

ID	Peptide	General information/range of concentration tested	N° AA	Sequence	*Theoretical				Antibiofilm activity	Antimicrobial activity	References
					Molecular weight	Total net charge	Hydrophobicity ⟨H⟩	Protein-binding potential (Boman index)	G +	G +	
23	Bac8c	It is based on bactenecin, a 12- amino acid cyclic peptide isolated from bovine neutrophils. It is one of the smallest natural cationic antimicrobial peptides and was developed by replacing some residues and linearizing; lower oral cytotoxicity; 64 μg/mL	8	RIWVIWRR	1.184.458	3	x	3.27 kcal/mol	*S. mutans* (UA159)	*S. mutans* (UA159)	(Ding et al., 2014)
24	CAMA	Both are antimicrobial cationic peptides (AMPs). Indolicidin is one of the shortest, a 13-residue tridecapeptide amide that is isolated from cytoplasmic granules of bovine neutrophils, and it has an extremely high tryptophan content. Cecropin (1–7)-melittin A (2–9) amide (CAMA) is a hybrid peptide that contains portions of the amino acid sequences for the silk moth peptide cecropin-A and the bee venom peptide melittin. Nisin is a 34-residue peptide and includes unusual amino acids (lanthionine and methyllanthionine residues); 640 mg/L	ns	Ns	x	x	x	x	*S. aureus* (ATCC43300)	*S. aureus* (ATCC43300)	(Dosler and Mataraci, 2013)
25	Indolicidin				x	x	x	x			
26	Nisin				x	x	x	x			
27	Chicken cathelicidin-2 (F2,5,12 W)	This is a chicken host defense peptide (HDP). The first 15 N-terminal amino acids of CATH-2 (cathelicidin-2) form the core element required for antibacterial activity and was subsequently improved by the tryptophan substitution of all three phenylalanine residues, resulting in peptide F2,5,12 W; 2.5–40 μM	15	RWGRWLRKIRRWRPK	2.144.611	8	0.195	5.52 kcal/mol	*S. epidermidis* (BM185, BM492)	*S. epidermidis* (BM185, BM492)	(Molhoek et al., 2011)
28	Cyclic lipodepsipeptide 1	It has a great structural flexibility and hydrophobicity derived from fusaricidins or the LI-Fs family of AMPs that are positively charged. Cyclic lipodepsipeptides were isolated from *Paenibacillus* sp. (In contrast to typical cationic antibacterial peptides, it has neutral amino acid sequences and a single positive charge located at the termini of their lipidic tails); 8–100 μg/mL	6	$_{D}$-AT-$_{D}$-VV-$_{Da}$T-$_{D}$-N	603.674	0	x	0.31 kcal/mol	*S. aureus* (ATCC29213, USA300, methicillin-resistant)	*S. aureus* (ATCC29213, USA300, methicillin-resistant)	(Bionda et al., 2014)
29	Cyclic lipopeptide 2		6	$_{D}$-A-Dap-$_{D}$-VV-$_{Da}$T-$_{D}$-N	x	x	x	x			
30	Cyclo(L-leucyl-L-prolyl)	An isolated cyclic dipeptide, hydrophilic; 10–50 μg/mL	ns	Ns	x	x	x	x	*S. mutans* (UA159)	*S. mutans* (UA159)	(Gowrishankar et al., 2014)
31	Nisin-NaF	It is a polycyclic cationic peptide produced by *Lactococcus lactis* and has been found to inhibit the growth and	25	NVNLLIYOARQCIX-ILAIHEEGSA-N	x	x	x	x	*S. mutans* (UA159)	*S. mutans* (UA159)	(Tong et al., 2011)

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Table 2 *(continued)*

ID	Peptide	General information/range of concentration tested	N° AA	Sequence	*Theoretical				Antibiofilm activity	Antimicrobial activity	References
					Molecular weight	Total net charge	Hydrophobicity ⟨H⟩	Protein-binding potential (Boman index)	G +	G +	
		spore germination of many Gram-positive and multidrug-resistant bacteria. The components in saliva do not affect the antibacterial activity of nisin. It has low toxicity and is odorless, colorless, and tasteless; 5000 U/mL									
32	P10	P60.4Ac retains the α-helical structure of the synthetic LL-37-derived peptide	24	LAREYKKIVEKLKRWLRQV-LRTLR	3.096.797	7	0.250	3.2 kcal/mol	*S. aureus* (LUH14616)	*S. aureus* (LUH14616)	(Haisma et al., 2014)
33	P60.4Ac	parent peptide and displays enhanced antimicrobial properties against Gram-negative bacteria and fungi compared to those of LL-37. P10 was designed by replacing one or more amino acids in the sequence of P60.4Ac in such a way that the α-helix was predicted to be retained; 1–16 μM	24	IGKEFKRIVERIKRFLRELVR-PLR	3.046.75	6	0.262	3.42 kcal/mol			
34	P15-CSP	Fusion peptides (two separate active domains) of competence-stimulating peptide (CSP), a quorum sensing peptide expressed by oral *Streptococcus mutans* and a synthetic peptide fragment of collagen type I; 10, 50, or 100 μg	53	GTPGPQGIAGQRGVVAEA-AAKEAAAK-EAAAKASGSLSTFFRLFNRS-FTQALGK	5.281.97	4	x	1.17 kcal/mol	*S. mutans* (UA159)		(Li et al., 2015)
35	PSN-1	Phylloseptin, mass of 2035.44 Da, isolated from the defensive skin secretion of the Paraguayan waxy monkey frog, *Phyllomedusa sauvagei*. The predominantly 19- amino acid residue is typically cationic and contains amphiphilic α-helices; 5 μM	19	FLSLIPHIVSGVASIAKHF	2.036.427	2	0.806	− 0.95 kcal/mol	*S. aureus* (NCTC10788)	*S. aureus* (NCTC10788)	(Zhang et al., 2010)
36	Talactoferrin-α	Talactoferrin-α (TLFα) is an human recombinant lactoferrin; 4–66 mg/mL	ns	Ns	x	x	x	x	Clinical isolates of *S. epidermidis* (ATCC55133, H100 and S101)	x	(Venkatesh et al., 2009)
37	β-peptoid–peptide hybrid 1d	Peptidomimetics, 1d (n = 8), Ac-[Lys-N-[(*S*)-1-phenylethyl]-alanine]n-NH2;	16	KKKKKKKKAAAAAAAA	1.612.007	8	− 0.340	1.86 kcal/mol	*S. epidermidis* (RP62A, ATCC35984)	*S. epidermidis* (RP62A, ATCC35984)	(Liu et al., 2013)
38	β-peptoid–peptide hybrid 2b	2b (n = 6), Ac-[hArg-N-[(*S*)-1-phenylethyl]-alanine]n-NH2; 3b	12	RRRRRRAAAAAA	1.381.617	6	− 0.350	6.55 kcal/mol			
39	β-peptoid–peptide hybrid 3b	(n = 6), Ac-[hArg-*N*-phenyl-β-alanine]n-NH2; 4c, d (n = 3,4), Ac-	12	RRRRRRAAAAAA	1.381.617	6	− 0.350	6.55 kcal/mol			
40	β-peptoid–peptide hybrid 4c	[Lys-N-[(*S*)-1-phenylethyl]-alanine-hArg-N-[(*S*)-1-phenylethyl]-alanine]n-	12	LLLAAARRRAAA	1.252.533	3	0.327	1.59 kcal/mol			
41	β-peptoid–peptide hybrid 4d	NH2. This strategy involves the incorporation of chiral hydrophobic peptoids and guanidinylated amino acid side chains while keeping the length relatively short. Simple alternating peptoid–peptide hybrid oligomers and the mixed amino/	16	LLLLAAAARRRRAAAA	1.664.039	4	0.327	1.59 kcal/mol			

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Table 2 *(continued)*

ID	Peptide	General information/range of concentration tested	N° AA	Sequence	*Theoretical				Antibiofilm activity	Antimicrobial activity	References
					Molecular weight	Total net charge	Hydrophobicity ⟨H⟩	Protein-binding potential (Boman index)	G +	G +	
		guanidino subtype of peptidomimetics were obtained; 2–16 μg/mL									
42	5-CC	It is a 5-kDa peptide fraction (AMP)	11	EVASFDKSKLK	1.251.427	1	0.051	2.29 kcal/mol	*S. epidermidis* (1457; DSM3269); *S. aureus* (ATCC29213)	*S. epidermidis* (1457; DSM3269); *S. aureus* (ATCC29213)	(Schillaci et al., 2010; Schillaci et al., 2012)
43		from the coelomocyte cytosol from *P. lividus* belonging to the sequence segment of a beta-thymosin of *P. lividus* whose molecular masses are 4592 Da, 1251.7 Da, 2293.2 Da, 2088.1 Da; 7.9–127 mg/mL	20	SFDKSKLKKAETQEKNTLPT	2.287.585	2	− 0.045	3.1 kcal/mol			
44			18	QEKNTLPTKETIEQEKTA	2.082.291	− 1	− 0.056	3.34 kcal/mol			

"X", not tested; "ns", not shown. *Theoretical: analyzed by computational model.

K and R are positively charged and hydrophilic, and they have a polar and basic chemical behavior. F (aromatic), L, V and A are non-polar, uncharged and hydrophobic. These AA (together) constitute 69% of composition of APB (G ±) group and deserve more attention.

The other possible AA that appeared in APB (G ±) composition represented ≤ 5% each one; They and their chemical characteristics are described below: The non-polar, uncharged, and hydrophobic compounds were glycine (G), isoleucine (I), and proline (P). The polar, uncharged and hydrophilic compounds included threonine (T), glutamine (Q), cysteine (C), serine (S), asparagine (N) and histidine (H). The polar, uncharged, and hydrophobic compounds were tryptophan (W) and tyrosine (Y), and the polar, charged (−), and hydrophilic compounds included aspartic acid (D) and glutamic acid (E). Additionally, there was the unnatural AA diaminopropionic acid (Dap).

The polar uncharged AA methionine (M) and the non-proteogenic ornithine (O) did not appear in significant amounts (Fig. 1).

The frequencies of the main AA that appeared or were repeated in each different peptide that carried them (not necessarily in sequence) (Table 5) showed that K was frequently observed bearing 1 single unit in 19% of the analyzed peptides, and 14% of them had 2 units. It was followed by F, with 38% bearing 2 units and by L, with 24% bearing 2 and 24% bearing 4 units. Additionally, V had 33% of the peptides bearing 3 units, A had 19% bearing just 1, and R had 14% bearing 3.

These results are comparable to the analysis conducted with all of the peptides (total without group segmentation, N = 59), except for the L and V frequencies (L with 5, 3, 4, and 6 units and V with 2 and 1).

2.1.2. APB with Gram-negative (G −) activity

The APB (G −) was the most expressive group in terms of number of molecules that could be analyzed, with 41 different peptides. Therefore, K was the most prevalent AA, present in 19% of them (with a maximum value of 50% of this AA in a single peptide), followed by R, with 11% (maximum value of 50%). They also featured L, 10%, I, 8%, V, 10% and F, 8% (with maximum values between 30 and 60% of this AA in a single peptide). Together, these six AA sum to 66% of the APB (G −) compositions and deserve more attention.

The other possible AA (16) that appeared in their composition individually constituted ≤ 5%, and the AA M did not appear in significant amounts (Fig. 2).

(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Compared with the frequencies of the main AA that appeared or were repeated in each different peptide that bore them (not necessary in sequence) (Table 6), K was frequently observed bearing 6 units in 17% of the analyzed peptides, followed by 1, 2, or 5 units, each with 12%. R had 19.5% bearing 4 units, 10% with 3, and 10% with 1. L had 29% of them bearing 2 units and 19.5% with 4. I had 27% of them bearing 2 units and 19.5% with 3. V had 29% of them bearing 3 units, 22% with 1, and 19.5% with 2 units. F had 11% bearing 2 units.

These results are comparable to the analysis carried out on all of the peptides (without group segmentation, N = 59), including the I results. The exception was the absence of A.

2.1.3. APB with Gram-positive (G +) activity

The APB (G +) group had 39 different peptides possible to be analyzed. In the (G +) group as well as in the (G −) group, K was the most prevalent AA, present in 18% of them (with a maximum value of 50% in a single peptide). It was followed by R, with 12%, and A, with 13% (maximum value of 50% in a single peptide for both), while in (G −), A was absent. The peptides also featured L at 9%, V at 7.5% and F at 7.5% (with maximum values between 30 and 60% in a single peptide). Additionally, in contrast to (G −), I does not appear here, nor in APB (G ±). Together, these six AAs sum to 67% of the APB (G +) constitution and deserve more attention.

The other possible AAs (16) that also appeared in their constitution accounted for < 5% individually. The AA M did not appear in a

Table 3
Peptide information in alphabetical order: peptides with Gram-negative (G −) antibiofilm relative activity tested.

ID	Peptide	General information/range of concentration tested	N° AA	Sequences	*Theoretical				Antibiofilm activity	Antimicrobial activity	References
					Molecular weight	Total net charge	Hydrophobicity ⟨H⟩	Protein-binding potential (Boman index)	G −	G −	
45	CAMA	They are both chimeric AMPs constructed from cecropin A (CA), melittin (ME), magainin 2 (MA) and HP (2 − 20), which is the N-terminal region of the *Helicobacter pylori* ribosomal protein L1 (RPL1). CA is a cationic peptide isolated from *Hyalaphora cecropia pupae*, MA is a cationic peptide found in the skin of the African clawed frog (*Xenopus laevis*), and HP exhibits antimicrobial activity, reflecting its relationship with the cecropin-like N-terminal peptides; 3–25 μM	20	KWKLFKK IGIGKFLHSAKKF	2.404.991	7	0.404	0.61 kcal/mol	*A. baumannii* (KCTC2508) and 19 clinical isolates resistant to ampicillin, cefotaxime, ciprofloxacin, tobramycin, erythromycin	*A. baumannii* (KCTC2508) and 19 clinical isolates resistant to ampicillin, cefotaxime, ciprofloxacin, tobramycin, erythromycin	(Gopal et al., 2014)
46	CAME		20	KWKLFKKI GIGAVLKVLTTG	2.200.766	5	0.553	− 0.48 kcal/mol			
47	HPMA		20	AKKVF KRLGIGKFLHSAKKF	2.303.848	7	0.277	1.15 kcal/mol			
48	HPME		20	AKKVFKRLGIGAVL-KVLTTG	2.099.623	5	0.426	0.05 kcal/mol			
49	CRAMP–vancomycin	CRAMP (cathelicidin-related antimicrobial peptide from mice) was conjugated with vancomycin using various aliphatic and aromatic linkers of different lengths with a minimal steric bulk of the linker group and without affecting the binding affinity; 0.25–0.40 μM	27	HKIGEKLKKIGQKIK-NFFQKLVPQPEQ	3.192.831	5	0.203	1.83 kcal/mol	*S. Typhimurium* (LT2)	*S. Typhimurium* (LT2)	(Mishra et al., 2015)
50	DJK-5	Small synthetic cationic peptides with a high proportion of hydrophobic residues (~50%). Synthetic L-amino acid variant of host defense peptides and designed D-enantiomeric protease-resistant peptides. Increased the antibiofilm activity of β-lactam antibiotics; 0.8–10 μg/mL	12	VQWRAIRVRVIR	1.551.909	4	0.463	3.26 kcal/mol	*P. aeruginosa* (PA14); *E. coli* (0157); *A.baumannii* (clinical isolate SENTRY C8); *K.pneumonie* (ATTC13883); *S.enterica* (serovar Typhimurium isolate 14028S)	*P. aeruginosa* (PA14); *E. coli* (0157); *A.baumannii* (clinical isolate SENTRY C8); *K.pneumonie* (ATTC13883); *S.enterica* (serovar Typhimurium isolate 14028S)	(de la Fuente-Nunez et al., 2015)
51	DJK-6		12	VQWRRIRVWVIR	1.667.043	4	0.625	3.21 kcal/mol			
52	1018		12	VRLIVAVRIWRR	1.536.938	4	0.623	2.38 kcal/mol			
53	GL13K	Designed based on the human parotid secretory protein (PSP; BPIFA2. Belongs to a family of bactericidal/permeability increasing (BPI) fold proteins that are expressed in the upper respiratory tract and oral cavity. Overall positive charge of + 5. Characterization of the amino acids responsible for antibiofilm activity identified a leucine residue in position 6. The amino acids around position 6 also appear to contribute, in particular amino acids 3 to 7.	13	GKIIKLKASLKLL	1.424.859	5	0.516	− 0.51 kcal/mol	*P. aeruginosa* (PAO1)	*P. aeruginosa* (PAO1)	(Hirt and Gorr, 2013)

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Table 3 *(continued)*

ID	Peptide	General information/range of concentration tested	N° AA	Sequences	*Theoretical				Antibiofilm activity	Antimicrobial activity	References
					Molecular weight	Total net charge	Hydrophobicity ⟨H⟩	Protein-binding potential (Boman index)	G −	G −	
		This region contains three hydrophobic and two charged amino acids, indicating that the correct balance of charge and hydrophobic features in that part of the peptide is necessary for full activity. The bactericidal activity of GL13K was achieved by replacing four amino acids with Lys residues; 32–100 μg/mL									
54	(IRIK)2 -NH2	They are short synthetic	8	IRIK-IRIK	1.039.371	4	x	2.65 kcal/mol	*S. aureus* (ATCC29737)	*S. aureus* (ATCC29737)	(Ong et al., 2013)
55	(IRVK)3-NH2	peptides with minimal resemblance to naturally occurring peptide sequences, with linear amphipathic β -sheet folding. Consist of short recurring (X1 Y 1 X 2 Y 2) n-NH 2 sequences based upon 1) the common occurrence of amphipathic dyad repeats in membrane spanning β -sheets, 2) the requirement for hydrophobic (X1 or X2 = Val and/or Ile or Phe or Trp) and cationic (Y1 or Y2 = Arg or Lys) residues to interact and disturb microbial cell walls and membranes and 3) the strong β-sheet folding propensities reported for Val, Ile, Phe and Trp. The peptides designed for this study were amidated at the C-terminal to confer a high net positive charge; 15–128 mg/L	12	IRVK-IRVK-IRVK	1.507.968	6	0.255	2.87 kcal/mol			
56	KR-20	They are cathelicidins, eukaryotic AMPs with cationic	20	KRIVQRIKDFLRNLV-PRTES	2.468.911	4	0.196	3.68 kcal/mol	*A. baumannii* (5711 and 5075)	*A. baumannii* (5711 and 5075)	(Feng et al., 2013)
57	KS-30	peptides with a positive net charge and a molecular weight	30	KSKEKIGKEFKRIVQ-RIKDFLRNLVPRTES	3.644.301	6	0.074	3.47 kcal/mol			
58	LL-37	in the range of 1–5 kDa; 32–128 μg/mL	37	LLGDFFRKSKEKIGK-EFKRIVQRIKDFLRN-LVPRTES	4.487.312	6	0.201	2.99 kcal/mol			
59	KT2	Two tryptophan-rich antimicrobial peptides that	17	NGVQPKYKWWKW-WKKWW	2.428.856	5	0.625	1.24 kcal/mol	*E. coli* (O157:H7)	*E. coli* (O157:H7)	(Anunthawan et al., 2015)
60	RT2	differ only by 4 K → R substitutions. Cationic amphipathic peptides with 30–40% α-helix content; 1 μM	17	NGVQPKYRWWRW-WRRWW	2.540.928	5	0.621	3.44 kcal/mol			
61	Lactoferrin-related	Human lactoferrin (hLF), bovine lactoferrin (bLF), iron-free bLF (apo-bLF) and iron-saturated	ns	Ns	x	x	x	x	*P. gingivalis* (ATCC33277T, ATCC53978,	*P. gingivalis* (ATCC33277T, ATCC53978,	(Wakabayashi et al., 2009)

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Table 3 *(continued)*

ID	Peptide	General information/range of concentration tested	N° AA	Sequences	*Theoretical				Antibiofilm activity	Antimicrobial activity	References
					Molecular weight	Total net charge	Hydrophobicity ⟨H⟩	Protein-binding potential (Boman index)	G −	G −	
		bLF (holo-bLF) were tested. Lactoferrin (LF) is an 80-kDa iron-binding glycoprotein of the transferrin family. The lactoferricin (LFcin) region seems to be the bactericidal domain (membrane disruption) of the LF molecule.							JCM8525); *P. intermedia* (ATCC25611, ATCC49046)	JCM8525); *P. intermedia* (ATCC25611, ATCC49046)	
62	bLactoferrin	Lactoferrin (LF) is an 80-kDa iron-binding glycoprotein of the transferrin family. Bovine LF (bLF) contains five N-linked glycosylation sites (Asn-233, − 281, − 368, − 476, and − 545), and the majority of glycans are located in the N-terminal region of LF (33-kDa fragment). This further supports LF retaining its tertiary structure after cleavage at the R284-to-S285 site; 0.01–0.04 mg/mL	ns	Ns	x	x	x	x	*P. gingivalis* (ATCC33277)		(Dashper et al., 2012)
63	rLactoferrin	Recombinant lactoferrin (rHL), an iron-binding glycoprotein, is one of the most abundant antimicrobial proteins found in airway secretions and plays an important role in human defense mechanisms. It was also tested in combination with antibiotics (amikacin, rifampicin, tobramycin, ceftazidime and ciprofloxacin) and demonstrated activity; 500 and 900 mg/L	ns	Ns	x	x	x	x	*B. multivorans* (LMG13010); *B. cenocepacia* (BC7); *B. dolosa* (LMG18941); (X)/(X)/(X)	(X)/(X); *B. dolosa* (LMG18941); (X)/(X); *B. dolosa* (LMG18943)	(Caraher et al., 2007)
64	LL-31	It is the only human member of the cathelicidin family of AMPs and its fragments with overlapping sequences. A truncated variant of LL-37, missing the six residues at the C-terminus. There was no strict correlation between the killing activity and the amount of α-helical structure; 20–100 μM	31	LLGDFFRKSKEKIGK-EFKRIVQRIKDFLRN-L	3.823.575	6	0.223	2.81 kcal/mol	*B. pseudomallei* (1026b, H777 and M10)	*B. pseudomallei* (1026b, H777 and M10)	(Kanthawong et al., 2012)
65	LL-37		37	LLGDFFRKSKEKIGK-EFKRIVQRIKDFLRN-LVPRTES	4.487.312	6	0.201	2.99 kcal/mol			
66	LL-31	It is a cationic peptide with high contents of basic and hydrophobic residues and forms an amphipathic α-helix in contact with lipid membranes, dodecylphosphocholine (DPC),	31	LLGDFFRKSKEKIGK-EFKRIVQRIKDFLRN-L	3.823.575	6	0.223	2.81 kcal/mol	*P. aeruginosa* (PAO1)	*P. aeruginosa* (PAO1)	(Nagant et al., 2012)
67	LL-37		37	LLGDFFRKSKEKIGK-EFKRIVQRIKDFLRN-LVPRTES	4.487.312	6	0.201	2.99 kcal/mol			

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Table 3 *(continued)*

ID	Peptide	General information/range of concentration tested	N° AA	Sequences	*Theoretical				Antibiofilm activity	Antimicrobial activity	References
					Molecular weight	Total net charge	Hydrophobicity ⟨H⟩	Protein-binding potential (Boman index)	G −	G −	
68	RK31	or SDS micelles. The removal of the first 6 residues at the N-terminal side greatly attenuated the toxicity of the peptide: LL7-37 and LL7-31 were much less toxic than LL-37 and LL-31; 5–10 μM	31	RKSKEKIGKEFKRIV-QRIKDFLRNLVPRT-ES	3.800.488	7	0.039	3.83 kcal/mol			
69	LL-37	See previous description.	37	LLGDFFRKSKEKIGK-EFKRIVQRIKDFLRN-LVPRTES	4.487.312	6	0.201	2.99 kcal/mol	*F. novicida* (BEI NR-13)	*F. novicida* (BEI NR-13)	(Amer et al., 2010)
70	Nisin-penicillin	It is a polycyclic cationic peptide produced by *Lactococcus lactis* and is minimally toxic, odorless, colorless, and tasteless. From top to bottom, all microbes in the biofilm were effectively killed by the combination of penicillin and nisin; 400 U/mL	25	NVNLLIYOARQCIXI-LAIHEEGSA	x	x	x	x	*E. faecalis* (ATCC29212)	*E. faecalis* (ATCC29212)	(Tong et al., 2014)
71	NRC-16	They are pleurocidin-like cationic AMPs that were	19	GWKKWLRKGAKHL-GQAAIK	2.176.621	7	0.241	1.32 kcal/mol	*P. aeruginosa* (1162, 3547, 4007, 3399, 1034)	*P. aeruginosa* (1162, 3547, 4007, 3399, 1034)	(Gopal et al., 2013)
72	NRC-17	identified from the witch flounder, *Glyptocephalus cynoglossus*. This structure has enough potential to provide the greatest degree of amphipathicity or hydrophobicity and the largest cationicity. NRC-16 also has amidated C-termini that greatly improve the microbicidal activity of the peptide; 4–16 μM	23	GWKKWLRKGAKHL-GQAAIKGLAS	2.504.989	7	0.285	0.91 kcal/mol			
73	T9 W	It is a simple substitution of amino acids in the middle position of the hydrophobic face of an amphipathic peptide with tryptophan, with a helical structure, 2267.8 MW. Considerably transformed into an antimicrobial peptide specifically targeting *Pseudomonas aeruginosa*.	16	RFRRLRKKWRKRLK-KI	2.268.863	11	− 0.111	6.07 kcal/mol	*P. aeruginosa* (ATCC27853)	*P. aeruginosa* (ATCC27853)	(Zhu et al., 2014)

"X", not tested; "ns", not shown. *Theoretical: analyzed by computational model.

Table 4
Peptide information in alphabetical order: peptides with fungus antibiofilm relative activity tested.

ID	Peptide	General information/range of concentration tested	N° AA	Sequence	*Theoretical				Models	Antibiofilm activity	Antimicrobial activity	References
					Molecular weight	Total net charge	Hydrophobicity ⟨H⟩	Protein-binding potential (Boman index)				
74	AS10	P318 is a shortened variant of the known cathelicidin-related	26	KIGEKLKKIAQKIKNFFQ-KLVPQPEQ	3.069.717	5	0.218	1.68 kcal/mol	*C. albicans* (SC5314, DAY286, CAF2–1); *C. glabrata* (BG2); *C. krusei* (IHEM6104); *C. dubliniensis* (NCPF3949)	(+); ns; ns; ns	(+);ns; ns; ns	(De Brucker et al., 2014)
75	P318	antimicrobial peptide from mice (CRAMP), discovered in the islets of Langerhans of the murine pancreas. AS10 is a shortened variant of P318 in which each amino acid of the native sequence was individually (10th aa) replaced by an alanine residue; 6.25–12.25 μM	26	KIGEKLKKIAQKIKNFFA-KLVAQPEQ	2.992.627	5	0.222	1.33 kcal/mol				
76	BL-DZ1	It was identified (14 kDa) as BL00275, accession number gi52082584, from *B. licheniformis*. It is stable at 75 °C for 30 min in the pH range between 3.0 and 11.0 but is sensitive to the enzymes trypsin and proteinase K; 1.60 mg/mL	ns	Ns	x	x	x	x	*C. albicans* (BH)	(+)	(+)	(Dusane et al., 2013)
77	Caspofungin and micafungin	(Lock strategy) Both are echinocandins, semi-synthetic lipopeptides with a chemical structure of cyclic hexapeptides connected to a fatty acid side chain; 5–25 mg/L	ns	Ns	x	x	x	x	*C. albicans* (ATCC3153, 66, 396, clinical isolation: 1119, 1126, 1137, 1150, 1151, 1156, 1160, 1163); *C. glabrata* (clinical isolation: 1, 767, 788, 924, 961, 1141)	(+); (+)	(+); (+)	(Cateau et al., 2011)
78	Gramicidin A	It is a linear pentadecapeptide with a molecular mass of approximately 1900 Da that consists of alternating L- and D-amino acids and is a hydrophobic peptide forming a β-bonded helix in a hydrophobic environment; 20 $mg.L^{-1}$	12	XGALAVVVWLWLWLW	x	x	x	x	*C. albicans* (ATCC3153)	(+)	(+)	(Yala et al., 2011)
79	(IKIK)2-NH2	It is a short β-sheet peptide based on AMP′ structures, (X1Y1X2Y)n, where X = hydrophilic amino acids, Y = cationic amino acids and n = number of repeat units; stable (to light, heat and aqueous solutions); 1000 mg/mL	8	IKIKIKIK	983.335	4	x	0.31 kcal/mol	*C. albicans* (ATCC); *F. solani* (ATCC)	(+); (−)	(+); (+)	(Wu et al., 2015)
80	Iturin and fengycin combination	Iturin, 1030.4–1111.5 *m/z*, and fengycin, 1450.3–1515.3 m/z, are cyclic lipopeptides (CLs). These compounds consist of a hydrophobic long alkyl chain linked to a hydrophilic polypeptide to form a cyclic structure. The presence of hydrophilic and hydrophobic moieties confers these CLs with the ability to accumulate	ns	Ns	x	x	x	x	*C. albicans* (MTCC1637, 4748 and 183)	(+)	(+)	(Rautela et al., 2014)

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Table 4 *(continued)*

ID	Peptide	General information/range of concentration tested	N° AA	Sequence	*Theoretical				Models	Antibiofilm activity	Antimicrobial activity	References
					Molecular weight	Total net charge	Hydrophobicity ⟨H⟩	Protein-binding potential (Boman index)				
		between fluid phases thus reducing the surface and interfacial tension (biosurfactants). The presence of Asn, Glu, Gln, Ala, Pro, Ile, Val, Tyr, Thr, Ser and the non-protein amino acid ornithine were observed. Heat stable; 1–6 mg/mL										
81	KABT-AMP	Both were used as a reference for the designed peptides. Containing	22	GIWKKWIKKWLKKLLKK-LWKKG	2.837.631	10	0.432	0.67 kcal/mol	*C. albicans* (SC5314)	(+)	(+)	(Lum et al., 2015)
82	Uperin 3.6	synthesized and naturally occurring peptides consecutively, it adopts a well-defined amphipathic α-helix, with a hydrophobic side above and a hydrophilic side below the polypeptide backbone. The three lysine residues in the sequence of uperin 3.6 are crucial for the antimicrobial activity.	17	GVIDAAKKVVNVLKNLF	1.828.209	3	0.444	0.01 kcal/mol				
83	Lactoferricin B	Lactoferricin B is derived from a multifunctional milk protein, lactoferrin, after proteolysis by pepsin. The influence of the addition of lactoferricin B to lens care solution was also evaluated to eradicate biofilm formation (positive) on a contact lens (32 μg/mL); 64 μg/mL	ns	Ns	x	x	x	x	*A. fumigatus* (JM3); *F. solani* (JW21); *C. albicans* (SJ11)	(+); (+); (+)	(+); (+); (+)	(Sengupta et al., 2012)
84	OSIP108 and derivatives	It is a decapeptide from *Arabidopsis thaliana* (induced in plants upon infection). A preliminary structure-activity relationship shows the importance of the order of the amino acids for the antibiofilm activity. D-OSP108 retains its antibiofilm activity, but cyclization reduced it; 6.25–100 μM	10	MLCVLQGLRE	1.161.459	0	0.722	0.39 kcal/mol	*C. albicans* (SC5314, CAI4, CAF2, DAY286, B2630 and B63195); *C. glabrata* (BG2); *C. dubliniensis* (NCPF3949); *C. krusei* (IHEM6104)	(+); (−); (−); (−)	(−); (−); (−); (−)	(Delattin et al., 2014)
85	Talactoferrin-α	Talactoferrin-α (TLFα) is an human recombinant lactoferrin; 4–66 $mg.ml^{-1}$	ns	Ns	x	x	x	x	*C. albicans* (ATCC32354 and MYA4441)	(+)	(−)	(Venkatesh et al., 2009)

"X", no information available; "ns", not shown; (+) indicates that it was active and (−) indicates that it was not active. *Theoretical: analyzed by computational model. *C. albicans: not necessarily active against all of them.

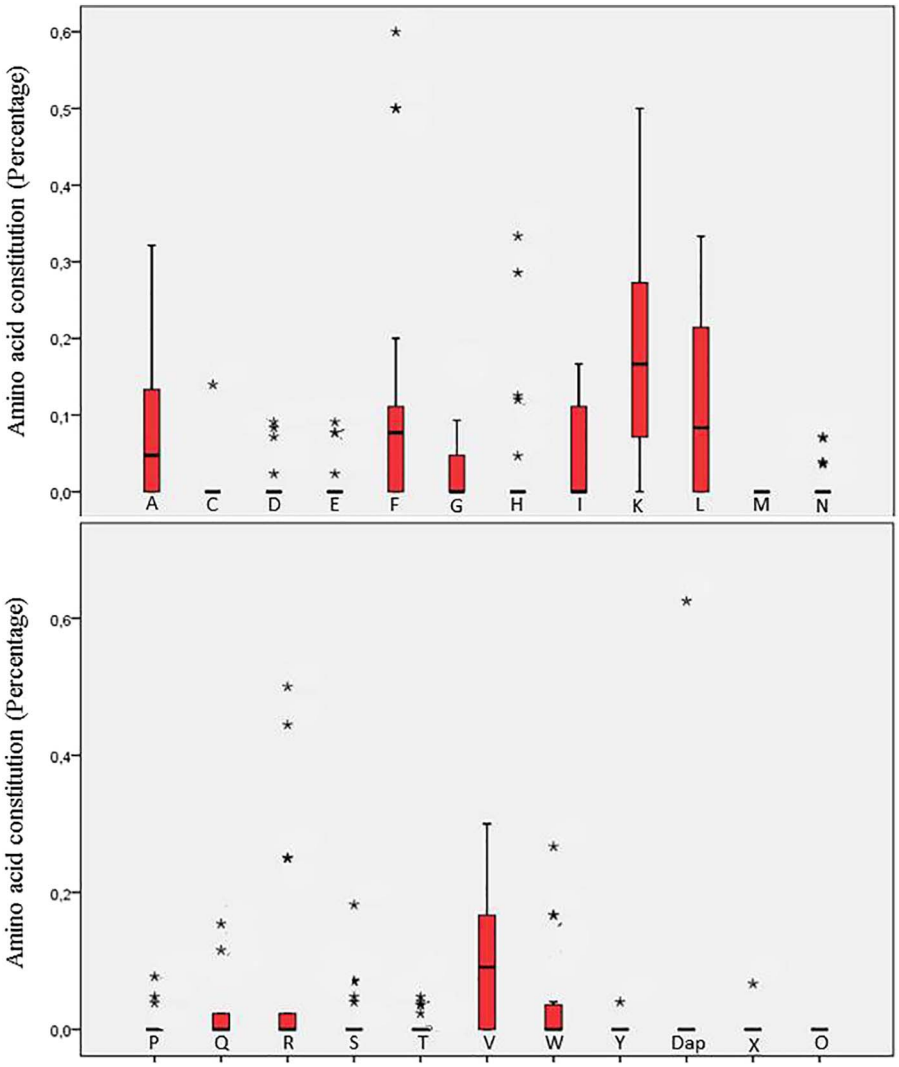

Fig. 1. Amino acid constitution of APB (G ±). The amino acids constitute the peptides from the APB (G ±) group (see Table 1) are shown as percent values as red bars on the box plot. Each letter corresponds to different amino acid; in particular, Dap = diaminopropionic acid, X = unknown or not shown, and O = non- proteogenic ornithine. (*,°) The values are isolated or extrapolated. The descriptive analysis was performed using the software SPSS Statistics v18.0 (N = 21). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

significant amount, repeating the result found for the (G −) group, and nor did O (Fig. 3).

The frequencies of the main AA that appeared or were repeated in the different peptides (not necessary in sequence) are shown in Table 7. K was frequently observed as 3 units in 16% of the analyzed peptides, followed by 1, 4, or 5 units, each with 13.5%. R had 15% with 3 units and 10% with 6 units. A had 25.6% with 1 single unit and 10% with 2 units. L had 20.5% with 2 units, 15.4% with 1, and 12.8% with 4 units. V had 23% with 2 units, 17.9% with 3, and 12% with 1 unit. Finally, F had 10% with 2 units.

Although the main AA seem to be the same between APB (G −) and (G +) (A (G +) and I (G −) as the exceptions), their frequencies were distinct. K appeared 50% < in (G +) than (G −). R showed the largest differences: (G +) 15% had 3 and 10% had 6 and (G −) 19.5% with 4, 10% with 3 and 10% with 1. L had the same 2 or 4 units as the mean frequencies in both (G +) and (G −), but only (G +) showed a significant frequency (16%) for 1 single unit. V and F showed similar behaviors between (G +) and (G −).

These results were comparable to the analysis conducted with all of the peptides (without group segmentation, N = 59), except for the R and V frequencies (R also with 4 units and V with 4).

3. Antibiofilm peptides against fungus (APF)

The same analysis method used for the APB was applied for the APF. They were not subdivided into yeasts or filamentous fungi because only two filamentous strains were tested.

Therefore, as in the APB, K was the most prevalent AA for APF, present in 24% of them (with a maximum value of 50% in a single peptide). L also appeared as the second most important participant, slightly more than I, with 15% and 14% participation, respectively. Both these AA corresponded to 53% of the total AA amounts of the APF. This range is singular due to the involvement of only three AA (two, considering the few differences between L and I) among the

Table 5
Frequencies of the most prevalent amino acids from each different APB (G ±): The table shows the number of times a single AA appeared in the peptide chain, not necessarily in sequence.

Amino acid (AA)	N° of AA appearances (units)	Absolute (f)	Relative (f)
Alanine-A	0	9	42.9
	1	4	19.0
	2	3	14.3
	3	1	4.8
	4	1	4.8
	5	1	4.8
	9	2	9.5
Arginine-R	0	15	71.4
	1	1	4.8
	2	1	4.8
	3	3	14.3
	4	1	4.8
Leucine-L	0	6	28.6
	1	3	14.3
	2	5	23.8
	4	5	23.8
	6	2	9.5
Lysine-K	0	4	19.0
	1	4	19.0
	2	3	14.3
	3	2	9.5
	4	2	9.5
	5	2	9.5
	6	2	9.5
	7	2	9.5
Phenylalanine-F	0	9	42.9
	1	3	14.3
	2	8	38.1
	3	1	4.8
Valine-V	0	7	33.3
	1	3	14.3
	2	3	14.3
	3	7	33.3
	5	1	4.8

(f): frequency. The relative frequencies sum to 100%.

most important constitutive AA, taking into consideration that in the APB, they were six.

Second, V participated with 8% of its amount, and G, A, and W with 6% each. Other AAs corresponded to ≤ 5%. Importantly, the AAs S, Y, T, H, O, and Dap did not appear significantly (≤ 5%), and this also varied for APB. In addition, M appears here, although just in one peptide (Fig. 4).

Furthermore, the frequencies of the main AA were not representative due to the small number of molecules included in the group of APF (Table 8).

These results are comparable to the analysis conducted with all the peptides (without group segmentation, N = 59), except for the absence of a frequency analysis and R, A, and F, as well as the presence of I, as well as in the APB (G −).

3.1. Number of amino acids

The number of amino acids in the peptide composition is crucial to its chemical complexity (Gautam et al., 2013; Heitz et al., 2009). The number of amino acids (AA) from the APB (G −) molecules was analyzed, with the smallest having 8 AA and the largest 37 AA. The mean and median were both 20 AA, as was the mode (five repeated cases), (Fig. 5). The APB (G +) molecules showed the smallest having 8 AA as the (G −) and the largest having 53 AA. Differently from (G −), the mean and median analysis were both 17 AA, with mode values of 12, 16, and 24 AA (two repeated cases each one), (Fig. 5). Moreover, the APB (g ±) molecules showed the smallest having 4 AA and the largest having 43 AA. The mean and median analysis indicated values of 12 AA, as was the mode (four repeated cases) (Fig. 5).

Furthermore, the APF smallest peptide was 8 AA, and the largest was 26 AA (from two variants). The median and mean analysis were both 17 AA, but the mode was 26 AA (just two repeated cases), (Fig. 5). The equivalent numbers for the AA range (small sequences ≤ 20 AA) was interesting for both the APB and APF activities.

4. Analysis of similarity

The AA disposition in the peptide chain is indispensable to establish all the intrinsically chemical characteristics and bioactivity. For example, a representative decapeptide, OSIP108, was preliminary assessed for an SAR study, and a scrambled version of it (S-OSIP108), containing the same AA but in random order, displayed no more activity, pointing also to the importance of the AA order (Delattin et al., 2014).

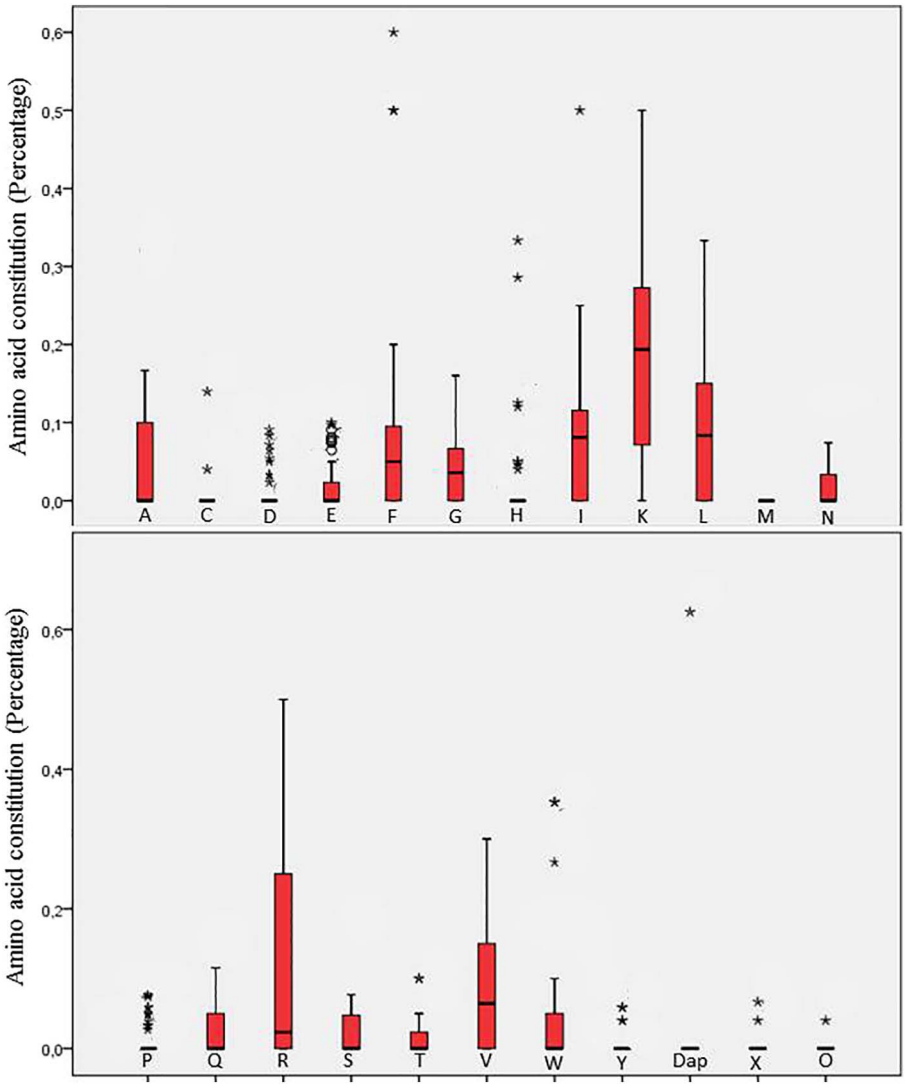

Fig. 2. Amino acids constitution of APB (G −). The amino acids that compose the peptides from the APB (G −) group (see Table 2) are shown as percent values as red bars on the box plot. Each letter corresponds to a different amino acid, with Dap = diaminopropionic acid, X = unknown or not shown, and O = non-proteogenic ornithine. (*,°) The values are isolated or extrapolated. The descriptive analysis was conducted using the software SPSS Statistics v18.0 (N = 41).

Accordingly, despite the similarity analysis of all the AA sequences, trends were found. Interestingly, as we can observe in the Fig. 6A, a major Gram-negative branch is formed after alignment. Such clustering indicates that a trend for the amino acid positions against Gram-negative bacteria seems to be clear. In particular, we can emphasize the conserved positions for the amino acids L, I and K, as noted in Fig. 6B. In general, no other significant relationship in clustering was found.

5. Peptide enantiomers

The AA enantiomer design is a wonderful strategy aiming protease resistance for example and improving the biological activity, including antibiofilm (Blower et al., 2015; Rabin et al., 2015).

However, it was noted that just a few studies reported it. Regarding the peptides 1018, DJK-5, DJK-6 (associated with β-lactam antibiotics) and gramicidin A were evaluated against different bacterial models, Gram negatives and positives (de la Fuente-Nunez et al., 2015; Yala et al., 2011; Ribeiro et al., 2015), and both of them exhibited enhanced antibiofilm activities. However, the reduced usage of the enantiomer strategy in other studies can be controversially associated with chirality or secondary structure changes leading to loss of activity (Chen et al., 2006; Juba et al., 2013; Hong et al., 1999).

6. Linear or cyclic chain

It is clear in medicinal chemistry that the chain forms of peptides have important effects on the structure-activity relationship. In accordance, some studies evidence the importance of cyclic peptides of a variety of activities as antimicrobial, cell permeability and antagonists of platelet fibrinogen (Lee and Hodges, 2003; Liang et al., 2014; Hayashi et al., 1998). However, only a few of the included molecules in this review were cyclic peptides (De et al., 2015; Gowrishankar et al., 2014; Tong et al., 2011; Tong et al., 2014; Cateau et al., 2011; Rautela et al., 2014; Bionda et al., 2016), while all others were linear ones, as exemplified in Fig. 7, making it impossible to further analyze this topic. Still, the majority of them were based on classic fungus products/

Table 6
Frequencies of the most prevalent amino acids from each different APB (G −): The table shows the number of times each AA appeared in the peptide chain, not necessarily in sequence.

Amino acid (AA)	N° of AA appearances (units)	Absolute (f)	Relative (f)
Arginine-R	0	20	48.8
	1	4	9.8
	2	2	4.9
	3	4	9.8
	4	8	19.5
	5	2	4.9
	6	1	2.4
Isoleucine-I	0	15	36.6
	1	5	12.2
	2	11	26.8
	3	8	19.5
	4	2	4.9
Leucine-L	0	12	29.3
	1	4	9.8
	2	12	29.3
	3	3	7.3
	4	8	19.5
	6	2	4.9
Lysine-K	0	8	19.5
	1	5	12.2
	2	5	12.2
	3	3	7.3
	4	4	9.8
	5	5	12.2
	6	7	17.1
	7	4	9.8
Phenylalanine-F	0	18	43.9
	1	7	17.1
	2	11	26.8
	3	3	7.3
	4	2	4.9
Valine-V	0	11	26.8
	1	9	22.0
	2	8	19.5
	3	12	29.3
	5	1	2.4

(f): frequency. The relative frequencies sum to 100%.

derivatives with strong biotic action, exception of Cyclo (L-leucyl-L-prolyl), (see table 2, ID 30).

However, the study of Dellatin and col. (2014) showed that OSIP108 (MLCVLQGLRE) cyclization reduced its antibiofilm activity in a preliminary structure-activity relationship, contributing to the perception of the good involvement of cyclic chains in the antimicrobial activity, but not as well as the antibiofilm.

In addition, specific studies such as one compilation addressing quorum sensing inhibitors (QSI) (Brackman and Coenye, 2015) described few peptides/enzymes including cyclic peptides (L-TyR-L-Pro and L-Phe-L-Pro) that inhibited or degraded the signal synthesis or a transduction cascade that plays a role in biofilm formation or maturation.

Thus, the results are still scarce and preliminary for further conclusions as well as the comparative studies available for this topic.

7. Secondary conformation

The secondary conformation of a peptide or protein structure is definitively linked to their behavior, SAR and stability (MacColl et al., 2001; Starkey et al., 1998; D'Ursi et al., 2007). Helices (Exemplified at Fig. 8) constitute the major secondary structural components of proteins and often play a crucial role in mediating protein–protein and protein–nucleic acid (DNA and RNA) interactions (Jadhav et al., 2013). However, there is still no relationship described between the secondary conformation and the antibiofilm activity.

The polar AA (G, N, H, S, T, Y, C, M and W) may interact strongly through hydrogen bonds or ionic forces affecting both the α-helix and β-sheet conformations, but the hydrophobics AA (A, L, I, F, V, G) seem to participate more intensively in β-sheet folding (Minor and Kim, 1994a; Minor and Kim, 1994b).

In accordance, the APB AMINO ACID COMPOSITION showed that at least four of these hydrophobic AA are present into their range of majorities, suggesting the possibility of their β-sheet conformation. However, some of the peptides listed here are AMPs or based on its concept, indicating an α-helix structure.

In addition, both the α-helix and β-sheet conformations were described by the references, and they were active against Gram-negative bacterial biofilms, Gram-positive or both. They were also active against fungus biofilms.

Due to the importance of the secondary structure, more investigations are necessary to correlate it with the SAR on biofilms. The linear structures were related but not the tertiary or quaternary.

8. Charge of the peptides

The charge motif has itself a fundamental role in the SAR of bioactive peptides due to a large possibility of electrostatic and hydrophobic interactions with the matrix complex components, as recently discussed for KT2 and RT2, positively charged peptides active against both Gram-negative and Gram-positive biofilms (Anunthawan et al., 2015). Still, the study of a cyclic lipopeptide derived from fusaricidin/LI-F used a positional-scanning combinatorial approach to revel the importance of hydrophobic as well as positively charged amino acids residues in antimicrobial and antibiofilm activities, for example (Bionda et al., 2016).

Additionally, a review of biosurfactants (a heterogeneous group of amphiphilic compounds that accumulate at the interface between liquid phases and therefore reduce surface and interfacial tension) evidence them as disruptors and inhibitors of microbial biofilms. It includes surfactins, polymixins, fengycins and fusaricidins both composed of hydrophilic peptides (aliphatic, branched or cyclic) attached to hydrophobic lipid or fatty acid, that alone or in combination with antibiotics showed a synergistic inhibition effect (Banat et al., 2014; Mataraci and Dosler, 2012).

Therefore, some peptides are described as cationic due their excess of L and A residues (Rabin et al., 2015), and the presence of these AAs are noticeable in both cited groups, see section AMINO ACID COMPOSITION. Thus, their basic and positively charged characteristics may interact with the matrix composition as previous mentioned. It is important to highlight that the biofilm matrix is highly diverse in composition and varies significantly according to the bacterial specie. In this sense, deep studies should be conducted to relate the character and charge of the bioactive peptide and action upon matrix (Flemming and Wingender, 2010).

After statistical analyses, we found that 82% of the APB(G ±) showed a positive charge (mean = 4) and 18% neutral. All of the APB (G −) showed a positive charge (mean = 6) although the APB(G +) showed only 87% positive charge (mean = 4), 7% of neutral and 7% of negative. These results are in accordance with their L and R

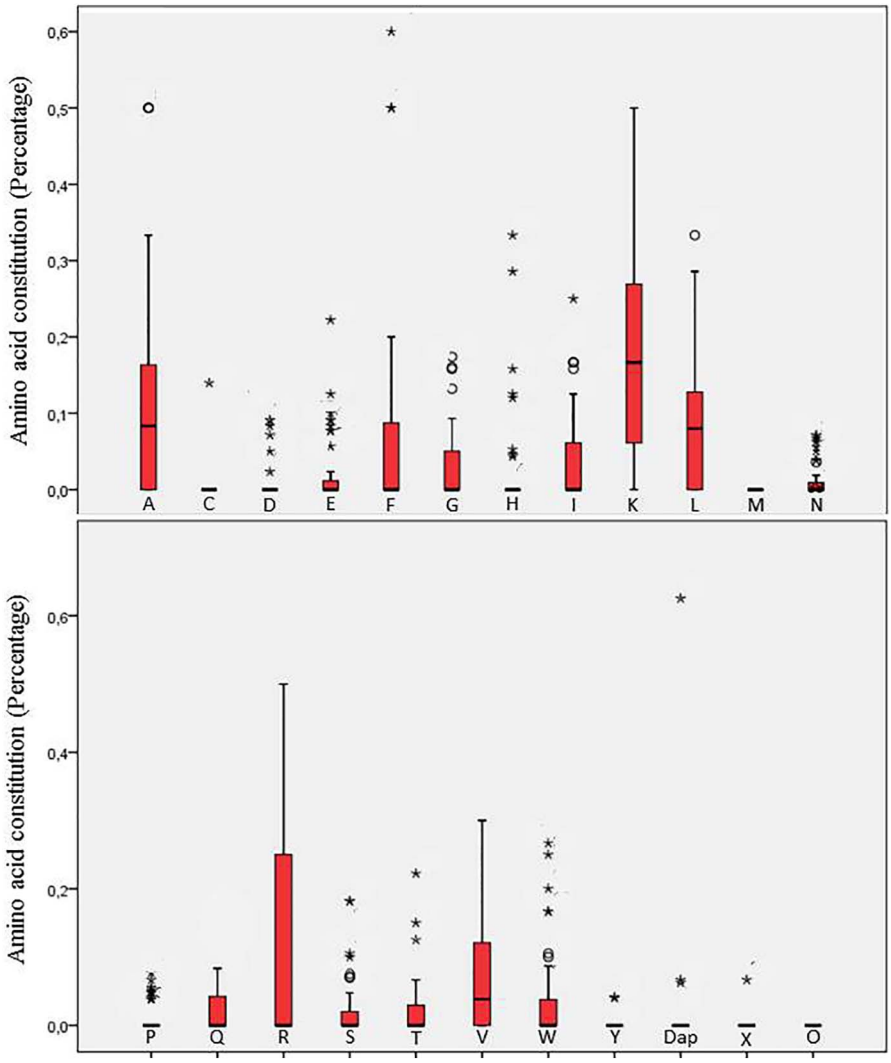

Fig. 3. Amino acid constitution of APB (G +). The amino acids that compose the peptides from the APB (G +) group (see Table 3) are shown in percentages as red bars on the box plot. Each letter corresponds to a different amino acid, with Dap = diaminopropionic acid, X = unknown or not shown, and O = non-proteogenic ornithine. (*,°) The values are isolated or extrapolated. The descriptive analysis was conducted using the software SPSS Statistics v18.0 (N = 39). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

contents. The APF follow in accordance to the majority of positive charge and a few neutral. Their AA composition analysis shows a mean nonpolar and hydrophobic AA.

Furthermore, the peptides reviewed were classified as charged by the theoretical analysis, and based on its calculated values, in general we can indicate that there is a slight trend for Gram negative in the net charges of 6 and 7.

9. Antibiofilm peptides without biotic activity

Antibiotics – biotic activity – have contributed to save millions of lives, but recently, the commercially available drugs have become no longer active against all pathogens, since bacteria and fungi developed strategies to overcome the antibiotic action. In this worrisome scenario, new strategies should be found to fight the resistant microorganisms. One of the alternatives found is antivirulence therapies, where the intention is to diminish the microbial virulence/pathogenicity to enable an immune system reaction. The idea behind this strategy is that microorganisms may suffer a low pressure to generate new resistant mechanisms without the biotic activity. In this sense, we found in literature only three peptides able to inhibit biofilm formation without antibiotic activity associating: P15-CSP (ID: 34), betaLactoferrin (ID: 62), and OSIP (ID:84).

Thus, the results are still scarce and preliminary for further conclusions.

10. Conclusions

The emergence of multi-drug resistant microorganisms to conventional antimicrobials has become more common and today is considered a major public health problem. We are facing a *post-antibiotic* era, where antibiotics are no longer effective against all microbial threats in healthcare institutions. Data presented by the American Centers for Diseases Control and Prevention point to over 2 million illnesses caused by bacteria and fungi that are resistant to at least some classes of antibiotics. In this regard, alternative strategies

Table 7
Frequencies of the most prevalent amino acids from each different APB (G +): how many times a single AA appeared in the peptide chain, not necessarily in sequence.

Amino acid (AA)	N° of AA appearances (units)	Absolute (f)	Relative (f)
Alanine-A	0	12	30.8
	1	10	25.6
	2	4	10.3
	3	3	7.7
	4	2	5.1
	5	1	2.6
	6	2	5.1
	8	2	5.1
	9	2	5.1
	13	1	2.6
Arginine-R	0	22	56.4
	1	3	7.7
	2	1	2.6
	3	6	15.4
	4	2	5.1
	5	1	2.6
	6	4	10.3
Leucine-L	0	14	35.9
	1	6	15.4
	2	8	20.5
	3	3	7.7
	4	5	12.8
	5	1	2.6
	6	2	5.1
Lysine-K	0	7	18.9
	1	5	13.5
	2	4	10.8
	3	6	16.2
	4	5	13.5
	5	5	13.5
	6	2	5.4
	7	2	5.4
	8	1	2.7
Phenylalanine-F	0	22	56.4
	1	5	12.8
	2	10	25.6
	3	1	2.6
	4	1	2.6
Valine-V	0	17	43.6
	1	5	12.8
	2	9	23.1
	3	7	17.9
	5	1	2.6

(f): frequency. The relative frequencies sum to 100%.

based preferably on the inhibition of microbial virulence, such as biofilms, instead of the inhibition of microbial growth, have become the object of consistent research lately, since virulence factors have an important pathological role in tissue colonization and invasion, apart from the fact that they are not essential for microbial survival (Silva et al., 2016). Therefore, there is a desperate need for the identification of new agents that inhibit the virulence factors and alter the course of multi-drug resistant microbial infections. In these context, peptides hold great promise since there are reports of antibiofilm activity in bacteria that are Gram-positive, Gram-negative and both together, and also from fungus, besides the activity of planktonic bacteria (Strempel et al., 2015). While the beneficial effects of peptides in antibiofilm activity have been well established in several fields, a structure-activity relationship (SAR) for this class is only emerging. So, in the proposed perspective, we will emphasize the SAR of a wide range of peptides (from different sources), targeting the antibiofilm activity (bacteria and fungus), as follows:

- The presence of K, R, L, V and F is essential for the antibiofilm activity;
- Active peptides contain between 12 at 20 amino acids;

For APBs with Gram-negative (G −) activity, the number of amino acids suggested is 20, with a frequency of up to 7 K, 5 R, 3 A, 2 V, 2 F and 2 L. The presence of 3 I is also favorable, diverging from (G +) where it was absent. We propose an ideal prototype structure as an application and future perspective with the amino acid sequence of

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-X20

wherein X1 = I, X2 = A or G, X3 = K or E or R, X4 = K or E, X5 = F or L or Y, X6 = K, X7 = K or R, X8 = I, X9 = V or A, X10 = Q or E, X11 = K or R, X12 = I, X13 = K, X14 = D or N or R, X15 = F or W, X16 = L or F, X17 = R or A or Q, X18 = N or K or E or Q, X19 = L or V, and X20 = V or L.

Although, for APBs with Gram-positive (G +) activity the SAR was not as evident as for Gram-negative (G −); the size suggested is 17 AA, wherein the presence of A, K and R are essential, diverging from (G −) where A was absent. In accordance with the information compiled in this review, Liu and col. (2013) demonstrated the antimicrobial and antibiofilm activity of the simple alternating-peptoid–peptide hybrid oligomers and the mixed amino/guanidino subtype of peptidomimetics against *S. epidermidis* (MRSE) (see Table 2, ID 37-41). Their effects were compared with that of vancomycin. They found that chiral and guanidinylated (i.e., hArg-rich) hybrids exhibited the fastest killing effects against low-growing cells and had more favorable antibiofilm properties than the analogues only containing lysine or lacking chirality in the peptoid residues. However, the results of cytotoxicity assays showed a clear correlation between the oligomer length, hArg-rich peptidomimetics and cell toxicity within each sub-class of peptides (Niu et al., 2012; Shi et al., 2016).

Due to the low number of findings, it was not possible to establish an SAR for antibiofilm peptides against fungus (APF), but a trend for the presence of K, L and I exist.

The production of peptidomimetics may be a great perspective. Various strategies have been employed, including stabilized peptides, conjugates, immobilized peptides, peptides congeners, mimetics, and hybridization. There are many different ways, methodologies and strategies to produce new compounds using peptides. These methodologies include the attachment of a chemical group to a peptide that mimics a natural substrate (Michael acceptors, aldehydes, epoxy ketones, halomethyketones), the synthesis of bi- or polyaryls that mimic α-peptides, α- and γ-AApeptides (*N*-**A**cylated-*N*-**A**minoethylamino acids), and amino acid residue linkages through non-peptide bonds. Almaaytah, 2015, for example, designed a novel hybrid peptide named BMAP27-Melittin and characterized its antimicrobial and antibiofilm activities.

Finally, we believe that these results could provide suitable information for the development of new peptides or peptidomimetics aiming pharmaceutical innovation through the development of potential antibiofilm agents in the war against resistant microorganisms.

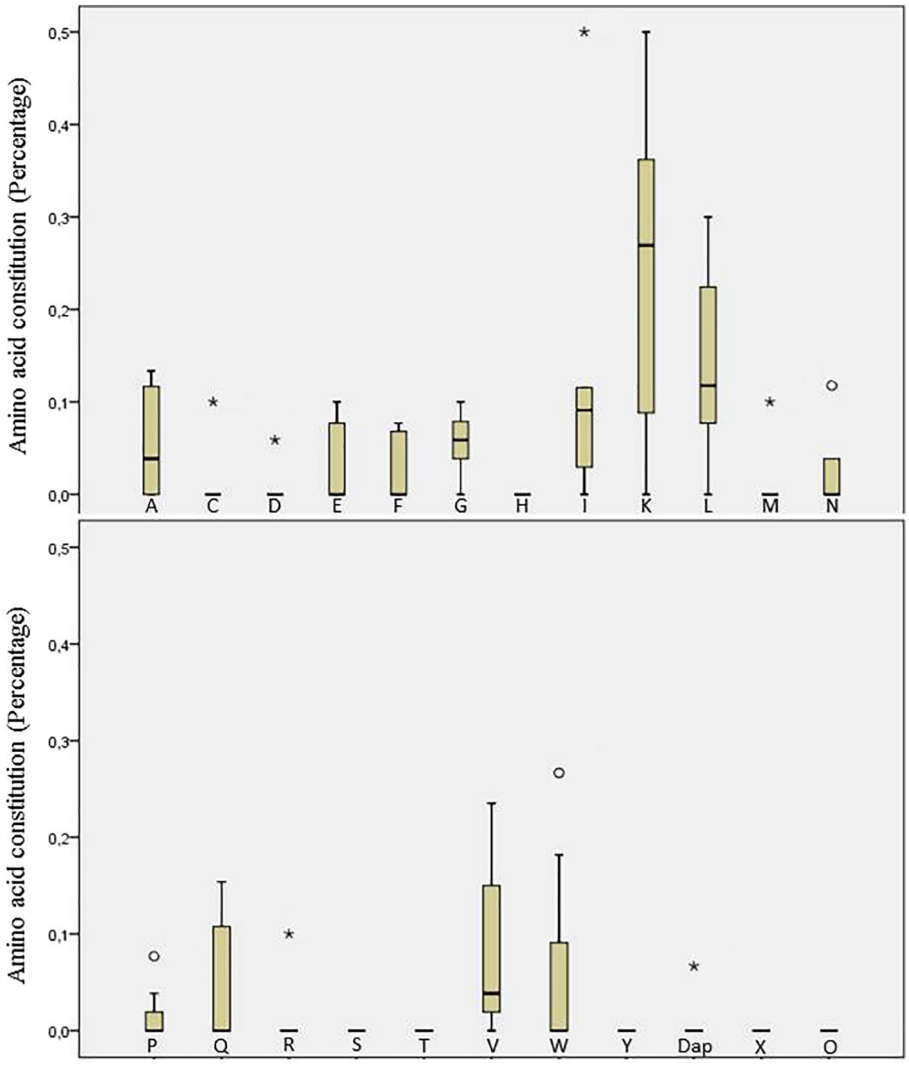

Fig. 4. Amino acid constitution of APF. The amino acids that compose the peptides from the APF group (see Table 4) are shown in percent values as green bars on the box plot. Each letter corresponds to a different amino acid, with Dap = -diaminopropionic acid, X = unknown or not shown, and O = non-proteogenic ornithine. (*,°) The values are isolated or extrapolated. The descriptive analysis was conducted using the software SPSS Statistics v18.0 (N = 7). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 8
Frequencies of the most prevalent amino acids from each different APF: The table shows the number of times a single AA appeared in the peptide chain, not necessarily in sequence.

Amino acid (AA)	N° of AA appearances (units)	Absolute (f)	Relative (f)
Isoleucine-I	0	2	28.6
	1	1	14.3
	2	1	14.3
	3	2	28.6
	4	1	14.3
Leucine-L	0	1	14.3
	2	3	42.9
	3	1	14.3
	4	2	28.6
Lysine-K	0	2	28.6
	3	1	14.3
	4	1	14.3
	7	2	28.6
	10	1	14.3

(f): frequency. The relative frequencies sum to 100%.

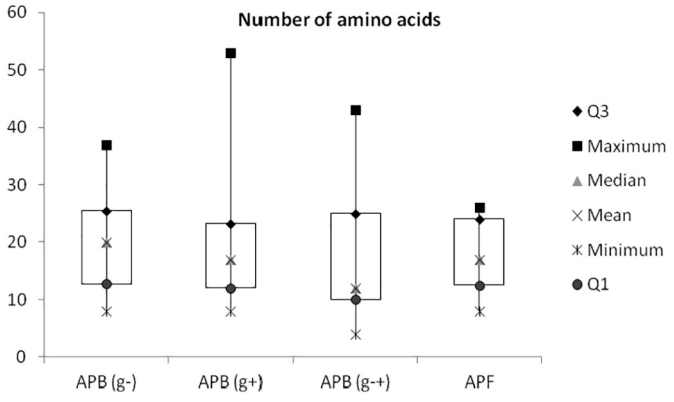

Fig. 5. Number of amino acids that constituted the size of the peptide chains. The numbers of amino acids, independently from each group (see Tables 1–4), are shown as real numbers in the y-axis on box plot. APB = Antibiofilm peptides against bacteria, (G −) = Gram negative, (G +) = Gram positive, (g ±) = both Gram negative and positive, APF = Antibiofilm peptides against fungus, Q1 = first interquartile range and Q3 = third interquartile range.

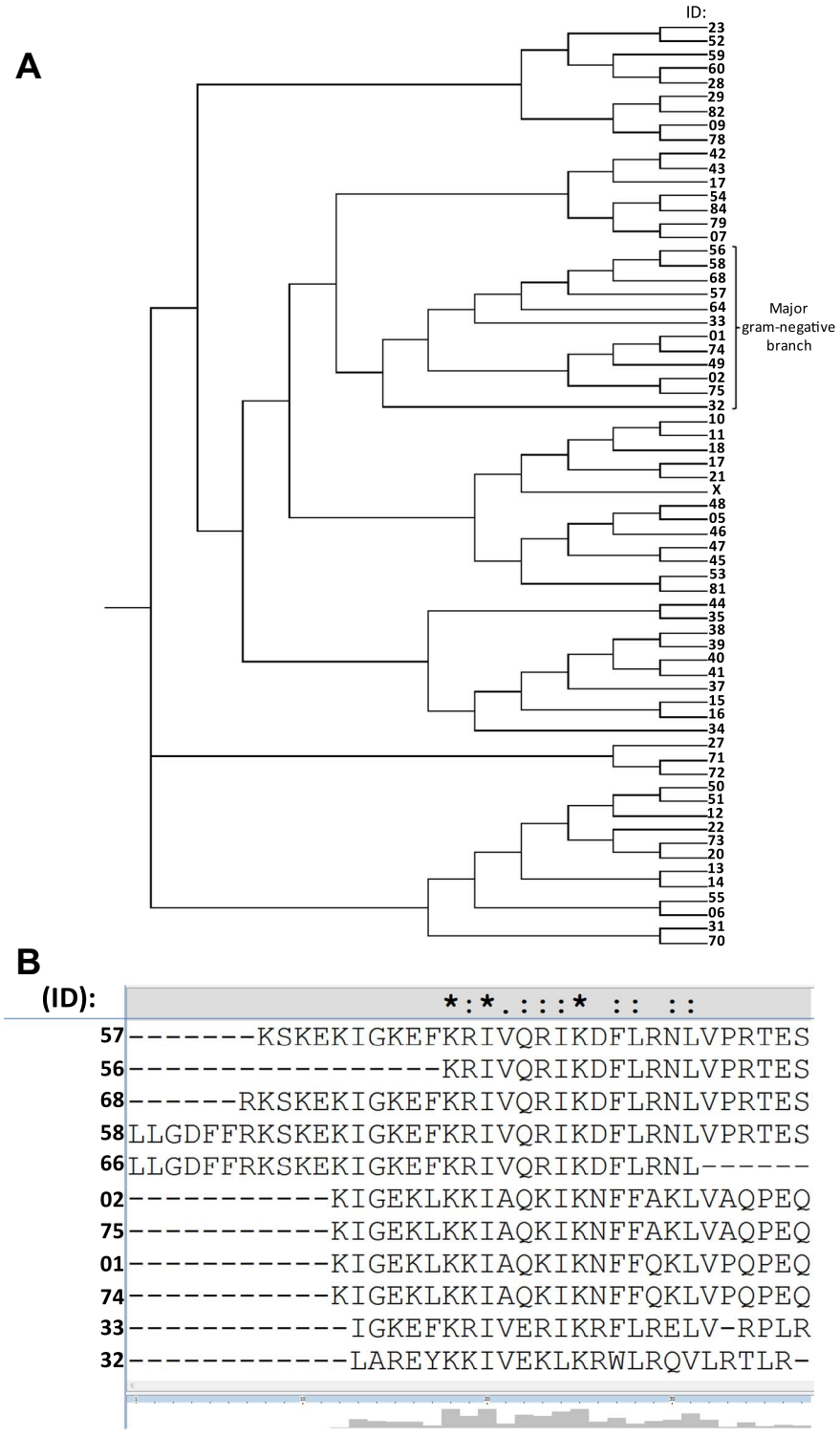

Fig. 6. (A) Neighbor-joining dendrogram of the phylogenetic relationships was constructed using the CLUSTALX software (Thompson et al., 1997) for sequence alignments and the neighbor-joining method (Saitou and Nei, 1987) with a bootstrap percentage values based on 1000 replications. (B) Amino acid alignment of the clustered Gram-negative bioactive peptides.

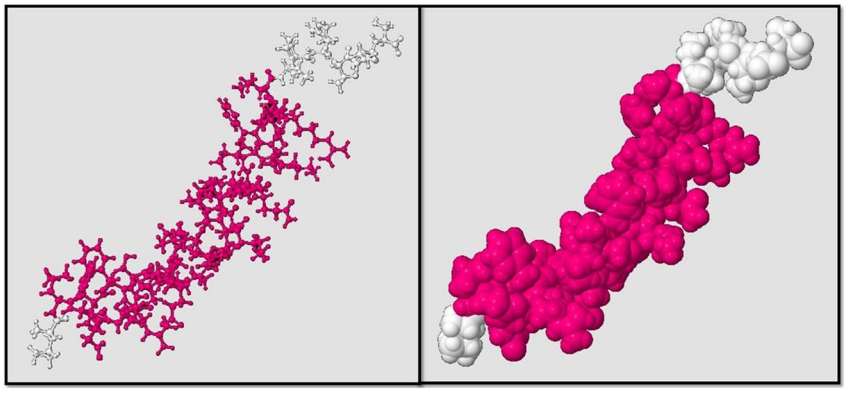

Fig. 7. 3D illustration of a linear chain of human cathelicidin LL-37 (ID58). Free 3D viewed from Protein Data Bank, accessed from http://www.rcsb.org/pdb/home/home.do.

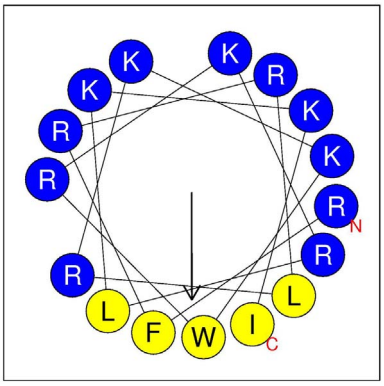

Fig. 8. Possible predicted alpha helical conformation (Table 3, ID73). In yellow, the segment containing an uninterrupted hydrophobic face, and in blue, the polar residues at the edge of the hydrophobic face. Data were analyzed and predicted as an algorithm for the HeliQuest webserver (Gautier et al., 2008). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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References

Almaaytah, A., Tarazi, S., Al-Fandi, M., Abuilhaija, A., Al-shar'i, N., Al-Balas, Q., Abu-Awad, A., Jun 2015. The design and functional characterization of the antimicrobial and antibiofilm activities of BMAP27-Melittin, a rationally designed hybrid peptide. Int. J. Pept. Res. Ther. 21, 165–177.

Amer, Lilian S., Bishop, Barney M., Hoek, van, Monique, L., May 28 2010. Antimicrobial and antibiofilm activity of cathelicidins and short, synthetic peptides against *Francisella*. Biochem. Biophys. Res. Commun. 396, 246–251.

Anunthawan, Thitiporn, de la Fuente-Nunez, Cesar, Hancock, Robert E.W., Klaynongsruang, Sompong, Jun 2015. Cationic amphipathic peptides KT2 and RT2 are taken up into bacterial cells and kill planktonic and biofilm bacteria. BBA-Biomembranes 1848, 1352–1358.

Bajou, Khalid, Herkenne, Stephanie, Thijssen, Victor L., D'Amico, Salvino, Ngoc-Quynh-Nhu, Nguyen, Bouche, Ann, Tabruyn, Sebastien, Srahna, Mohammed, Carabin, Jean-Yves, Nivelles, Olivier, Paques, Cecile, Cornelissen, Ivo, Lion, Michelle, Noel, Agnes, Gils, Ann, Vinckier, Stefan, Declerck, Paul J., Griffioen, Arjan W., Dewerchin, Mieke, Martial, Joseph A., Carmeliet, Peter, Struman, Ingrid, Jul 2014. PAT-1 mediates the antiangiogenic and profibrinolytic effects of 16K prolactin. Nat. Med. 20, 741–747.

Banat, Ibrahim M., Rienzo, De, Diaz, Mayri A., Quinn, Gerry A., Dec 2014. Microbial biofilms: biosurfactants as antibiofilm agents. Appl. Microbiol. Biotechnol. 98, 9915–9929.

Berge, Gerd, Eliassen, Liv Tone, Camilio, Ketil Andre, Bartnes, Kristian, Sveinbjornsson, Baldur, Rekdal, Oystein, Aug 2010. Therapeutic vaccination against a murine lymphoma by intratumoral injection of a cationic anticancer peptide. Cancer Immunol. Immunother. 59, 1285–1294.

Bionda, N., Pastar, I., Davis, S.C., Cudic, P., Apr 2014. In vitro and in vivo activities of novel cyclic lipopeptides against staphylococcal biofilms. Protein Pept. Lett. 21, 352–356.

Bionda, Nina, Fleeman, Renee M., de la Fuente-Nunez, Cesar, Rodriguez, Maria C., Reffuveille, Fany, Shaw, Lindsey N., Pastar, Irena, Davis, Stephen C., Hancock, Robert E.W., Cudic, Predrag, Jan 27 2016. Identification of novel cyclic lipopeptides from a positional scanning combinatorial library with enhanced antibacterial and anti-biofilm activities. Eur. J. Med. Chem. 108, 354–363.

Bjarnsholt, T., Ciofu, O., Molin, S., Givskov, M., Hoiby, N., Oct 2013. Applying insights from biofilm biology to drug development — can a new approach be developed? Nat. Rev. Drug Discov. 12, 791–808.

Blower, R.J., Barksdale, S.M., van Hoek, M.L., Jul 2015. Snake cathelicidin NA-CATH and smaller helical antimicrobial peptides are effective against *Burkholderia thailandensis*. PLoS Negl. Trop. Dis. 9.

Brackman, Gilles, Coenye, Tom, 2015. Quorum sensing inhibitors as anti-biofilm agents. Curr. Pharm. Des. 21 (2015), 5–11.

Burton, E., Gawande, P.V., Yakandawala, N., LoVetri, K., Zhanel, G.G., Romeo, T., Friesen, A.D., Madhyastha, S., May 2006. Antibiofilm activity of GlmU enzyme inhibitors against catheter-associated uropathogens. Antimicrob. Agents Chemother. 50, 1835–1840.

Campoccia, Davide, Montanaro, Lucio, Arciola, Carla Renata, Nov 2013. A review of the biomaterials technologies for infection-resistant surfaces. Biomaterials 34, 8533–8554.

Caraher, E.M., Gumulapurapu, K., Taggart, C.C., Murphy, P., McClean, S., Callaghan, M., Sep 2007. The effect of recombinant human lactoferrin on growth and the antibiotic susceptibility of the cystic fibrosis pathogen *Burkholderia cepacia* complex when cultured planktonically or as biofilms. J. Antimicrob. Chemother. 60, 546–554.

Cateau, Estelle, Berjeaud, Jean-Marc, Imbert, Christine, Apr 2011. Possible role of azole and echinocandin lock solutions in the control of Candida biofilms associated with silicone. Int. J. Antimicrob. Agents 37, 380–384.

Chen, Y.X., Vasil, A.I., Rehaume, L., Mant, C.T., Burns, J.L., Vasil, M.L., Hancock, R.E.W., Hodges, R.S., Feb 2006. Comparison of biophysical and biologic properties of alpha-helical enantiomeric antimicrobial peptides. Chem. Biol. Drug Des. 67, 162–173.

Chernysh, S., Kim, S.I., Bekker, G., Pleskach, V.A., Filatova, N.A., Anikin, V.B., Platonov, V.G., Bulet, P., 2002. Antiviral and antitumor peptides from insects. In: Proceedings of the National Academy of Sciences of the United States of America 99 (Oct 1 2002), pp. 12628–12632.

Choi, Hyemin, Lee, Dong Gun, Dec 2012. Antimicrobial peptide pleurocidin synergizes with antibiotics through hydroxyl radical formation and membrane damage, and exerts antibiofilm activity. BBA-Gen. Subjects 1820, 1831–1838.

Ciocca, D.R., Cayado-Gutierrez, N., Maccioni, M., Cuello-Carrion, F.D., Nov 2012. Heat Shock Proteins (HSPs) based anti-cancer vaccines. Curr. Mol. Med. 12, 1183–1197.

Dashper, Stuart G., Pan, Yu, Veith, Paul D., Chen, Yu-Yen, Toh, Elena C.Y., Liu, Sze Wei, Cross, Keith J., Reynolds, Eric C., Mar 2012. Lactoferrin inhibits *Porphyromonas gingivalis* proteinases and has sustained biofilm inhibitory activity. Antimicrob. Agents Chemother. 56, 1548–1556.

De Brucker, K., Delattin, N., Robijns, S., Steenackers, H., Verstraeten, N., Landuyt, B., Luyten, W., Schoofs, L., Dovgan, B., Frohlich, M., Michiels, J., Vanderleyden, J., Cammue, B.P.A., Thevissen, K., Sep 2014. Derivatives of the mouse cathelicidin-related antimicrobial peptide (CRAMP) inhibit fungal and bacterial biofilm formation. Antimicrob. Agents Chemother. 58, 5395–5404.

De, Zoysa, Heruka, Gayan, Cameron, Alan James, Hegde, Veena V., Raghothama, Srinivasarao, Sarojini, Vijayalekshmi, Jan 22 2015. Antimicrobial peptides with potential for biofilm eradication: synthesis and structure activity relationship studies of Battacin peptides. J. Med. Chem. 58, 625–639.

Delattin, Nicolas; De Brucker, Katrijn; Craik, David J.; Cheneval, Olivier; Froehlich, Mirjam; Veber, Matija; Girandon, Lenart; Davis, Talya R.; Weeks, Anne E.; Kumamoto, Carol A.; Cos, Paul; Coenye, Tom; De Coninck, Barbara; Cammue, Bruno P. A.; and Thevissen, Karin. Plant-derived decapeptide OSIP108 interferes with *Candida albicans* biofilm formation without affecting cell viability. Antimicrob. Agents Chemother. 58 (May 2014): 2647–2656.

Di Luca, Mariagrazia, Maccari, Giuseppe, Nifosi, Riccardo, Apr 2014. Treatment of microbial biofilms in the post- antibiotic era: prophylactic and therapeutic use of

antimicrobial peptides and their design by bioinformatics tools. Pathog. Dis. 70, 257–270.
Ding, Yonglin, Wang, Wei, Fan, Meng, Tong, Zhongchun, Kuang, Rong, Jiang, WenKai, Ni, Longxing, Feb 2014. Antimicrobial and anti-biofilm effect of Bac8c on major bacteria associated with dental caries and *Streptococcus mutans* biofilms. Peptides 52, 61–67.
Dinh, Thuy T.T., Kim, Do-Hee, Luong, Huy X., Lee, Bong-Jin, Kim, Young-Woo, Sep 15 2015. Antimicrobial activity of doubly-stapled alanine/lysine-based peptides. Bioorg. Med. Chem. Lett. 25, 4016–4019.
Dosler, S., Mataraci, E., Nov 2013. In vitro pharmacokinetics of antimicrobial cationic peptides alone and in combination with antibiotics against methicillin resistant *Staphylococcus aureus* biofilms. Peptides 49, 53–58.
D'Ursi, Annamaria, Caliendo, Giuseppe, Perissuti, Elisa, Santagada, Vincenzo, Severino, Beatrice, Albrizio, Stefania, Bifulco, Giuseppe, Spisani, Susanna, Temussi, Piero A., Jun 2007. Conformation-activity relationship of peptide T and new pseudocyclic hexapeptide analogs. J. Pept. Sci. 13, 413–421.
Dusane, D.H., Damare, S.R., Nancharaiah, Y.V., Ramaiah, N., Venugopalan, V.P., Kumar, A.R., Zinjarde, S.S., May 2013. Disruption of microbial biofilms by an extracellular protein isolated from epibiotic tropical marine strain of *Bacillus licheniformis*. PLoS One 8.
Dusane, Devendra H., Hosseinidoust, Zeinab, Asadishad, Bahareh, Tufenkji, Nathalie, 2014. Alkaloids modulate motility, biofilm formation and antibiotic susceptibility of uropathogenic *Escherichia coli*. PLoS One 9, 1–9.
Faruqi, Mariam, Mar 2013. PEPTIDES activating autophagy. Nat. Rev. Drug Discov. 12 (190–190).
Feng, X.R., Sambanthamoorthy, K., Palys, T., Paranavitana, C., Nov 2013. The human antimicrobial peptide LL-37 and its fragments possess both antimicrobial and anti-biofilm activities against multidrug-resistant *Acinetobacter baumannii*. Peptides 49, 131–137.
Flemming, Hans-Curt, Wingender, Jost, Sep 2010. The biofilm matrix. Nat. Rev. Microbiol. 8, 623–633.
de la Fuente-Nunez, C., Korolik, V., Bains, M., Nguyen, U., Breidenstein, E.B.M., Horsman, S., Lewenza, S., Burrows, L., Hancock, R.E.W., May 2012. Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide. Antimicrob. Agents Chemother. 56, 2696–2704.
de la Fuente-Nunez, Cesar, Reffuveille, Fany, Mansour, Sarah C., Reckseidler-Zenteno, Shauna L., Hernandez, Diego, Brackman, Gilles, Coenye, Tom, Hancock, Robert E.W., 2015. D-Enantiomeric peptides that eradicate wild-type and multidrug-resistant biofilms and protect against lethal pseudomonas aeruginosa infections. Chem. Biol. 22 (196), 1280–1282 (Sep 17 2015).
Ganz, T., Sep 2003. Defensins: antimicrobial peptides of innate immunity. Nat. Rev. Immunol. 3, 710–720.
Gautam, Ankur, Chaudhary, Kumardeep, Kumar, Rahul, Sharma, Arun, Kapoor, Pallavi, Tyagi, Atul, Raghava, Gajendra P.S., Open Source Drug Discovery, Consort, Mar 22 2013. In silico approaches for designing highly effective cell penetrating peptides. J. Transl. Med. 11.
Gautier, Romain, Douguet, Dominique, Antonny, Bruno, Drin, Guillaume, Sep 15 2008. HELIQUEST: a web server to screen sequences with specific alpha-helical properties. Bioinformatics 24, 2101–2102.
Gawande, P.V., Leung, K.P., Madhyastha, S., May 2014. Antibiofilm and antimicrobial efficacy of dispersinB(A (R))-KSL-W peptide-based wound gel against chronic wound infection associated bacteria. Curr. Microbiol. 68, 635–641.
Glinel, K., Thebault, P., Humblot, V., Pradier, C.M., Jouenne, T., May 2012. Antibacterial surfaces developed from bio-inspired approaches. Acta Biomater. 8, 1670–1684.
Gopal, R., Lee, J.H., Kim, Y.G., Kim, M.S., Seo, C.H., Park, Y., Jun 2013. Anti-microbial, anti-biofilm activities and cell selectivity of the NRC-16 peptide derived from witch flounder, *Glyptocephalus cynoglossus*. Mar. Drugs 11, 1836–1852.
Gopal, R., Kim, Y.G., Lee, J.H., Lee, S.K., Chae, J.D., Son, B.K., Seo, C.H., Park, Y., Mar 2014. Synergistic effects and antibiofilm properties of chimeric peptides against multidrug-resistant *Acinetobacter baumannii* strains. Antimicrob. Agents Chemother. 58, 1622–1629.
Gowrishankar, Shanmugaraj, Poornima, Balan, Pandian, Shunmugiah Karutha, May 2014. Inhibitory efficacy of cyclo(L-leucyl-L-prolyl) from mangrove rhizosphere bacterium-*Bacillus amyloliquefaciens* (MMS-50) toward cariogenic properties of *Streptococcus mutans*. Res. Microbiol. 165, 278–289.
Haisma, E.M., de Breij, A., Chan, H., van Dissel, J.T., Drijfhout, J.W., Hiemstra, P.S., El Ghalbzouri, A., Nibbering, P.H., Aug 2014. LL-37-derived peptides eradicate multidrug-resistant staphylococcus aureus from thermally wounded human skin equivalents. Antimicrob. Agents Chemother. 58, 4411–4419.
Hancock, Robert E.W., Sahl, Hans-Georg, Dec 2006. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat. Biotechnol. 24, 1551–1557.
Haney, E.F., Mansour, S.C., Hilchie, A.L., de la Fuente-Nunez, C., Hancock, R.E.W., Sep 2015. High throughput screening methods for assessing antibiofilm and immunomodulatory activities of synthetic peptides. Peptides 71, 276–285.
Hayashi, Y., Katada, J., Sato, Y., Igarashi, K., Takiguchi, Y., Harada, T., Muramatsu, M., Yasuda, E., Uno, I., Mar 1998. Discovery and structure-activity relationship studies of a novel and specific peptide motif, Pro-X-X-X-Asp-X, as a platelet fibrinogen receptor antagonist. Bioorg. Med. Chem. 6, 355–364.
Heitz, Frederic, Morris, May Catherine, Divita, Gilles, May 2009. Twenty years of cell-penetrating peptides: from molecular mechanisms to therapeutics. Br. J. Pharmacol. 157, 195–206.
Hirt, H., Gorr, S.U., Oct 2013. Antimicrobial peptide GL13K is effective in reducing biofilms of pseudomonas aeruginosa. Antimicrob. Agents Chemother. 57, 4903–4910.
Hoiby, Niels, Bjarnsholt, Thomas, Givskov, Michael, Molin, Soren, Ciofu, Oana, Apr 2010. Antibiotic resistance of bacterial biofilms. Int. J. Antimicrob. Agents 35, 322–332.
Hong, S.Y., Oh, J.E., Lee, K.H., Dec 1999. Effect of D-amino acid substitution on the stability, the secondary structure, and the activity of membrane-active peptide. Biochem. Pharmacol. 58, 1775–1780.
Hwang, I.S., Hwang, J.S., Hwang, J.H., Choi, H., Lee, E., Kim, Y., Lee, D.G., Jan 2013. Synergistic effect and antibiofilm activity between the antimicrobial peptide coprisin and conventional antibiotics against opportunistic bacteria. Curr. Microbiol. 66, 56–60.
Jadhav, Sandip V., Bandyopadhyay, Anupam, Gopi, Hosahudya N., 2013. Protein secondary structure mimetics: crystal conformations of alpha/gamma(4)-hybrid peptide 12-helices with proteinogenic side chains and their analogy with alpha- and beta-peptide helices. Org. Biomol. Chem. 11 (2013), 509–514.
Jorge, Paula, Lourenco, Analia, Pereira, Maria Olivia, 2012. New trends in peptide-based anti-biofilm strategies: a review of recent achievements and bioinformatic approaches. Biofouling 28, 1033–1061.
Juba, M., Porter, D., Dean, S., Gillmor, S., Bishop, B., Jul 2013. Characterization and performance of short cationic antimicrobial peptide isomers. Biopolymers 100, 387–401.
Kanthawong, Sakawrat, Bolscher, Jan G.M., Veerman, Enno C.I., van Marle, Jan, de Soet, Hans J.J., Nazmi, Kamran, Wongratanacheewin, Surasakdi, Taweechaisupapong, Suwimol, Jan 2012. Antimicrobial and antibiofilm activity of LL-37 and its truncated variants against *Burkholderia pseudomallei*. Int. J. Antimicrob. Agents 39, 39–44.
Kvist, M., Hancock, V., Klemm, P., Dec 2008. Inactivation of efflux pumps abolishes bacterial biofilm formation. Appl. Environ. Microbiol. 74, 7376–7382.
Laverty, G., McCloskey, A.P., Gilmore, B.F., Jones, D.S., Zhou, J., Xu, B., Sep 2014. Ultrashort cationic naphthalene-derived self-assembled peptides as antimicrobial nanomaterials. Biomacromolecules 15, 3429–3439.
Lee, D.L., Hodges, R.S., 2003. Structure-activity relationships of de novo designed cyclic antimicrobial peptides based on gramicidin S. Biopolymers 71 (2003), 28–48.
Leuschner, C., Hansel, W., 2004. Membrane disrupting lytic peptides for cancer treatments. Curr. Pharm. Des. 10 (2004), 2299–2310.
Li, X., Contreras-Garcia, A., LoVetri, K., Yakandawala, N., Wertheimer, M.R., De Crescenzo, G., Hoemann, C.D., Dec 2015. Fusion peptide P15-CSP shows antibiofilm activity and pro-osteogenic activity when deposited as a coating on hydrophilic but not hydrophobic surfaces. J. Biomed. Mater. Res. A 103, 3736–3746.
Liang, Yanyu, Tang, Shan, Zheng, Jishen, Nov 2014. Cell-permeable cyclic peptides. Prog. Chem. 26, 1793–1800.
Lim, Kaiyang, Chua, Ray Rong Yuan, Saravanan, Rathi, Basu, Anindya, Mishra, Biswajit, Tambyah, Paul Anantharajah, Ho, Bow, Leong, Susanna Su Jan, Sep 11 2013. Immobilization studies of an engineered arginine-tryptophan rich peptide on a silicone surface with antimicrobial and antibiofilm activity (vol 5, pg 6412, 2013). ACS Appl. Mater. Interfaces 5 (8821–8821).
Liu, Y., Knapp, K.M., Yang, L., Molin, S., Franzyk, H., Folkesson, A., Jan 2013. High in vitro antimicrobial activity of beta-peptoid-peptide hybrid oligomers against planktonic and biofilm cultures of *Staphylococcus epidermidis*. Int. J. Antimicrob. Agents 41, 20–27.
Lum, Kah Yean, Tay, Sun Tee, Le, Cheng Foh, Lee, Vannajan Sanghiran, Sabri, Nadia Hanim, Velayuthan, Rukumani Devi, Hassan, Hamimah, Sekaran, Shamala Devi, May 12 2015. Activity of novel synthetic peptides against Candida albicans. Sci. Rep. 5.
MacColl, R., Eisele, L.E., Stack, R.F., Hauer, C., Vakharia, D.D., Benno, A., Kelly, W.C., Mizejewski, G.J., Oct 3 2001. Interrelationships among biological activity, disulfide bonds, secondary structure, and metal ion binding for a chemically synthesized 34-amino-acid peptide derived from alpha-fetoprotein. BBA-Gen. Subjects 1528, 127–134.
Mah, Thien-Fah, 2012a. Biofilm-specific antibiotic resistance. Future Microbiol 7, 1061–1072 (Sep).
Mah, Thien-Fah, 2012b. Regulating antibiotic tolerance within biofilm microcolonies. J. Bacteriol. 194, 4791–4792 (Sep).
Martinez-Hoyer, Sergio, Sole-Sanchez, Sonia, Aguado, Fernando, Martinez-Martinez, Sara, Serrano-Candelas, Eva, Luis Hernandez, Jose, Iglesias, Mar, Redondo, Miguel, Juan, Casanovas, Oriol, Messeguer, Ramon, Perez-Riba, Merce, Jul 2015. A novel role for an RCAN3-derived peptide as a tumor suppressor in breast cancer. Carcinogenesis 36, 792–799.
Mataraci, Emel, Dosler, Sibel, Dec 2012. In vitro activities of antibiotics and antimicrobial cationic peptides alone and in combination against methicillin-resistant staphylococcus aureus biofilms. Antimicrob. Agents Chemother. 56, 6366–6371.
Minor, D.L., Kim, P.S., 1994a. Context is a major determinant of beta-sheet propensity. Nature 371, 264–267 (Sep 15).
Minor, D.L., Kim, P.S., 1994b. Measurement of the beta-sheet-forming propensities of amino-acids. Nature 367, 660–663 (Feb 17).
Mishra, N.M., Briers, Y., Lamberigts, C., Steenackers, H., Robijns, S., Landuyt, B., Vanderleyden, J., Schoofs, L., Lavigne, R., Luyten, W., Van der Eycken, E.V., 2015. Evaluation of the antibacterial and antibiofilm activities of novel CRAMP-vancomycin conjugates with diverse linkers. Org. Biomol. Chem. 13, 7477–7486.
Molhoek, E.M., van Dijk, A., Veldhuizen, E.J.A., Haagsman, H.P., Bikker, F.J., May 2011. A cathelicidin-2-derived peptide effectively impairs *Staphylococcus epidermidis* biofilms. Int. J. Antimicrob. Agents 37, 476–479.
Nagant, C., Pitts, B., Nazmi, K., Vandenbranden, M., Bolscher, J.G., Stewart, P.S., Dehaye, J.P., Nov 2012. Identification of peptides derived from the human antimicrobial peptide LL-37 active against biofilms formed by *Pseudomonas aeruginosa* using a library of truncated fragments. Antimicrob. Agents Chemother. 56, 5698–5708.
Nefedov, Alexey V., Sadygov, Rovshan G., Nov 7 2011. A parallel method for enumerating amino acid compositions and masses of all theoretical peptides. BMC Bioinformatics 12.
Niu, Y., Hu, Y., Wang, R.E., Li, X., Wu, H., Chen, H., Cai, J., 2012. Protein Interactions. Janeza Trdine 9, 51000 Rijeka, Croatia: Copyright © 2012 InTech.
Ong, Z.Y., Gao, S.J., Yang, Y.Y., Aug 2013. Short synthetic beta-sheet forming peptide

amphiphiles as broad spectrum antimicrobials with antibiofilm and endotoxin neutralizing capabilities. Adv. Funct. Mater. 23, 3682–3692.
Pasikowski, Pawel, Gozdziewicz, Tomasz, Stefanowicz, Piotr, Artym, Jolanta, Zimecki, Michal, Szewczuk, Zbigniew, Dec 2011. A novel immunosuppressory peptide originating from the ubiquitin sequence. Peptides 32, 2418–2427.
Pennington, Michael W., Chang, Shih Chieh, Chauhan, Satendra, Huq, Redwan, Tajhya, Rajeev B., Chhabra, Sandeep, Norton, Raymond S., Beeton, Christine, Jan 2015. Development of highly selective Kv1.3-blocking peptides based on the sea anemone peptide ShK. Mar. Drugs 13, 529–542.
Rabin, Nira, Zheng, Yue, Opoku-Temeng, Clement, Du, Yixuan, Bonsu, Eric, Sintim, Herman O., 2015. Agents that inhibit bacterial biofilm formation. Future Med. Chem. 7 (2015), 647–671.
Rautela, Ria, Singh, Anil Kumar, Shukla, Abha, Cameotra, Swaranjit Singh, May 2014. Lipopeptides from *Bacillus* strain AR2 inhibits biofilm formation by *Candida albicans*. Anton. Leeuw. Int. J. Gen. Mol. Microbiol. 105, 809–821.
Ribeiro, Suzana Meira, de la Fuente-Nunez, Cesar, Baquir, Beverlie, Faria-Junior, Celio, Franco, Octavio L., Hancock, Robert E.W., Jul 2015. Antibiofilm peptides increase the susceptibility of carbapenemase-producing *Klebsiella pneumoniae* clinical isolates to beta-lactam antibiotics. Antimicrob. Agents Chemother. 59, 3906–3912.
Saitou, N., Nei, M., Jul 1987. The neighbor-joining method — a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.
Sanchez-Margalet, V., Martin-Romero, C., Santos-Alvarez, J., Goberna, R., Najib, S., Gonzalez-Yanes, C., Jul 2003. Role of leptin as an immunomodulator of blood mononuclear cells: mechanisms of action. Clin. Exp. Immunol. 133, 11–19.
Schillaci, D., Arizza, V., Parrinello, N., Di Stefano, V., Fanara, S., Muccilli, V., Cunsolo, V., Haagensen, J.J.A., Molin, S., Jan 2010. Antimicrobial and antistaphylococcal biofilm activity from the sea urchin *Paracentrotus lividus*. J. Appl. Microbiol. 108, 17–24.
Schillaci, D., Vitale, M., Cusimano, M.G., Arizza, V., 2012. Fragments of beta-thymosin from the sea urchin *Paracentrotus lividus* as potential antimicrobial peptides against staphylococcal biofilms. Thymosins Health Dis. 1270, 79–85.
Schillaci, Domenico, Cusimano, Maria Grazia, Cunsolo, Vincenzo, Saletti, Rosaria, Russo, Debora, Vazzana, Mirella, Vitale, Maria, Arizza, Vincenzo, 2013. Immune mediators of sea-cucumber *Holothuria tubulosa* (Echinodermata) as source of novel antimicrobial and anti-staphylococcal biofilm agents. AMB Express 3, 2013.
Schillaci, D., Cusimano, M.G., Spinello, A., Barone, G., Russo, D., Vitale, M., Parrinello, D., Arizza, V., Oct 2014. Paracentrin 1, a synthetic antimicrobial peptide from the sea-urchin *Paracentrotus lividus*, interferes with staphylococcal and *Pseudomonas aeruginosa* biofilm formation. AMB Express 4.
Sengupta, J., Saha, S., Khetan, A., Sarkar, S.K., Mandal, S.M., Oct 2012. Effects of lactoferricin B against keratitis-associated fungal biofilms. J. Infect. Chemother. 18, 698–703.
Shi, Yan, Teng, Peng, Sang, Peng, She, Fengyu, Wei, Lulu, Cai, Jianfeng, Mar 2016. gamma-AApeptides: design, structure, and applications. Acc. Chem. Res. 49, 428–441.
Silva, Laura Nunes, et al., July 20, 2016. Plant natural products targeting bacterial virulence factors. Chem. Rev. 116 (16), 9162–9236.
Starkey, J.R., Dai, S., Dratz, E.A., Dec 8 1998. Sidechain and backbone requirements for anti-invasive activity of laminin peptide 11. BBA-Protein Struct. M. 1429, 187–207.
Strempel, Nikola, Strehmel, Janine, Overhage, Joerg, 2015. Potential application of antimicrobial peptides in the treatment of bacterial biofilm infections. Curr. Pharm. Des. 21 (2015), 67–84.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., Dec 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.
Tong, Zhongchun, Zhou, Lin, Jiang, Wenkai, Kuang, Rong, Li, Jie, Tao, Rui, Ni, Longxing, Oct 2011. An in vitro synergetic evaluation of the use of nisin and sodium fluoride or chlorhexidine against *Streptococcus mutans*. Peptides 32, 2021–2026.
Tong, Zhongchun, Zhang, Yuejiao, Ling, Junqi, Ma, Jinglei, Huang, Lijia, Zhang, Luodan, Feb 20 2014. An in vitro study on the effects of nisin on the antibacterial activities of 18 antibiotics against *Enterococcus faecalis*. PLoS One 9.
Venkatesh, M., Rong, L., Raad, I., Versalovic, J., Jul 2009. Novel synergistic antibiofilm combinations for salvage of infected catheters. J. Med. Microbiol. 58, 936–944.
Wakabayashi, H., Yamauchi, K., Kobayashi, T., Yaeshima, T., Iwatsuki, K., Yoshie, H., Aug 2009. Inhibitory effects of lactoferrin on growth and biofilm formation of *Porphyromonas gingivalis* and *Prevotella intermedia*. Antimicrob. Agents Chemother. 53, 3308–3316.
Wang, Guangshun, Li, Xia, Wang, Zhe, Jan 2009. APD2: the updated antimicrobial peptide database and its application in peptide design. Nucleic Acids Res. 37, D933–D937.
Wang, Guangshun, Mishra, Biswajit, Epand, Raquel F., Epand, Richard M., Sep 2014. High-quality 3D structures shine light on antibacterial, anti-biofilm and antiviral activities of human cathelicidin LL-37 and its fragments. BBA-Biomembranes 1838, 2160–2172.
Wang, Guangshun, Li, Xia, Wang, Zhe, Jan 4 2016. APD3: the antimicrobial peptide database as a tool for research and education. Nucleic Acids Res. 44, D1087–D1093.
Wu, Hong, Ong, Zhan Yuin, Liu, Shaoqiong, Li, Yan, Wiradharma, Nikken, Yang, Yi Yan, Ying, Jackie Y., Mar 2015. Synthetic beta-sheet forming peptide amphiphiles for treatment of fungal keratitis. Biomaterials 43, 44–49.
Yala, Jean-Fabrice, Thebault, Pascal, Hequet, Arnaud, Humblot, Vincent, Pradier, Claire-Marie, Berjeaud, Jean-Marc, Feb 2011. Elaboration of antibiofilm materials by chemical grafting of an antimicrobial peptide. Appl. Microbiol. Biotechnol. 89, 623–634.
Yoshikawa, Masaaki, Oct 2015. Bioactive peptides derived from natural proteins with respect to diversity of their receptors and physiological effects. Peptides 72, 208–225.
Zaïri, A., Ferrières, L., Latour-Lambert, P., Beloin, C., Tangy, F., Ghigo, J.M., Hani, K., 2014. In vitro activities of dermaseptins K4S4 and K4K20S4 against *Escherichia coli*, *Staphylococcus aureus*, and Pseudomonas aeruginosa planktonic growth and biofilm formation. Antimicrob. Agents Chemother. 58, 2221–2228.
Zhang, R.W., Zhou, M., Wang, L., McGrath, S., Chen, T.B., Chen, X.H., Shaw, C., Jul 2010. Phylloseptin-1 (PSN-1) from *Phyllomedusa sauvagei* skin secretion: a novel broad-spectrum antimicrobial peptide with antibiofilm activity. Mol. Immunol. 47, 2030–2037.
Zhu, X., Ma, Z., Wang, J.J., Chou, S.L., Shan, A.S., Dec 2014. Importance of tryptophan in transforming an amphipathic peptide into a pseudomonas aeruginosa-targeted antimicrobial peptide. PLoS One 9.

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4. Chapter 2. Main article : article 2

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Capsicumicine, a peptide from *Capsicum baccatum* pepper displays powerful antibiofilm activity by a novel mechanism of action: matrix anti-assembly.

Rafael Gomes Von Borowski[1,2], Sophie Chat[1], Rafael Schneider[1,2], Emmanuel Giudice[1], Simone Cristina Baggio Gnoatto[2], Alexandre José Macedo[2,3]*, and Reynald Gillet[1]*

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Abstract

Biofilm forming bacteria are considered an important source of nosocomial infections and antibiotic resistance and tolerance. These bacteria are enclosed in a complex matrix leading to adherence to medical devices and tissues as well as protection against antibiotics and the immune system. Hence, the progress of antibiofilm strategies targeting biofilm matrix is extremely relevant in fighting multi-drug resistant and tolerant bacteria. Plants are constantly submitted to a wide range of pathogens and to defend themselves they synthesize protective factors such as peptides. These peptides are common components of the *Capsicum* red pepper seeds. Here, we investigated and identified a new antibiofilm peptide named capsicumicine. We demonstrated that capsicumicine prevents methicillin resistant *S. epidermidis* adhesion, biofilm establishment and maintenance. We discovered that its activity is due to a new extracellular mechanism of action called "anti-matrix self-assembly". We evidenced that capsicumicine disturbs the matrix structuration leading to the lost of functionality. Importantly, this peptide is non-antibiotic and non-cytotoxic, providing a new alternative to prevent biofilm infections.

Keywords: biofilm, matrix, peptides, resistance.

Introduction

Antimicrobial failure is a worldwide challenge, endorsed in a currently global action plan (Who e Organization, 2015). The lack of novel antibiotics and their inappropriate uses are resulting in an increase of multi-drug resistant strains (Who e Organization, 2018). This process is favored by biofilm development hence microorganisms enclosed in the matrix display up to 1000 times higher antibiotic resistance than the planktonic ones (Ceri et al., 1999; Conibear et al., 2009; Penesyan et al., 2015) making the biofilm matrix itself a new important target. Biofilms are organized microbial clusters made of a self-assembled matrix, usually attached to an abiotic (*e.g.* medical devices or teeth) or biotic surface (*e.g.* host tissue or suspended in mucus or in chronic wounds) (Flemming e Wingender, 2010; Taglialegna et al., 2016). Since they are embedded into this self-assembled matrix these bacteria have an increased tolerance and persistence to antibiotics, disinfectants and host defenses, and are therefore harder to treat (Hoiby et al., 2010; Beloin et al., 2014). Advantages over planktonic form include: high physiological and biochemical changes, beneficial quorum sensing, higher mutation rates (up to 100 times) and the development of persisters (Davies, 2003; Le e Otto, 2015; Brauner et al., 2016; Levin-Reisman et al., 2017; Defraine et al., 2018). Therefore, the development of antivirulence strategies such as efficient antibiofilm agents is crucial against the current antibiotic crisis. In this scenario, peptides and peptidomimetics are rising as important arsenal (De La Fuente-Nunez et al., 2015; Strempel et al., 2015; Silva, 2016; Von Borowski et al., 2017; Gomes Von Borowski et al., 2018).

In this context, *Staphylococcus epidermidis* is the most frequent coagulase negative *Staphylococcus* (CoNS) infection causing disease (Rogers et al., 2009; Uçkay et al., 2011), being capable to survive on surfaces for months (Neely e Maley, 2000; Otto, 2008). *Staphylococcus epidermidis* correspond to 30% of health care-associated bloodstream infections and is significantly associated to medical devices infections (15-40% of prosthetic valve endocarditis (Lalani et al., 2006; Nishizaki et al., 2013), 30-43% of prosthetic orthopedic devices infections (Teterycz et al., 2010; Otto, 2014; Abad e Haleem, 2018). Over 150 million intravascular catheters that are used per year in the USA, 250,000 catheter-related infections are estimated to arise (Maki et al., 2006; Cdcp, 2011; Rupp, 2014). This bacteria is developing antibiotic multi-resistance such as elevated glycopeptide minimal inhibitory concentrations (Sieradzki et al., 1999; Lazaris et al., 2017; Lee et al., 2018) and 73–88% of *S. epidermidis* isolates display resistance to oxacillin, fluoroquinolones, macrolides, clindamycin and trimethoprim–sulfamethoxazole (Streit et al., 2004; Hope et al., 2008; Flamm et al., 2016). While *S. epidermidis* expresses many virulence factors such as toxins, proteases, enzymes, surface and extracellular proteins and capsule (Fey e Olson, 2010) the biofilm formation is the most important mechanism contributing to infection (Otto, 2008; 2014). The extracellular matrix is a complex physicochemical barrier that represents one of the highest difficulties to treat biofilms (Flemming e Wingender, 2010).

Here, we report the discovery of a new antibiofilm peptide (capsicumicine) derived from the red pepper *Capsicum baccatum* that prevents methicillin resistant *S. epidermidis* adhesion, biofilm establishment and maintenance. Notably we demonstrate that capsicumicine antibiofilm activity is due to an extracellular mechanism of action, shifting the matrix assembly. Importantly, capsicumicine is non-antibiotic and non-cytotoxic, providing a new alternative to fight against biofilm infections.

Results

Capsicumicine prevents biofilm formation without antibiotic activity. Three different peptides P1 (RVQSEEGEDQISQRE), P2 (RAEAFQTAQALPGLCRI) and P3 (RSCQQQIQQAQQLSSCQQYLKQ) were

selected and synthesized based on promising natural antibiofilm peptides (NAP) from *Capsicum baccatum* var. *pendulum* as previously described (Von Borowski, RG et al; in progress). Briefly, we chose the most stable fragments after NAP enzymatic digestion and Maldi-MS fragmentation. In order to select the most active compound, we exposed them to strong biofilm forming *S. epidermidis* RP62A (ATCC 35984). The remaining biofilm was quantified after 24h using crystal violet method. P3, named capsicumicine, was noticeably the most active peptide with strong antibiofilm activity. Biofilm decreasing was observed at all tested concentrations but especially at 10µM more than 90% of biofilm reduction was detected, independently of cell growth inhibition. This effect is not dose response dependent (Figure 1a). One relevant element on ideal strategy for antibiofilm drug development is the absence of antibiotic effect (Batoni et al., 2016), therefore, to evidence capsicumicine effect on growth we examined colony forming units (CFU) of *S. epidermidis* RP62A (ATCC 35984) exposed to the peptide. Accordingly, *S. epidermidis* CFU was not affected in the presence of capsicumicine evidencing that biofilm inhibition is not due to bactericidal activity (Figure 1b). Furthermore, in order to localize the peptide, we used capsicumicine conjugated to fluorescein isothiocyanate (capsicumicine-FITC) and confocal fluorescence microscopy (CFM) images. The CFM images analysis shows that capsicumicine-FITC does not enter neither into bacterial cells nor into the walls or membranes, butremains associated with extracellular matrix components (Figure 1c,d).

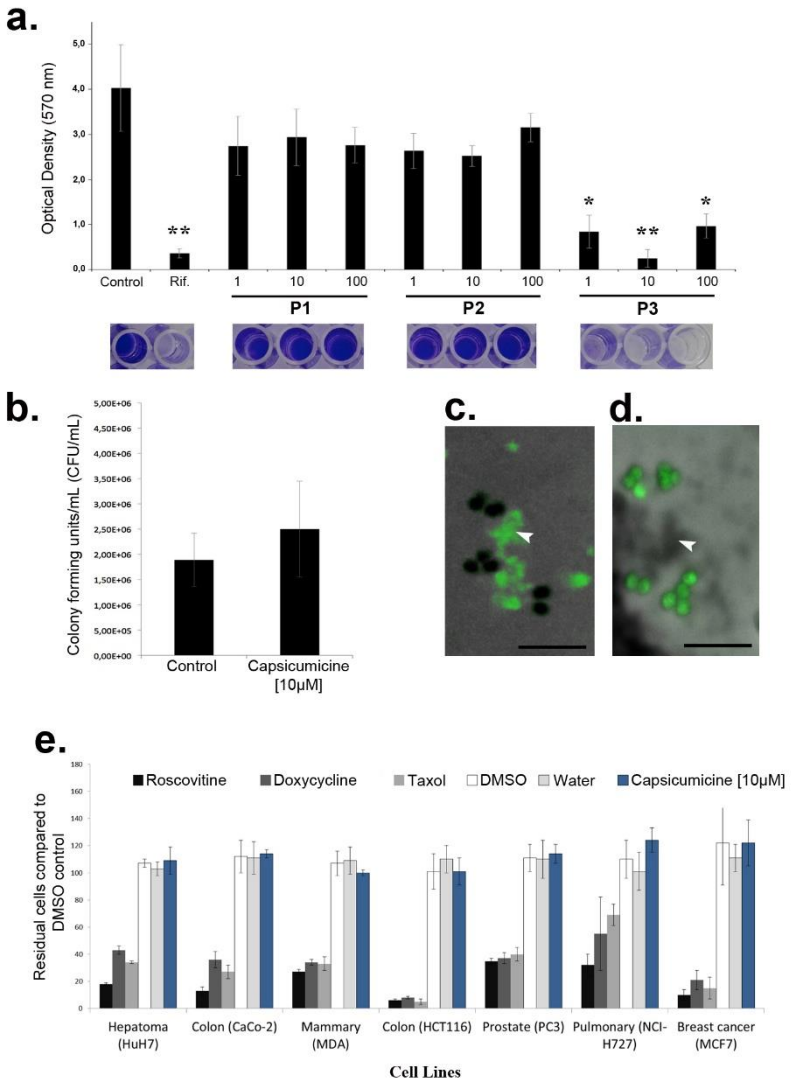

Figure 1. Antibiofilm activity of the peptides. a) Antibiofilm activity: black bars correspond to *Staphylococcus epidermidis* (ATCC 35984) biofilm quantification (OD = 570 nm) after 24h. Peptides P1, P2 and P3 (capsicumicine) were tested at 1, 10 or 100 µM. "Control", correspond to bacteria without peptides exposition and "Rif." correspond to antibiotic control rifampicin at 16µg/mL. Statistical analysis was performed using Student's *t*-test compared to control, where (*) $p<0.05$ and (**) $p<0.01$. b) Colony forming units (CFU) after capsicumicine exposition at 10µM for 24h. Control received vehicle (water) instead of peptide and the result is shown as colony forming unit per mL (CFU/mL). c-d) Confocal fluorescence microscopy images (CFM). c) *S. epidermidis* after capsicumicine fluorescein isothiocyanate (capsicumicine-FITC) exposition at 10µM; matrix in green (fluorescence) and bacteria cells in black spheres (no fluorescence). d) Control *S. epidermidis* after the exposition to an antibacterial peptide-FITC; matrix in grey (no fluorescence) and bacteria cells in green spheres (fluorescence). White arrows point matrix contents. e) Capsicumicine cytotoxicity evaluation in mammalian cells. The number of normal cells is presented as residual cells percentage (%) compared to the average of controls (DMSO and water, show as white and light blue, respectively). Whereas, 100% represent no cytotoxicity or inhibition of cell growth, below 25/30% is considered cytotoxic and 0% represents acute cytotoxicity. The three first bars, black-grey bars, show cytotoxic controls (roscovitine, doxycycline and taxol) and the blue bar shows capsicumicine (10µM) exposed cells. Cells

lines are described under the bars. It was used automated system image-based cellular content analysis (HCS / HCA).

Capsicumicine effects on the eradication of structured biofilms. To verify the interaction between the capsicumicine and already assembled matrix we exposed a single concentration (100µM) of peptide to a 24h *S. epidermidis* pre-existing biofilm and quantified the total biomass after 24h of exposition, using crystal violet method. At this concentration, capsicumicine presents only approximately 15% of disruption of pre-existing biofilm (Figure S1). This indicates that capsicumicine acts only during the first stages of biofilm formation.

Absence of cytotoxicity of capsicumicine in mammalian cells. In order to ensure capsicumicine future safe applicability, we verified biological cytotoxicity in 7 different mammalian lines (HuH7, CaCo-2, MDA, HCT116, PC3, NCI-H727 and MCF7), applying automated system image-based cellular content analysis (HCS / HCA). Thereby, cells treated with capsicumicine had exactly the same performance as untreated controls, evidencing the absence of cytotoxicity (Figure 1e).

Capsicumicine impairs initial attachment, aggregation and biofilm accumulation independently of cell interaction. To reveal capsicumicine activity along the first stages of biofilm development, we analyzed polystyrene coupons after 1, 4 and 24h of biofilm cultures in presence or absence of the peptide. Scanning electron microscopy (SEM) analysis shows bacteria attachment decreasing after 1h of capsicumicine exposure (Figure 2a). Additionally, biofilm accumulation and cell aggregation profiles are strongly reduced after 4 and 24h, demonstrating that capsicumicine prevents *S. epidermidis* coupons adhesion (Figure 2a). Notably this action remains after 24h of incubation (Figure 2a). In order to study capsicumicine possible mechanisms of action, we then selected some genes involved in different stages of biofilm development (*atlE*, *aap*, *agrC*, *icaA*, *leuA*, *saeR*, *saeS* and *sarA*) (Figure 2b) (Table S1, primers information) and analyzed its fold change by quantitative real-time PCR (qRT-PCR). Since exposed bacteria remain planktonic, we compared the relative gene expression of them to planktonic control cells. Capsicumicine exposed cells show the same fold change as the planktonic control cells for all tested genes (Figure 2b). This finding indicates that capsicumicine antibiofilm mechanism of action is not linked to cellular but rather to extracellular interactions.

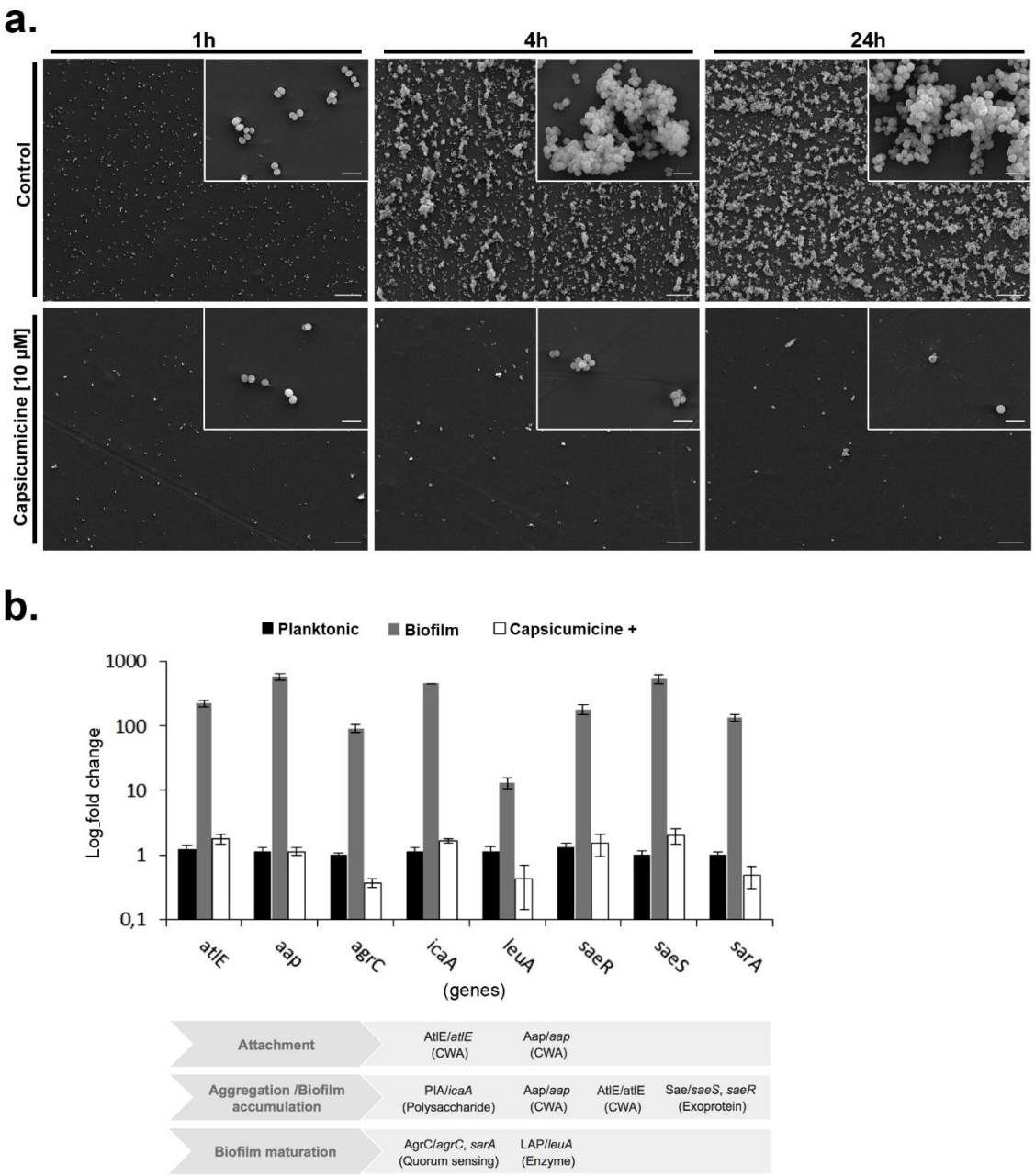

Figure 2. Scanning electron microscopy (SEM) and qRT-PCR analysis. a) SEM images of polystyrene coupons after 1, 4 or 24h of culture with *Staphylococcus epidermidis* (ATCC 35984). On the top, growth CONTROL without peptide. On the bottom capsicumicine EXPOSED cultures (10µM). Large images are shown at magnification of x500 and overlapping small images at x5,000; Scale bars showing 10µm. b) Relative (log fold change) gene expression (mean, ±SEM) of encoding genes involved in biofilm formation from *S. epidermidis* (ATCC 35984). Planktonic control is shown as black bars; biofilm control as grey bars and capsicumicine + (exposed group) as white bars. The *ssrA* gene and planktonic control were used as reference. CWA is cell wall-anchored proteins.

Capsicumicine disturbs *S. epidermidis* matrix assembly. Since capsicumicine antibiofilm activity was not associated to bacterial direct interaction nor the modulation of some gene expression, we set out to investigate the interactions between the peptide and the extracellular matrix by a set of microscopic approaches. Whereas in the biofilm control we macroscopically observe that homogenous whitish adhered layer covers the wall and the bottom of the well (Figure 3a, middle), in the presence of capsicumicine we find non-adhered heterogeneous agglutinates with whitish flocculent-like aspect (Figure 3a, right). Transmitted light microscopy (TL) images match with the macroscopic description. Biofilm control shows a large amount of overlapped attached cells, surrounded by matrix and besides enormous bacteria clusters (Figure 3b). Conversely, the capsicumicine exposed cells form a suspended agglutinate (Figure 3b). Complementary, confocal fluorescence microscopy (CFM) images confirm the presence of labelled-capsicumicine in the matrix, displaying a smoothie cloud-like aspect (Figure 3c). Supporting this, scanning and transmission electron microscopies (SEM, TEM) enable crucial ultra-structural descriptions. Biofilm matrix control shows denser assembled structures, globular-like features (Figure 3d-e). In contrast, in the presence of capsicumicine, biofilm matrix is clearly less dense and displays thin fibrillary oligomer structures, branched-like features (Figure 3d-e). Therefore, matrix conformation changes occur in presence of capsicumicine through biofilm assembly, as evidenced by different image techniques. In addition, no modifications of the cellular morphologies were observed compared to control, reinforcing the hypothesis of a lack of interactions between peptide and bacteria.

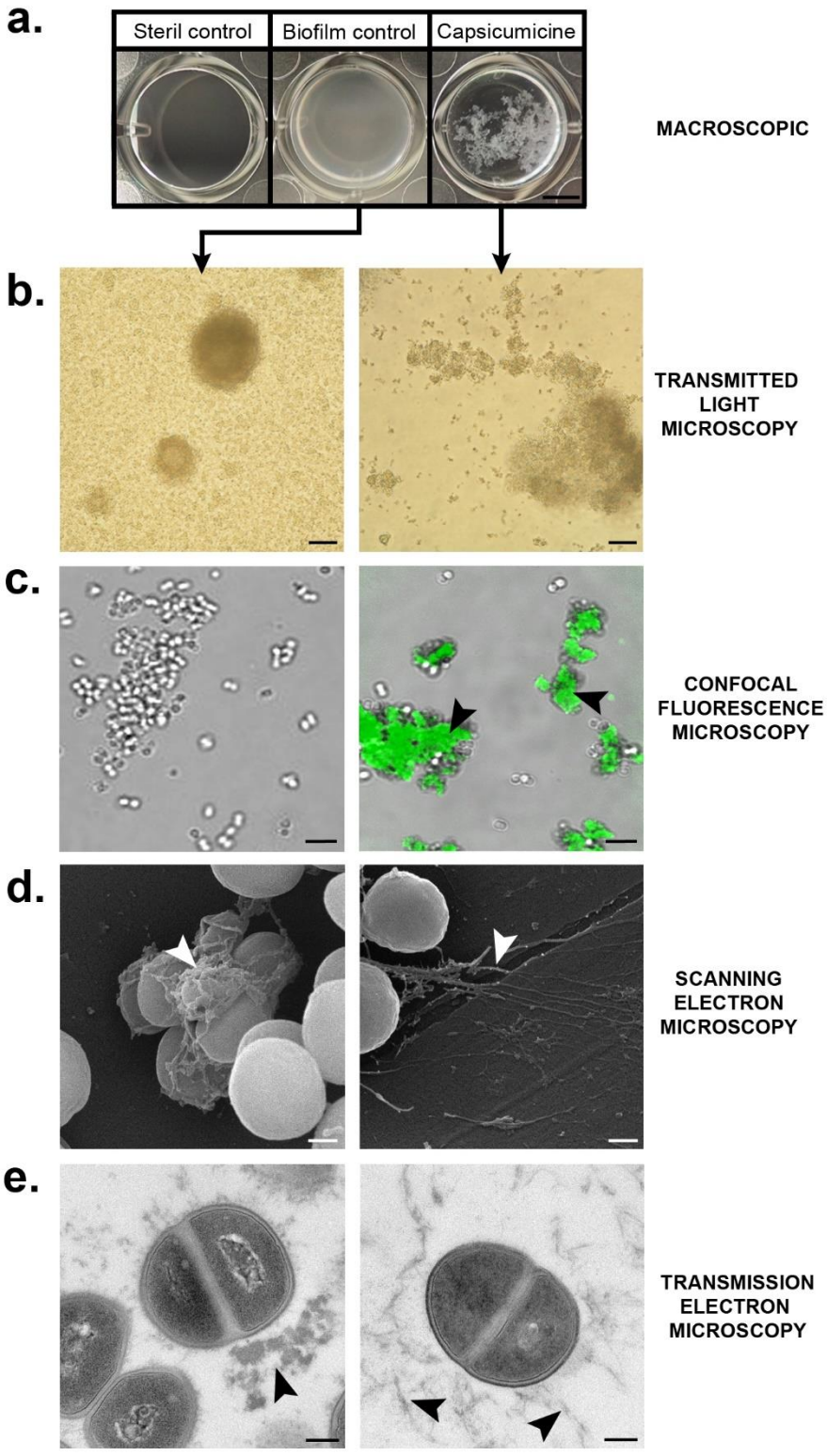

Figure 3. Different set of microscopies images of *Staphylococcus epidermidis* (ATCC 35984) biofilm. These images explore the state of organization of the biofilm matrix in the presence or absence of capsicumicine after 24h. On the left, biofilm control (without capsicumicine) and on the right, capsicumicine exposed culture: a) Pictures from the bottom of 24 wells plate. “Steril control” showing no bacteria nor biofilm formation, “Biofilm control” showing homogenous adhered layers of biofilm formation in absence of capsicumicine and “capsicumicine” showing non-adhered bacteria

and agglutinates in the presence of capsicumicine. b) Transmitted light microscopy: biofilm control with large amount of overlapped attached cells surrounded by matrix besides bacteria clusters. Capsicumicine exposed culture with non-adhered cells but suspended like agglutinate. c) Confocal fluorescence microscopy: biofilm control with no fluorescence detected and capsicumicine-FITC showing matrix green fluorescence. d) Scanning electron microscopy: biofilm control showing matched dense globular-like feature matrix. And, capsicumicine exposed culture showing matched fibrillary oligomer structures, branched-like feature. e) Transmission electron microscopy: biofilm matrix control shows denser assembled structures. At capsicumicine presence, biofilm matrix less dense, displaying thin fibrillary oligomer structures. Arrows highlight matrix.

Capsicumicine shifts molecular self-assembly of artificial matrix. To confirm capsicumicine interaction with *Staphylococcus* matrix assembly in absence of metabolic or regulatory influences, we then used an artificial matrix model (adapted from Stewart et al. (2015)). Briefly, after checking the ideal pH for matrix assembly, the reaction begins and is monitored by optical density (OD 600 nm) measurement in function of time (Real time molecular self-assembly - RTMSA) in presence or absence of capsicumicine. As a negative control we used a peptide of approximately the same size as capsicumicine, called PA-1 (Liu et al., 2016). In the presence of capsicumicine the OD increases, establishing that molecular self-assembly is more speedily established than controls. This correlation is maintained throughout the reaction time (Figure 4a). Remarkably, capsicumicine assembled matrix profile is also visually different from controls displaying larger agglutinates (Figure 4c-e). However, controls assembled matrix profiles are all similar (Figure 4c,d). This demonstrates a central interaction between matrix and capsicumicine, leading to molecular self-assembly shifts.

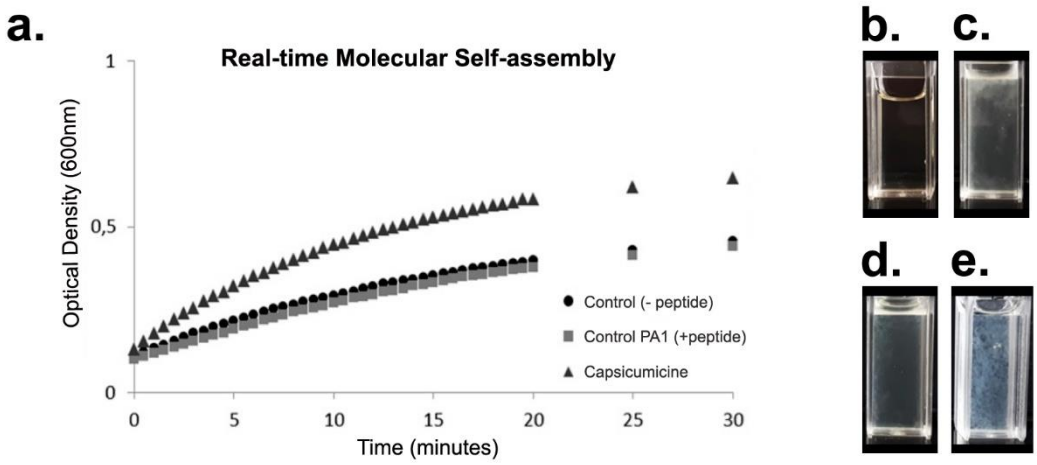

Figure 4. Real time molecular self-assembly curves of synthetic *Staphylococcus* matrix. a) The graph displays optical density (OD 600nm) in function of the time (minutes). Black triangles represent synthetic matrix in presence of capsicumicine. Black spheres represent positive control synthetic matrix. Grey squares represent peptide negative control (PA1) with synthetic matrix. b-e) shows synthetic matrix tubs in different conditions: b) calibration reaction, containing all reaction compounds before polymerization; c) Positive control reaction, after polymerization; d) Negative peptide control reaction with PA1, after polymerization and e) Capsicumicine reaction, after polymerization. Peptides were tested at the same final concentration.

Capsicumicine interacts with exopolysaccharides. To display the potential affinity of the peptide with these essential components of the matrix, we exposed *S. epidermidis* cultures to capsicumicine, capsicumicine-FITC or peptide antibiotic-FITC and calcofluor and analyzed them on confocal fluorescence microscopy (CFM). Calcofluor was used to target matrix polysaccharides (blue) and FITC to localize the peptides (green). An antibacterial peptide-FITC was used as peptide control (Schneider et al; in progress), displaying green fluorescence into the cells but not detectable in the matrix (Figure 5e,f). Figure 5a shows CFM images of substantial polysaccharides amounts on the matrix (blue) in the presence of capsicumicine. Calcofluor is also detected in cells because of the wall saccharides components. Additionally, figure 5c shows considerable amounts of capsicumicine-FITC exclusively on the matrix. These results point to a suitable affinity between capsicumicine and exopolysaccharides once they are both significantly localized on the matrix.

In order to explore capsicumicine features compatible with carbohydrate-binding-like domains (CBM), we used chitin, chitosan and PIA binding proteins (Table S2) BLAST and amino acids alignments with capsicumicin, using UniProt tools (The uniprot consortium, 2017) and Cazy information crossing (Lombard *et al.*, 2014). In fact, capsicumicine presents CBM homology with all tested proteins (Figure S2), reinforcing the idea that capsicumicine interacts with these saccharides.

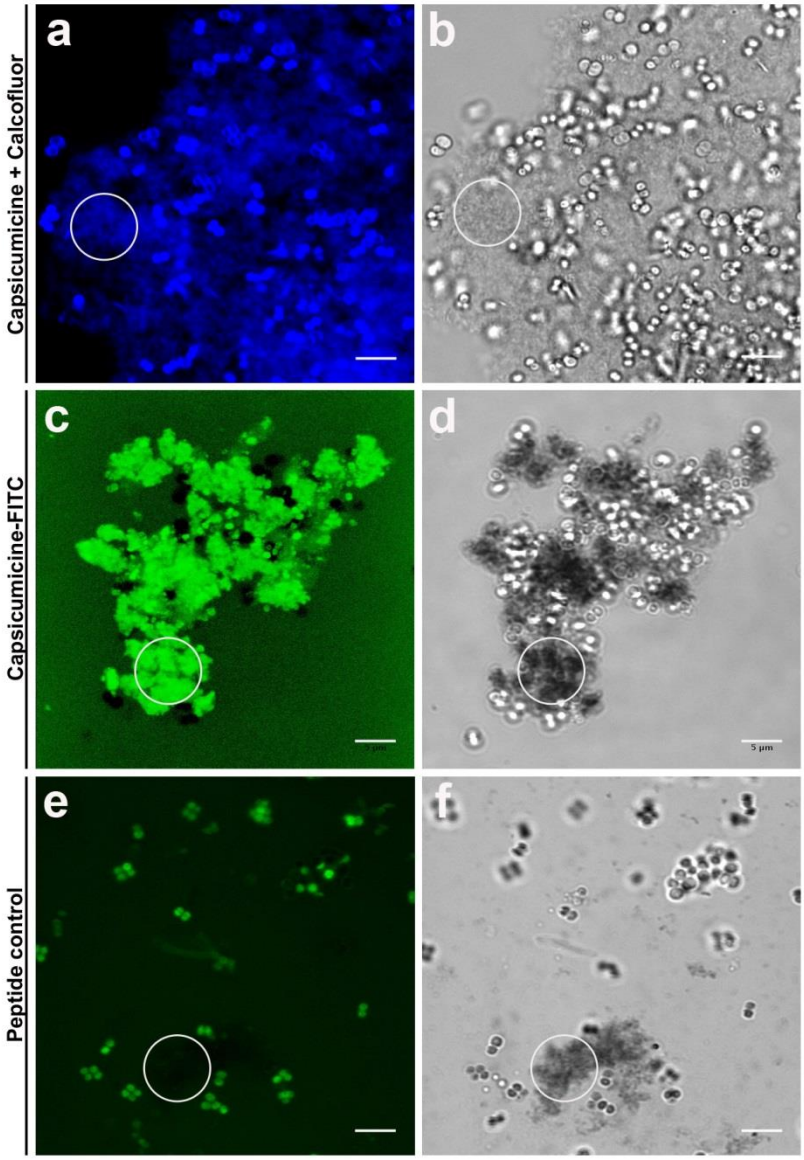

Figure 5. Confocal fluorescence microscopy (CFM) of *Staphylococcus epidermidis* (ATCC 35984). Calcofluor was used to evidence matrix polysaccharides (blue) and FITC to localize the peptides (green). a,b) Culture exposed to capsicumicine and calcofluor; visualized by fluorescence mode (a) or transmitted light (b). c-d) Culture exposed to P3-FITC; visualized by fluorescence mode (c) or transmitted light (d). e-f) Peptide negative control: culture exposed to an antimicrobial peptide-FITC (Schneider et al; in progress); visualized by fluorescence mode (e) or transmitted light (f). Dotted circles display extracellular matrix contents.

Discussion

Considering that there are no antibiofilm drugs available, natural peptides have been increasingly prominent as antivirulence alternatives allies against bacterial tolerance (Stewart, 2015; Feuillie et al., 2017; Grassi et al., 2017; Von Borowski et al., 2017). This study demonstrates that capsicumicine, a peptide from red pepper *C. bacattum*, possesses significant antibiofilm activity. It prevents the establishment and maintenance of biofilm architecture through a new mechanism of action that we called "matrix anti-assembly" (MAA). MAA differs from matrix disassembly (Roy et al., 2018) because it acts on the initial phase of matrix assembly, preventing its functional assembly rather than de-structuring once established. In fact, established biofilms are known to be harder to treat than initial biofilm because they show a very complex structure (Lewis, 2001; Jabbouri e Sadovskaya, 2010). In general, more complex is the structure more energy is required to de-establish it (Fleming e Rumbaugh, 2017). Also, MAA can be established in response to diverse physicochemical stimuli. Here, we discuss the main points linking capsicumicine to MAA mechanism in *Staphylococcus epidermidis*, an emerging nosocomial pathogen.

Bacterial surface proteins are capable of passively interact with abiotic surfaces such as medical devices, generating an initial and reversible adhesion (*e.g.*, due to electrostatic and hydrophobic interactions, Van der Waals forces, hydrodynamic forces and others) (Speziale et al., 2014; Even et al., 2017; Armbruster e Parsek, 2018). Then, due to these weak interactions bacteria require a matrix production to remain attached (Otto, 2008; Otto, 2013). During this process, physicochemical interactions drive to matrix molecular and colloidal self-assembly, establishing a chain of dense architecture leading to a stable adhesion (Figure 6A) (Dorken et al., 2012; Schwarz-Linek et al., 2012; Stewart et al., 2015). On the other hand, capsicumicine interacts with the extracellular matrix and modifies the self-assembly chain, resulting in a less dense nonfunctional matrix that consequently prevents biofilm formation (Figure 6B).

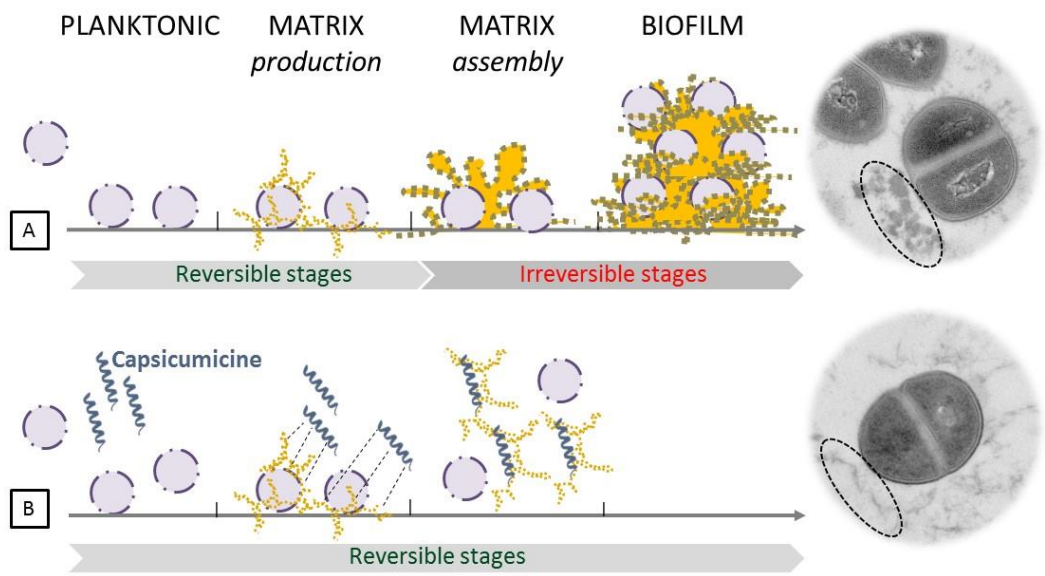

Figure 6. Capsicumicine antibiofilm mechanism of action: the matrix anti-assembly (MAA). The illustration highlights the formation and assembly (structuring) stages of the extracellular matrix on abiotic surface, in absence or presence of capsicumicine. A) Biofilm matrix development without capsicumicine: planktonic cells are able to passively interact with the surface and start the extracellular matrix production to stablish first adhesion. Then, matrix self-assembly take place leading to irreversible biofilm structuring and adhesion. The extracellular matrix is dense assembled and globular-like feature as shown in the TEM image (on the right). B) Biofilm matrix development in presence of capsicumicine (MAA): planktonic cells are still able to passively interact with the surface and start the extracellular matrix production to stablish first adhesion. At this time the capsicumicine acts as a magnet through physicochemical forces, attracting extracellular components such as the PIA and shifting the self-assembly. This anti assembly action lead to the disturbance of the matrix polymeric state. Thus, this disturb leads to the lost of adhesion and aggregation functionality and cells remain planktonic. The extracellular matrix displays now thin fibrillary oligomer structures, branched-like feature as shown in the TEM image (on the right). The arrow below the illustration containing the green writing indicates reversible stages and the writing in red, indicates irreversible steps.

The amino-acid sequence of capsicumicine peptide (RSCQQQIQQAQQLSSCQQYLKQ) relates it to antibiofilm peptides. Accordingly, it displays contents of arginine (R), lysine (K), alanine (A), leucine (L) and isoleucine (I), a linear chain, size and abiotic effect (Sharma et al., 2016; Von Borowski et al., 2017). The presence of several glutamine (Q) and cysteine (C) residues confers great helical stability (Thévenet et al., 2012; Shen et al., 2014; Lamiable et al., 2016) (Figure S3). Notably, capsicumicine exhibits good stability since its bioactivity remains over 24 hours under culture conditions. Moreover, capsicumicin displays homologies with all tested carbohydrate-binding-like domain (CBM) proteins (Table S2, Figure S2), possibly allowing capsicumicine to bind saccharides. CBM is a contiguous amino acid sequence with a discreet fold having carbohydrate-binding activity. Chitin is precursor of chitosan and has further protein data available to study, so we rely on it mostly. The chitin-binding domain (CBD) is a well conserved amino acid stretch found in plants, bacteria and fungi binding specifically to N-acetyl glucosamine, an homologous structure of PIA (Lombard *et al.*, 2014; Suginta *et al.*, 2016; El-Gebali *et*

al., 2019). Notably, icaA is a PIA-synthetase from the same strain used in this study. Furthermore, several residue characteristics to recognize putative CBD are found in capsicumicin such as the conservation of polar and hydrophobic residues (Suetake *et al.*, 2000; Hemmi *et al.*, 2003) and features conserved cysteine residues responsible for structural conformation. Still, conserved serine residue that stabilizes the interaction and an aromatic amino acid pocket responsible for sugar binding follows this serine residue (Wright *et al.*, 1991; Yin *et al.*, 2014). Thus, this set of CBM homology possibly allows capsicumicine function to saccharide-link.

The Capsicumicine matrix anti-assembly (MAA) mechanism of action

This mechanism is proposed based on a set of intermolecular and cooperative forces triggered by capsicumicine leading to the disturbance of matrix assembly.

Intermolecular forces

Staphylococcus matrix is mainly composed of polysaccharides (PIA/PNAG) but also of proteins (AtlE, Aap, Empb), teichoic acids and extracellular DNA (Rohde et al., 2010; Arciola et al., 2012; Paharik e Horswill, 2016). PIA is a homoglycan of beta-1,6-linked 2-amino-2-deoxy-D-glucopyranosyl residues, containing positive charged amino groups (PNAG, due to partial de-N-acetylation($GlcNH_3$)) as well as negative charges (resulting from O-succinylation). Thus, it confers electric charges lability to the matrix.

Capsicumicine has mostly neutral amino acids (13 glutamines, Q and 3 serines, S) capable to generate electronegative zones (dipole-dipole) due to the amide group of the Q and the hydroxyl group of S. This high electronegativity suggests that capsicumicine may interact with the positive free charges (Van der Waals forces) of the polysaccharides (PIA/PNAG) (Vuong et al., 2004; Otto, 2008). Capsicumicine may certainly exercise polar, non-ionic forces and perform suitable interactions with the matrix that alter its self-assembly. Whereas, strong interactions such as ionic forces may trigger unwanted effects (Sharma et al., 2016) such as repulsion or sequestration by the matrix (Vuong et al., 2004; Chan et al., 2005; Brancatisano et al., 2014; Batoni et al., 2016), capsicumicine "ideal interactions" represent a relevant approach.

Polymeric cooperative forces

In living systems, biomolecules perform their functions in the presence of various macromolecules with different shapes and sizes where polymeric cooperative physicochemical forces may act (Banani et al., 2017). These are noncovalent and non-specific interactions, dependently of size and shape such as *(i)* depletion forces (DF) and *(ii)* subsequent molecular crowding (MC). These forces lead to bridging, aggregation and rheological variation (Dorken et al., 2012; Kudlay et al., 2012; Even et al., 2017) such as we observe in the presence of capsicumicine (Figures 3 and 4) and may contribute to MAA.

(i) Depletion forces (DF)

In a suspension containing molecules of different sizes and shapes, DF is the pressure exerted for small particles that result in an attractive force between the macromolecules. DF is expressed only in crowded environments like biofilms, driving the assembly and final shape of these structures (Marenduzzo et al., 2006). Hence, in the presence of capsicumicine we notice prominent differences in matrix profiles both *in vivo* and *in vitro* (Figures 3 and 4). We suppose that capsicumicine associated

DF may coagulate and align multiple particles in a polymer solution, forming fibers and parallel bundles (Zhou, 2008; O'brien et al., 2011) justifying the observed brunched-like profile (Figure 3d-e) (Sakaguchi et al., 2018). As a result, capsicumicine probably alters the osmotic DF, facilitating matrix molecular self-assembly (Fantoni e Santos, 2014).

(ii) Molecular crowding (MC)

MC is characterized by the decrease in accessible volume owing to high macromolecule concentration (Aumiller et al., 2014; Banani et al., 2017). In this sense, capsicumicine improves polymerization kinetics (Figure 4) possibly acting as MC agent. It may decrease the reaction free energy leading to reduction of the entropic forces driving to segregation (Polson e Kerry, 2018). For example, a large study show different modulation influences by some molecular crowders (Dextran, Ficoll, PEG and human serum albumin) which have chemical similarities with capsicumicine such as hydrophilicity, linear open-chain and neutrality. They displayed enhanced cooperativity in the domain separation suggesting that the observed increase in refolding kinetics and decrease in competing aggregation pathways might be a direct outcome of such a phenomenon (Biswas et al., 2018).

Therefore, the set intermolecular and cooperative forces of capsicumicine probably perform their influences on biofilm environment disturbing the matrix self-assembly at molecular and colloidal levels. Consequently, it shifts matrix functionality leading to antibiofilm activity.

Biofilm genetic profile

Capsicumicine exposed bacteria display the same biomolecular profile (fold change) as untreated planktonic ones for all tested genes, indicating an antibiofilm extracellular mechanism of action. This demonstrates that capsicumicine activity is independent of cell death or bacterial interactions. Bacteria remain in their planktonic state without undergoing pressure for expression of biofilm formation factors and this is in accordance with the proposed MAA mechanism.

Importantly, non-antibiotic strategies suggest less susceptibility to the development of resistance phenomena than conventional antibiotics because microorganisms suffer a milder evolutionary pressure to generate resistance without the biotic activity (Rasko e Sperandio, 2010; Krachler e Orth, 2013; Travier et al., 2013).

Even more, capsicumicine has no cytotoxicity towards mammalian cells. This is certainly due to the intermolecular forces that do not play any affinity for eukaryotic membranes. This great selectivity is extremely relevant aiming its biological uses. In contrast, the applicability of some antibiofilm peptides such as AMPs is hindered by the absence of specificity, targeting eukaryotic cells and causing severe damages (Strempel et al., 2015; Forde et al., 2016).

Finally, this study proofs the applicability of the novel capsicumicine peptide as a strong antibiofilm agent, preventing biofilm establishment and maintenance. Significantly, capsicumicine decreases *S. epidermidis* adhesion and aggregation. Especially, we evidence its innovative and promising matrix anti-assembly mechanism of action, less susceptible to the development of bacteria resistance. Therefore, non-bactericidal peptides are encouraging to find new applications as antivirulence therapy and biomaterials coating, alone or in combination with other drugs to fight against bacteria resistance and tolerance concern (Mandava et al., 2012; Krachler e Orth, 2013; Wright, 2016).

Materials and Methods

Peptides. All peptides were synthesized by Biomatik™ and ProteoGenix™ both containing a purity grade greater than 95% in salt suitable for cell culture. Mass spectral and HPLC analysis were provided as quality control. They were all solubilized in ultra-pure sterile water for the assays.

Bacterial Strain and growth conditions. *Staphylococcus epidermidis* ATCC 35984 was grown overnight on blood agar (Thermo Scientific, Oxoid PB5039A) at 37°C. A bacterial suspension of 3×10^8 colony-forming units (CFU)/mL in tryptone soya broth (TSB, Oxoid Ltd., England, UK) or 0.9% NaCl was used in the assays. Lysogenic broth (LB, Oxoid Ltd., England, UK) agar was used to colony forming units (CFU) assay.

Biofilm formation. All assays were at least performed as technical and biological triplicates using 1, 10 or 100µM of peptides. *Biofilm inhibition:* a protocol adapted from Zimmer and collaborators (2013) Trentin et al.(2015) employing crystal violet in 96-well poly(vinyl chloride) microtiter plates (Falcon; Becton Dickinson Labware, Oxnard, CA) was used. Briefly, 100µL of the bacterial suspension, 100µL of the peptide solution (at different concentrations) or vehicle (to controls) and 50µL of tryptone soya broth (TSB, Oxoid Ltd., England). Following 37°C for 1, 4 or 24h of incubation, the content of the wells was removed and the wells were washed three times with sterile saline. The remaining contents were heat-fixed at 60°C for 1h. The adherent biofilm layer formed was stained with 0.4% crystal violet for 15min at room temperature and then washed three times with distilled water. The stain bound to the cells was solubilized with absolute ethanol (Sigma–Aldrich Co., USA) and absorbance was measured at 570nm (Powerwave™ XS Plate Reader, BIO-TEK instruments®, Inc.). The biofilm formation controls represent 100% of biofilm formation. *Biofilm eradication:* biofilm was pre-formed as described before, during 24h at 37°C, without treatment. After biofilm formation, the wells were washed to remove the planktonic cells and the peptides solutions and controls were added and incubated for 24h. The eradication was verified by evaluating the remaining content by crystal violet as previous described.

Bacterial growth assays. *Microtiter plates:* bacterial growth was evaluated by difference between the optical density absorbance at 600nm measured at the end and the beginning of the incubation time (37°C, 1, 4 or 24h) in 96-well poly(vinyl chloride) microtiter plates. Rifampicin 16µg/mL (Sigma–Aldrich Co., USA) was used as a control for bacterial growth inhibition. *Colony-forming units (CFU/mL):* after incubation (37°C, 24h) the CFU was calculated to determine bactericidal effect of peptide solution. Untreated growth control was considered 100% of planktonic cells. All assays were at least performed as technical and biological triplicates.

Microscopic analysis. *S. epidermidis* ATCC 35984 biofilm was cultured as previous described.

Scanning electron microscopy (SEM): sterile polystyrene coupons (10 X 4mm) were co-culture in presence or absence of capsicumicine for 1, 4 and 24 hours. After, the coupons were washed with sterile NaCl 0.9% and fixed with glutaraldehyde 2.5%, paraformaldehyde 2%, cacodylate 0,1M buffer (pH 7.2). Afterwards, they were washed with cacodylate 0,1M buffer with sucrose 0.2 M and dehydrated with increasing concentrations of ethanol and dehydrated samples were then subjected to Critical Point Drying (Leica EM CPD 300). Finally, they were sputtered with palladium (Leica EM ACE 200) and analyzed by JEOL JSM 7100 F EDS EBSD Oxford microscope, at 10 kV.

Transmission electron microscopy (TEM): all the content of the wells was suitable detached (1, 4 and 24h cultures in presence or absence of capsicumicine), recovered, centrifuged at 10,000 g, 15min, 4°C and washed with sterile NaCl 0.9%. Fixation was performed at 4°C with sodium cacodylate 0.1M, paraformaldehyde 2%, glutaraldehyde 2.5% and lysine 75mM. After that, samples were washed with sodium cacodylate 0.1M, sucrose 0.2M and contrasted with osmium tetroxide 1%, potassium ferrocyanide 1.5%. Dehydration was done with gradual solution of ethanol and infiltration with increasing concentration of LR White® resin (Delta Microscopies). Then, LR White® resin inclusion and polymerization were made during 24h at 60°C in O_2 absence. Thin sections (80nm) were collected onto 200 mesh carbon grids, and visualized with a Tecnai Sphera operating at 200kV (FEI, Eindhoven, Netherlands) equipped with a 4x4 k CCD UltraScan camera (Gatan, Pleasanton, USA).

Confocal fluorescence microscopy (CFM): Capsicumicine-fluorescein isothiocyanate (capsicumicine - FITC, 10µM) was used to detect capsicumicine peptide. After incubation (1, 4 and 24h) all the content of the wells was suitable detached, recovered, centrifuged at 11,000g, 2 min, 4°C and washed with sterile NaCl 0.9%. This suspension was visualized directly or after Calcofluor 2mg/mL (Fluorescent Brightener 28, Sigma) addition. To illustrate bacterial cells permeable by a peptide (control) we used an antimicrobial peptide also labeled with FITC (Schneider, R. et al; 2019). Those antimicrobial peptides are seen on/in bacteria although not in the extracellular matrix. Images were acquired with Leica SP8 DMI 6000 CS (resonant scanner) confocal microscope with hybrid detector. ImageJ software was used for image analysis.

Quantitative Reverse Transcriptase PCR (qRT-PCR): the RNAs were isolated from planktonic cells (control), biofilm cells (control) or total cells (exposed to capsicumicine at 10µM), after 4, 24h cultures. It was applied TRIzol™ Max™ Bacterial RNA Isolation Kit (Invitrogen™) and TURBO™ DNase treatment (Ambion®) according to manufacturer's instructions. Concentration and purity of total RNA was spectrophotometrically assessed using SimpliNano™ (Biochrom, USA) and PCR reaction was performed to certify the complete absence of DNA. It was considered 50% of yield for Reverse Transcriptase Reaction (M-MLV, Promega®): 1000ng of RNA = 500ng cDNA. Then, we used 10ng of cDNA and 0.2µM of primers per qRT-PCR reaction, previously verified. Reactional volumes were calculated according to the manufacturer's instructions (SYBR® Select Master Mix, Applied Biosystems Inc; USA). Primers (Table S1) were designed through the Primer3 program (Thermo Fisher® Primers) and according to literature. They were produced by Eurofins Genomic. It was used Applied Biosystems StepOnePlus™ equipment and software. The relative transcript levels were determined by $2^{-\Delta\Delta ct}$ (Livak e Schmittgen, 2001). To validate the selected biofilm encoding genes we compared planktonic control to biofilm control. We found purposeful differences between biofilm and planktonic controls, as expected.

Real time molecular self-assembly (RTMSA) assay: after checking the starting point (pH=7.2) of the assembly reaction for *staphylococcal* synthetic matrix (Stewart et al., 2015), we recorded the optical density (OD = 600nm) in function of the time, each 30sec until 30min. Molecular self-assembly reactions were calculated to a final volume of 4mL, considering 0.3% chitosan (medium molecular weight, 75-85% of deacetylation, Sigma), 0.15% bovine serum albumin (BSA, Sigma), 0.015% lambda DNA (Sigma) in tryptone soya broth (TSB, Oxoid Ltd., England, UK). The concentration of tested peptides was calculated in µM to the final volume of 4mL (100µM). Before get the pH starting point of assembly reaction, a calibration record was done using the same reactional tube containing all reagents (auto zero). The pH adjustments were made using acetic acid and NaOH and the reaction temperature was around 30°C.

Cytotoxicity assay: the assays were performed in a robotic platform (ImPACcell, BIOSIT, Université de Rennes 1) dedicated to multiparameter high-throughput image analysis (HCS: High Content Screening and HCA: High Content Analysis), using 7 different mammalian lines: HuH7, CaCo-2, MDA, HCT116, PC3, NCI-H727 and MCF7. The number of normal cells is presented as residual cells percentage (%) compared to the average of DMSO control. Whereas, 100% represent no cytotoxicity or inhibition of cell growth, below 25/30% is considered cytotoxic and 0% represents acute cytotoxicity. This platform are equipped with Olympus right microscope (Spot NB camera and Simple PCI software, Compix), Right Zeiss AxioImager M1 microscope (Marzhauser, Zeiss NB camera and AxioVision software) and the robots Arrayscan VTI Cellomics /Thermofisher, Hamilton Starlet, Hamilton Nimbus and Spotter Scienion.

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References:

ABAD, C. L.; HALEEM, A. Prosthetic Joint Infections: an Update. **Curr Infect Dis Rep,** v. 20, n. 7, p. 15, May 2018. ISSN 1523-3847. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/29789958 >.

ARCIOLA, C. R. et al. Biofilm formation in Staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. **Biomaterials,** v. 33, n. 26, p. 5967-5982, Sep 2012. ISSN 0142-9612. Disponível em: < <Go to ISI>://WOS:000306720400001 >.

ARMBRUSTER, C. R.; PARSEK, M. R. New insight into the early stages of biofilm formation. **Proc Natl Acad Sci U S A,** v. 115, n. 17, p. 4317-4319, 04 2018. ISSN 1091-6490. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/29632199 >.

AUMILLER, W. M.; DAVIS, B. W.; KEATING, C. D. Phase separation as a possible means of nuclear compartmentalization. **Int Rev Cell Mol Biol,** v. 307, p. 109-49, 2014. ISSN 1937-6448. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/24380594 >.

BANANI, S. F. et al. Biomolecular condensates: organizers of cellular biochemistry. **Nat Rev Mol Cell Biol,** v. 18, n. 5, p. 285-298, 05 2017. ISSN 1471-0080. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28225081 >.

BATONI, G.; MAISETTA, G.; ESIN, S. Antimicrobial peptides and their interaction with biofilms of medically relevant bacteria. **Biochimica Et Biophysica Acta-Biomembranes,** v. 1858, n. 5, p. 1044-1060, May 2016. ISSN 0005-2736. Disponível em: < <Go to ISI>://WOS:000374603600015 >.

BELOIN, C. et al. Novel approaches to combat bacterial biofilms. **Curr Opin Pharmacol,** v. 18C, p. 61-68, Sep 2014. ISSN 1471-4973. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25254624 >.

BISWAS, S. et al. Mixed Macromolecular Crowding: A Protein and Solvent Perspective. **ACS Omega,** v. 3, n. 4, p. 4316-4330, Apr 2018. ISSN 2470-1343. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/30023892 >.

BRANCATISANO, F. L. et al. Inhibitory effect of the human liver-derived antimicrobial peptide hepcidin 20 on biofilms of polysaccharide intercellular adhesin (PIA)-positive and PIA-negative strains of Staphylococcus epidermidis. **Biofouling,** v. 30, n. 4, p. 435-46, 2014. ISSN 1029-2454. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/24645694 >.

BRAUNER, A. et al. Distinguishing between resistance, tolerance and persistence to antibiotic treatment. **Nat Rev Microbiol,** v. 14, n. 5, p. 320-30, 04 2016. ISSN 1740-1534. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/27080241 >.

CDCP. Center for Disease Control and Prevention. Vital signs: central line-associated blood stream infections -United States, 2001, 2008, and 2009. **MMWR Morb Mortal Wkly Rep,** v. 60, n. 8, p. 243-8, Mar 2011. ISSN 1545-861X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/21368740 >.

CERI, H. et al. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. **J Clin Microbiol,** v. 37, n. 6, p. 1771-6, Jun 1999. ISSN 0095-1137. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/10325322 >.

CHAN, C.; BURROWS, L. L.; DEBER, C. M. Alginate as an auxiliary bacterial membrane: binding of membrane-active peptides by polysaccharides. **J Pept Res,** v. 65, n. 3, p. 343-51, Mar 2005. ISSN 1397-002X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/15787964 >.

CONIBEAR, T. C.; COLLINS, S. L.; WEBB, J. S. Role of mutation in Pseudomonas aeruginosa biofilm development. **PLoS One,** v. 4, n. 7, p. e6289, Jul 2009. ISSN 1932-6203. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/19606212 >.

DAVIES, D. Understanding biofilm resistance to antibacterial agents. **Nat Rev Drug Discov,** v. 2, n. 2, p. 114-22, Feb 2003. ISSN 1474-1776. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12563302 >.

DE LA FUENTE-NUNEZ, C. et al. D-Enantiomeric Peptides that Eradicate Wild-Type and Multidrug-Resistant Biofilms and Protect against Lethal Pseudomonas aeruginosa Infections (vol 22, pg 196, 2015). **Chemistry & Biology,** v. 22, n. 9, p. 1280-1282, Sep 17 2015. ISSN 1074-5521. Disponível em: < <Go to ISI>://WOS:000364012100013 >.

DEFRAINE, V.; FAUVART, M.; MICHIELS, J. Fighting bacterial persistence: Current and emerging anti-persister strategies and therapeutics. **Drug Resist Updat,** v. 38, p. 12-26, 05 2018. ISSN 1532-2084. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/29857815 >.

DORKEN, G. et al. Aggregation by depletion attraction in cultures of bacteria producing exopolysaccharide. **J R Soc Interface,** v. 9, n. 77, p. 3490-502, Dec 2012. ISSN 1742-5662. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/22896568 >.

EVEN, C. et al. Recent advances in studying single bacteria and biofilm mechanics. **Adv Colloid Interface Sci,** v. 247, p. 573-588, Sep 2017. ISSN 1873-3727. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28754382 >.

FANTONI, R.; SANTOS, A. Depletion force in the infinite-dilution limit in a solvent of nonadditive hard spheres. **J Chem Phys,** v. 140, n. 24, p. 244513, Jun 2014. ISSN 1089-7690. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/24985660 >.

FEUILLIE, C. et al. Molecular interactions and inhibition of the staphylococcal biofilm-forming protein SdrC. **Proc Natl Acad Sci U S A,** v. 114, n. 14, p. 3738-3743, 04 2017. ISSN 1091-6490. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28320940 >.

FEY, P. D.; OLSON, M. E. Current concepts in biofilm formation of Staphylococcus epidermidis. **Future Microbiol,** v. 5, n. 6, p. 917-33, Jun 2010. ISSN 1746-0921. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/20521936 >.

FLAMM, R. K. et al. Linezolid Surveillance Results for the United States (LEADER Surveillance Program 2014). **Antimicrob Agents Chemother,** v. 60, n. 4, p. 2273-80, Apr 2016. ISSN 1098-6596. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/26833165 >.

FLEMING, D.; RUMBAUGH, K. P. Approaches to Dispersing Medical Biofilms. **Microorganisms,** v. 5, n. 2, Apr 2017. ISSN 2076-2607. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28368320 >.

FLEMMING, H.-C.; WINGENDER, J. The biofilm matrix. **Nature Reviews Microbiology,** v. 8, n. 9, p. 623-633, Sep 2010. ISSN 1740-1526. Disponível em: < <Go to ISI>://WOS:000280855500009 >.

FORDE, É. et al. Differential In Vitro and In Vivo Toxicities of Antimicrobial Peptide Prodrugs for Potential Use in Cystic Fibrosis. **Antimicrob Agents Chemother,** v. 60, n. 5, p. 2813-21, 05 2016. ISSN 1098-6596. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/26902766 >.

GOMES VON BOROWSKI, R. et al. Promising Antibiofilm Activity of Peptidomimetics. **Front Microbiol,** v. 9, p. 2157, 2018. ISSN 1664-302X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/30271394 >.

GRASSI, L. et al. Combination Strategies to Enhance the Efficacy of Antimicrobial Peptides against Bacterial Biofilms. **Front Microbiol,** v. 8, p. 2409, 2017. ISSN 1664-302X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/29375486 >.

HOIBY, N. et al. Antibiotic resistance of bacterial biofilms. **International Journal of Antimicrobial Agents,** v. 35, n. 4, p. 322-332, Apr 2010. ISSN 0924-8579. Disponível em: < <Go to ISI>://WOS:000274869800003 >.

HOPE, R. et al. Non-susceptibility trends among staphylococci from bacteraemias in the UK and Ireland, 2001-06. **J Antimicrob Chemother,** v. 62 Suppl 2, p. ii65-74, Nov 2008. ISSN 1460-2091. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/18819981 >.

JABBOURI, S.; SADOVSKAYA, I. Characteristics of the biofilm matrix and its role as a possible target for the detection and eradication of Staphylococcus epidermidis associated with medical implant infections. **FEMS Immunol Med Microbiol,** v. 59, n. 3, p. 280-91, Aug 2010. ISSN 1574-695X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/20528930 >.

KRACHLER, A. M.; ORTH, K. Targeting the bacteria-host interface: strategies in anti-adhesion therapy. **Virulence,** v. 4, n. 4, p. 284-94, May 2013. ISSN 2150-5608. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/23799663 >.

KUDLAY, A.; CHEUNG, M. S.; THIRUMALAI, D. Influence of the shape of crowding particles on the structural transitions in a polymer. **J Phys Chem B,** v. 116, n. 29, p. 8513-22, Jul 2012. ISSN 1520-5207. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/22616622 >.

LALANI, T. et al. Prosthetic valve endocarditis due to coagulase-negative staphylococci: findings from the International Collaboration on Endocarditis Merged Database. **Eur J Clin Microbiol Infect Dis,** v.

25, n. 6, p. 365-8, Jun 2006. ISSN 0934-9723. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/16767483 >.

LAMIABLE, A. et al. PEP-FOLD3: faster de novo structure prediction for linear peptides in solution and in complex. **Nucleic Acids Res,** v. 44, n. W1, p. W449-54, Jul 2016. ISSN 1362-4962. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/27131374 >.

LAZARIS, A. et al. Novel multiresistance cfr plasmids in linezolid-resistant methicillin-resistant Staphylococcus epidermidis and vancomycin-resistant Enterococcus faecium (VRE) from a hospital outbreak: co-location of cfr and optrA in VRE. **J Antimicrob Chemother,** v. 72, n. 12, p. 3252-3257, Dec 2017. ISSN 1460-2091. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28961986 >.

LE, K. Y.; OTTO, M. Quorum-sensing regulation in staphylococci-an overview. **Front Microbiol,** v. 6, p. 1174, 2015. ISSN 1664-302X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/26579084 >.

LEE, J. Y. H. et al. Global spread of three multidrug-resistant lineages of Staphylococcus epidermidis. **Nat Microbiol**, Sep 2018. ISSN 2058-5276. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/30177740 >.

LEVIN-REISMAN, I. et al. Antibiotic tolerance facilitates the evolution of resistance. **Science,** v. 355, n. 6327, p. 826-830, 02 2017. ISSN 1095-9203. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28183996 >.

LEWIS, K. Riddle of biofilm resistance. **Antimicrob Agents Chemother,** v. 45, n. 4, p. 999-1007, Apr 2001. ISSN 0066-4804. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/11257008 >.

LIU, P. et al. Genetic Selection of Peptide Aptamers That Interact and Inhibit Both Small Protein B and Alternative Ribosome-Rescue Factor A of Aeromonas veronii C4. **Front Microbiol,** v. 7, p. 1228, 2016. ISSN 1664-302X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/27588015 >.

_____. Targeting Inhibition of SmpB by Peptide Aptamer Attenuates the Virulence to Protect Zebrafish against. **Front Microbiol,** v. 8, p. 1766, 2017. ISSN 1664-302X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28955325 >.

LIVAK, K. J.; SCHMITTGEN, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. **Methods,** v. 25, n. 4, p. 402-8, Dec 2001. ISSN 1046-2023. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/11846609 >.

MAKI, D. G.; KLUGER, D. M.; CRNICH, C. J. The risk of bloodstream infection in adults with different intravascular devices: a systematic review of 200 published prospective studies. **Mayo Clin Proc,** v. 81, n. 9, p. 1159-71, Sep 2006. ISSN 0025-6196. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/16970212 >.

MANDAVA, S. H. et al. Infection retardant coated inflatable penile prostheses decrease the incidence of infection: a systematic review and meta-analysis. **J Urol,** v. 188, n. 5, p. 1855-60, Nov 2012. ISSN 1527-3792. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/22999690 >.

MARENDUZZO, D.; FINAN, K.; COOK, P. R. The depletion attraction: an underappreciated force driving cellular organization. **J Cell Biol,** v. 175, n. 5, p. 681-6, Dec 2006. ISSN 0021-9525. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/17145959 >.

NEELY, A. N.; MALEY, M. P. Survival of enterococci and staphylococci on hospital fabrics and plastic. **J Clin Microbiol,** v. 38, n. 2, p. 724-6, Feb 2000. ISSN 0095-1137. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/10655374 >.

NISHIZAKI, Y. et al. Japanese features of native valve endocarditis caused by coagulase-negative staphylococci: case reports and a literature review. **Intern Med,** v. 52, n. 5, p. 567-72, 2013. ISSN 1349-7235. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/23448766 >.

O'BRIEN, E. P. et al. Influence of Nanoparticle Size and Shape on Oligomer Formation of an Amyloidogenic Peptide. **J Phys Chem Lett,** v. 2, n. 10, p. 1171-1177, May 2011. ISSN 1948-7185. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/21691423 >.

OTTO, M. Staphylococcal biofilms. **Bacterial Biofilms,** v. 322, p. 207-228, 2008 2008. ISSN 0070-217X. Disponível em: < <Go to ISI>://WOS:000258523900010 >.

_____. Staphylococcal Infections: Mechanisms of Biofilm Maturation and Detachment as Critical Determinants of Pathogenicity. **Annual Review of Medicine, Vol 64,** v. 64, p. 175-188, 2013 2013. ISSN 0066-4219. Disponível em: < <Go to ISI>://WOS:000316384400013 >.

_____. Physical stress and bacterial colonization. **FEMS Microbiol Rev,** v. 38, n. 6, p. 1250-70, Nov 2014. ISSN 1574-6976. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25212723 >.

PAHARIK, A. E.; HORSWILL, A. R. The Staphylococcal Biofilm: Adhesins, Regulation, and Host Response. **Microbiol Spectr,** v. 4, n. 2, 04 2016. ISSN 2165-0497. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/27227309 >.

PENESYAN, A.; GILLINGS, M.; PAULSEN, I. T. Antibiotic discovery: combatting bacterial resistance in cells and in biofilm communities. **Molecules,** v. 20, n. 4, p. 5286-98, Mar 2015. ISSN 1420-3049. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/25812150 >.

POLSON, J. M.; KERRY, D. R. Segregation of polymers under cylindrical confinement: effects of polymer topology and crowding. **Soft Matter,** v. 14, n. 30, p. 6360-6373, Aug 2018. ISSN 1744-6848. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/30028460 >.

RASKO, D. A.; SPERANDIO, V. Anti-virulence strategies to combat bacteria-mediated disease. **Nat Rev Drug Discov,** v. 9, n. 2, p. 117-28, Feb 2010. ISSN 1474-1784. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/20081869 >.

ROGERS, K. L.; FEY, P. D.; RUPP, M. E. Coagulase-negative staphylococcal infections. **Infect Dis Clin North Am,** v. 23, n. 1, p. 73-98, Mar 2009. ISSN 1557-9824. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/19135917 >.

ROHDE, H. et al. Structure, function and contribution of polysaccharide intercellular adhesin (PIA) to Staphylococcus epidermidis biofilm formation and pathogenesis of biomaterial-associated infections. **Eur J Cell Biol,** v. 89, n. 1, p. 103-11, Jan 2010. ISSN 1618-1298. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/19913940 >.

ROY, R. et al. Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. **Virulence,** v. 9, n. 1, p. 522-554, 01 2018. ISSN 2150-5608. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28362216 >.

RUPP, M. E. Clinical characteristics of infections in humans due to Staphylococcus epidermidis. **Methods Mol Biol,** v. 1106, p. 1-16, 2014. ISSN 1940-6029. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/24222451 >.

SAKAGUCHI, A.; HIGASHIGUCHI, K.; MATSUDA, K. Bundle formation of supramolecular fibers of amphiphilic diarylethene by depletion force. **Chem Commun (Camb),** v. 54, n. 34, p. 4298-4301, Apr 2018. ISSN 1364-548X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/29632919 >.

SCHWARZ-LINEK, J. et al. Phase separation and rotor self-assembly in active particle suspensions. **Proc Natl Acad Sci U S A,** v. 109, n. 11, p. 4052-7, Mar 2012. ISSN 1091-6490. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/22392986 >.

SHARMA, A. et al. dPABBs: A Novel in silico Approach for Predicting and Designing Anti-biofilm Peptides. **Sci Rep,** v. 6, p. 21839, Feb 2016. ISSN 2045-2322. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/26912180 >.

SHEN, Y. et al. Improved PEP-FOLD Approach for Peptide and Miniprotein Structure Prediction. **J Chem Theory Comput,** v. 10, n. 10, p. 4745-58, Oct 2014. ISSN 1549-9626. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/26588162 >.

SIERADZKI, K. et al. Heterogeneously vancomycin-resistant Staphylococcus epidermidis strain causing recurrent peritonitis in a dialysis patient during vancomycin therapy. **J Clin Microbiol,** v. 37, n. 1, p. 39-44, Jan 1999. ISSN 0095-1137. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/9854061 >.

SILVA, L. N. E. A. Plant Natural Products Targeting Bacterial Virulence Factors. ***Chemical Reviews*****,** v. 116.16, p. 9162-9236, July 20, 2016 2016.

SPEZIALE, P. et al. Protein-based biofilm matrices in Staphylococci. **Front Cell Infect Microbiol,** v. 4, p. 171, 2014. ISSN 2235-2988. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/25540773 >.

STEWART, E. J. et al. Artificial biofilms establish the role of matrix interactions in staphylococcal biofilm assembly and disassembly. **Sci Rep,** v. 5, p. 13081, Aug 2015. ISSN 2045-2322. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/26272750 >.

STEWART, P. S. Prospects for Anti-Biofilm Pharmaceuticals. **Pharmaceuticals (Basel),** v. 8, n. 3, p. 504-11, Aug 2015. ISSN 1424-8247. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/26343685 >.

STREIT, J. M. et al. Assessment of pathogen occurrences and resistance profiles among infected patients in the intensive care unit: report from the SENTRY Antimicrobial Surveillance Program (North America, 2001). **Int J Antimicrob Agents,** v. 24, n. 2, p. 111-8, Aug 2004. ISSN 0924-8579. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/15288308 >.

STREMPEL, N.; STREHMEL, J.; OVERHAGE, J. Potential Application of Antimicrobial Peptides in the Treatment of Bacterial Biofilm Infections. **Current Pharmaceutical Design,** v. 21, n. 1, p. 67-84, 2015 2015. ISSN 1381-6128. Disponível em: < <Go to ISI>://WOS:000345434600008 >.

TAGLIALEGNA, A. et al. Staphylococcal Bap Proteins Build Amyloid Scaffold Biofilm Matrices in Response to Environmental Signals. **PLoS Pathog,** v. 12, n. 6, p. e1005711, 06 2016. ISSN 1553-7374. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/27327765 >.

TETERYCZ, D. et al. Outcome of orthopedic implant infections due to different staphylococci. **Int J Infect Dis,** v. 14, n. 10, p. e913-8, Oct 2010. ISSN 1878-3511. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/20729115 >.

THÉVENET, P. et al. PEP-FOLD: an updated de novo structure prediction server for both linear and disulfide bonded cyclic peptides. **Nucleic Acids Res,** v. 40, n. Web Server issue, p. W288-93, Jul 2012. ISSN 1362-4962. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/22581768 >.

TRAVIER, L. et al. Escherichia coli Resistance to Nonbiocidal Antibiofilm Polysaccharides Is Rare and Mediated by Multiple Mutations Leading to Surface Physicochemical Modifications. **Antimicrobial Agents and Chemotherapy,** v. 57, n. 8, p. 3960-3968, Aug 2013. ISSN 0066-4804. Disponível em: < <Go to ISI>://WOS:000321761800064 >.

UÇKAY, I. et al. Meticillin resistance in orthopaedic coagulase-negative staphylococcal infections. **J Hosp Infect,** v. 79, n. 3, p. 248-53, Nov 2011. ISSN 1532-2939. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/21955452 >.

VON BOROWSKI, R. G.; MACEDO, A. J.; GNOATTO, S. C. B. Peptides as a strategy against biofilm-forming microorganisms: Structure-activity relationship perspectives. **Eur J Pharm Sci,** v. 114, p. 114-137, Nov 2017. ISSN 1879-0720. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/29133243 >.

VUONG, C. et al. Polysaccharide intercellular adhesin (PIA) protects Staphylococcus epidermidis against major components of the human innate immune system. **Cell Microbiol,** v. 6, n. 3, p. 269-75, Mar 2004. ISSN 1462-5814. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/14764110 >.

WHO; ORGANIZATION, W. H. **WHO: Global action plan on antimicrobial resistance** 2015.

_____. **WHO: Antibiotic resistance** 2018.

WRIGHT, G. D. Antibiotic Adjuvants: Rescuing Antibiotics from Resistance. **Trends in Microbiology,** v. 24, n. 11, p. 862-871, Nov 2016. ISSN 0966-842X. Disponível em: < <Go to ISI>://WOS:000386644800004 >.

ZHOU, H. X. Effect of mixed macromolecular crowding agents on protein folding. **Proteins,** v. 72, n. 4, p. 1109-13, Sep 2008. ISSN 1097-0134. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/18506780 >.

Supplementary Material:

Table S1. Primers used in this study. They were previously designed through the Primer3 program (Thermo Fisher® Primers) and standardized by our team.

Gene	Forward primer 5'-3'	Reverse primer 5'-3'	Amplicon
atlE	TACCAGGGTTTGCAGGATTC	GGCGCTAAATTCATTGGAAA	85pb
aap	AGGCCGTACCAACAGTGAAT	ATGGGCAAACGTAGACAAGG	100pb
agrC	TCATCAATATCGCATTCATCG	CCTAAACCGCGATTATCACC	136pb
icaA	TTATCAATGCCGCAGTTGTC	CCGTTGGATATTGCCTCTGT	104pb
leuA	GATGATCTCGGAATGGCAGT	TGAGGCATTTCCTGCTCTTT	108pb
saeR	GCTAACACTGTCAATGTCCACA	AGGCCCCACACAGTTGTAAT	92pb
saeS	GGCGTCAATTTGTTGTGCTA	AGGGCATAGGTATCGTTCCA	140pb
sarA	TTTGCTTCTGTGATACGGTTGT	CGTAATGAACACGATGAAAGAACT	107pb
gyrB	ATCAACATCGGCATCAGTCA	GCATTTGGTACGGGTATTGG	87pb
rrsA	AAGCAACGCGAAGAACCTTA	ATGCACCACCTGTCACTCTG	95pb

Table S2. Carbohydrate-binding-domain proteins homologs to capsicumicine. This proteins are available in UniProtKB database and were used to blast and amino acids alignment for similarity analysis.

Entry name	Accession n°.
ICAA_STAEQ Intercellular adhesion protein icaA	Q5HKQ0.1
A0A3A0IKV7_STAEP Poly-beta-1,6 N-acetyl-D-glucosamine synthase	A0A3A0IKV7
ICAD_STAEQ Poly-beta-1,6 N-acetyl-D-glucosamine synthase prot. icaD	Q5HKP9
A7Z8H9_BACVZ Chitosanase	A7Z8H9
A0A0N0MLT5_9ACTN Chitosanase	A0A0N0MLT5
A0A0U5QL95_STAEP Chitosanase	A0A0U5QL95
CBP2_MOROL Chitin-binding protein 2	C0HKC5
A0A1R0GTZ5_9FUNG Chitin-synthase 8	A0A1R0GTZ5
A0A194V113_9PEZI Chitin biosynthesis protein CHS5	A0A194V113

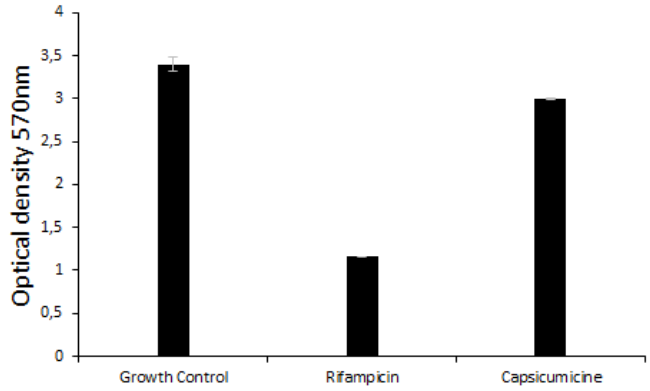

Figure S1. Biofilm eradication test. Black bars correspond to *Staphylococcus epidermidis* (ATCC 35984) biofilm quantification (OD = 570 nm) after 24h. Capsicumicine was tested at 100 µM. "Growth Control", correspond to bacteria without peptide exposition and "Rifampicin" correspond to antibiotic control rifampicin.

Figure S2. Capsicumicine and carbohydrate-binding-domain proteins homologs amino acids alignment. a) It shows the icaA fragment from 1 to 60 (protein accession number Q5HKQ0.1). b) It shows capsicumicine fragment from 1 to 21. c) It shows the chitosanase fragment from 1 to 60 and 61 to 120 (protein accession number A7Z8H9). d) It shows capsicumicine fragment from 1 to 15 and 16 to 22. e) It shows the chitin-synthase 8 fragment from 1801 to 1860 and 1921 to 1967 (protein accession number A0A1R0GTZ5). f) It shows capsicumicine fragment from 1 to 14 and 16 to 22. (*) means equal content, (.) indicates amino acid similarity and (:) high amino acid similarity. The violet color designates a polar characteristic of the amino acids. This analysis is supported and available at UniProt database.

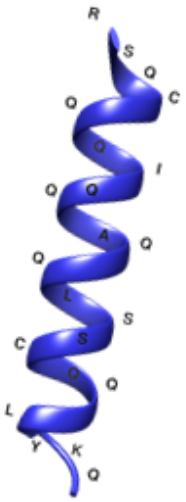

Figure S3. Structural prediction of capsicumicine. This peptide shows a high probability of presenting helical conformation with a small-unorganized terminal portion. Its structure was predicted using computational framework PEP-FOLD3. PEP-FOLD3 is available at http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3.

5. Chapter 3. International patent

All the international patent content is under responsibility of Sociétés d'Accélération du Transfert de Technologies (SATT), Rennes metropole in France. European Patent Registration nº EP19305205.7.

6. Chapter 4. Perspectives : article 3

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Promising Antibiofilm Activity of Peptidomimetics

Rafael Gomes Von Borowski [1,2], Simone Cristina Baggio Gnoatto [2], Alexandre José Macedo [2] and Reynald Gillet [1]**

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Pathogenic biofilms are a global health care concern, as they can cause extensive antibiotic resistance, morbidity, mortality, and thereby substantial economic loss. Scientific efforts have been made over the past few decades, but so far there is no effective treatment targeting the bacteria in biofilms. Antimicrobial peptidomimetics have been proposed as promising potential anti-biofilm agents. Indeed, these structurally enhanced molecules can mimic the action of peptides but are not susceptible to proteolysis or immunogenicity, the characteristic limitations of natural peptides. Here, we provide insights into antibiofilm peptidomimetic strategies and molecular targets, and discuss the design of two major peptidomimetics classes: AApeptides (*N*-acylated-*N*-aminoethyl-substituted peptides) and peptoids (*N*-substituted glycine units). In particular, we present details of their structural diversity and discuss the possible improvements that can be implemented in order to develop antibiofilm drug alternatives.

Keywords: antibiotic resistance, biofilm, peptides, peptidomimetics, AApeptides, peptoids

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INTRODUCTION

The increased resistance of biofilms to antibiotics is a global health care problem (Costerton et al., 1999; Hall and Mah, 2017). Biofilms are well-organized microbial clusters which produce a matrix from a series of compounds that include extracellular DNA (eDNA), proteins, and polysaccharides. These compounds are either attached to a surface (when originating on medical devices or teeth) or are suspended (in mucus or in chronic wounds) (Flemming and Wingender, 2010). Their form confers advantages over planktonic cells to the matrix-enclosed microorganisms, including improved biocide tolerance, host immune defense, and persistence. These advantages are caused by vast physiological and biochemical changes, including slow cell growth, beneficial quorum sensing, and higher mutation rates (Davies, 2003). Indeed, chronic bacterial infections are themselves encouraged by the accumulation of bacteria in the biofilm-producing biopolymer matrix. Since they are embedded into the matrix, these bacteria have an increased tolerance to antibiotics, chemical disinfectants, and/or host defenses, and are much harder to treat than infections without biofilm (Høiby et al., 2010; Beloin et al., 2014).

The most relevant clinical biofilm-forming bacteria are the gram-negative *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa,* along with gram-positive *Staphylococcus aureus* and the less virulent *S. epidermidis* (Jabbouri and Sadovskaya, 2010; de la Fuente-Núñez et al., 2013; Chen et al., 2014; Culotti and Packman, 2014; Longo et al., 2014; Andrea et al., 2018). These microorganisms can form biofilms on virtually any medical device, including cardiac pacemakers and prosthetic heart valves, endotracheal tubes, urinary catheters,

central venous catheters, prostheses, orthopedic devices, contact lenses, and dentures (Baquero and Coque, 2011). This ability is possible due the broad genetic variability of the microbial populations found in health care institutions. This genetic spectrum, also implying phenotypic variations, occurs within the same species. This makes it difficult to develop a therapy or even a general surface material that could deter the growth and adhesion of these microorganisms (Cegelski et al., 2008). Medical devices are an important cause of human infections, for instance turning *S. epidermidis* into an important emerging pathogen responsible for most infections in central venous catheters. This results in the need to remove and replace the medical device, increasing costs and patient suffering (Maki et al., 2006). Not only do bacteria have the individual capacity to form biofilm, but in biofilm some strains will have increase their horizontal transfers of plasmids carrying antibiotic resistance genes, thus increasing mutation frequency (Savage et al., 2013). For all of these reasons, pathogenic biofilms have a huge clinical impact in terms of economic losses, morbidity, and mortality.

Therefore, bacterial biofilms are promising targets for combatting this problem of antibiotic resistance. The successful development of antibiofilm compounds will therefore be an important tool for controlling human infections (Miquel et al., 2016).

In this context, peptides have been proposed as an important direction to follow, either for creating alternative drug therapies or for developing new anti-infective surfaces (Riool et al., 2017).

Peptides are fundamental molecules made up of 2–50 amino acids, with many biological functions. Indeed, their versatile chemical features such as malleability and multifunctionality, make them good models for the synthesis of new bioactive compounds (Von Borowski et al., 2017). Antimicrobial peptides (AMPs) are very interesting molecules to be explored in the search for antibiofilm agents to replace conventional antibiotics. This is because they are relatively easy to produce while exhibiting broad-spectrum antimicrobial activity, with a distinct mode of action that means that they are less prone to developing resistance (de la Fuente-Núñez et al., 2013; Strempel et al., 2015; Andrea et al., 2018). However, although natural peptides are indispensable for the structure, functioning, and metabolism of each living organism, their regulation is mediated by molecular interactions, proteolysis, and immunogenic responses (Avan et al., 2014). Thus low stability and availability limits their therapeutic relevance. On the other hand, peptidomimetics are chemically modified expressly to limit the drawbacks of natural peptides. The underlying strategy is to create small peptide-like molecules that still have the inherent abilities of natural ones (so that the advantageous biological effects remain), but which are more stable and available, with improved selectivity and/or potency (Grauer and König, 2009; Croft and Purcell, 2011). In this context, rather than joining the amino acids to a bioisosteric group to mimic the original amide, a very efficient chemical strategy is to replace the peptide bond, the -CO-NH- amide (Niu et al., 2013). Although quite a number of amide bond replacements have been reported, our review focuses here on the *N*-acylated-*N*-aminoethyl amino acids (AApeptides) and on peptoids. These specific strategies were chosen as they each have pronounced chemical diversity, can mimic both the primary and secondary structures of peptides, resist proteolysis, and show good activity against pathogenic biofilms. By presenting these promising candidates, we pave the way for the design of more active and safer innovative molecules.

PEPTIDOMIMETICS ARE AN IMPROVEMENT OVER NATURAL PEPTIDES

Despite their inherent robust and promising bioactivity, there are drawbacks to the use of natural peptides, including their high clearance and their susceptibility to proteolysis or immunogenicity, both of which can cause unwanted effects (Von Borowski et al., 2017). Inspired by natural peptides, chemists have developed a variety of structurally diverse synthetic mimics with key physicochemical natures (*i.e.*, cationic charges and amphiphilicity) which they call *peptidomimetics*.

These molecules can be obtained in different ways. Peptidomimetics can be made by manipulating the amino acid backbone of native peptides in order to enrich structural diversity, making them extraordinarily useful. They can also be prepared through the coupling of stable unnatural amino acids generated via modifications such as amine alkylation. For example, poly-N-substituted glycines allow for the generation of peptoids that differ from peptides only in their side chains, making them protease-resistant (Miller et al., 1995). Another strategy is the isosteric replacement of the amino group by for example an oxygen or sulfur atom. This changes the H-bonding pattern, significantly affecting the secondary structure and folding properties of peptides. Another possible approach for getting peptidomimetics that have new secondary structures and biological activities involves taking natural peptides and performing C-α configuration inversion, α-hydrogen replacement (by the alkyl or other groups), and replacing the α-carbon atom by heteroatom, mostly nitrogen (Avan et al., 2014). Several of these strategies have been used to synthesize peptidomimetics which appear to be as promising against biofilms as naturally occurring AMPs. In fact, many synthetic antibacterial peptidomimetics are currently undergoing clinical trials, including the membrane-disrupting compound LTX-109, the cationic steroid compound CSA-13, and the novel peptidomimetic brilacidin (see https://clinicaltrials.gov/). Recently, short peptidomimetics made of Arg and N-alkyl/aryl pyrazole residues were shown to have good antimicrobial and anti-inflammatory activities, increased proteolytic stability against trypsin digestion, and antimicrobial activity, even in the presence of physiological salts (Ahn et al., 2017). Another series of AMP mimetics were synthesized by incorporating a 3′-amino-[1,1′-biphenyl]-3-carboxylic acid backbone in MSI-78, a peptide currently in Phase-III clinical trials (Kuppusamy et al., 2018).

AApeptides as a Peptidomimetic Strategy

AApeptides are oligomers of *N*-acylated-*N*-aminoethyl-substituted amino acids that are derived from chiral peptide

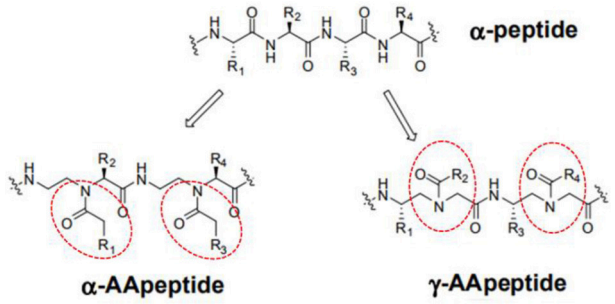


FIGURE 1 | Structure illustration of an α-peptide and its corresponding AApeptide models, adapted from Niu et al. (2013). The α- and γ-*N*-acylated-*N*-aminoethyl amino acid amide bond replacement structures are identified by dotted circles in red. R corresponds to possible radicals.

nucleic acid (PNA) backbones (Shi et al., 2016). The chiral side chain is connected to either the α-C or γ-C of the carbonyl group, while acylation is used to introduce the other side chain to the central N, as illustrated in **Figure 1** (Sang et al., 2017). Compared to their original peptide counterparts, AApeptides have the same backbone lengths and functional group counts, and the same number of nitrogen atoms involved in secondary or tertiary amide bonds. In addition, they mimic the original amino acid side-chain positions, so they have the same activity. However, their backbones are more flexible, and since the AApeptides have tertiary amide bonds that can be involved in cis/trans configurations, they should have interesting hydrogen bonding properties and conformational flexibilities (Niu et al., 2013).

Antibiofilm AApeptides

In a recent study, Teng et al. (2016) described an acyclic model based on host defense peptides (HDPs) with charged and non-charged radicals (**Table 1**, ID 1). The global structure has a cationic hydrophobic residue composed of ornithine, and adamantyl or aromatic rings. This model was used to produce a global amphiphilic AApeptide that targets membrane disruption for antibiotic activity. The molecule displays high Gram-negative antifouling activity and low cytotoxicity effects. In addition, it was hypothesized that if radical 1 (R1) was a cationic group, and R2, R3, and R4 were hydrophobic, the global structure should be both an HDP and amphipathic, which will result in the killing of bacteria via membrane disruption. Accordingly, the hydrophobicities of R2, R3, and R4 were modified by inserting various groups (such as adamantyl, biphenyl, CF3, t-butyl) into the aromatic rings, then testing the resulting compounds against clinically relevant bacteria. A reduction in R2–R4 hydrophobicity correlates to decreased molecule killing capacities.

In order to verify the selectivity of the compounds, cationic residues such as lysine, ornithine, and arginine were added, and the hemolysis profiles assessed. Lysine decreases hemolytic and antibacterial activities, ornithine increases them, and arginine has no effect. Often amphipathic agents are cytotoxic, but at a concentration of 25 μg/mL, the compounds did not show noticeable cytotoxicity against either the HK-2 renal epithelial cell line or the K562 human erythroleukemic one. **Table 1** details the most promising AApeptide, "Compound 13," which was tested for antibiofilm activity at concentrations below the minimal inhibitory concentration (MIC). At this level, a 50–75% reduction of biofilm formation was seen via crystal violet staining in both *E. coli* (ATCC 25922) and *A. baumannii*.

In another study, Padhee et al. (2015) assessed the biofilm antifouling and eradication activities of peptidomimetics based on the structures of daptomycin and polymyxin B. The main compound, YL-36, is a cyclic γ-AApeptide having both charged and neutral radicals (**Table 1**, ID 2). YL-36 was designed by joining lipid tails from amphiphilic building blocks with the cyclic rings, with ornithine as cationic residues. Lipo-cyclic structures turn out to have a broader-spectrum of antimicrobial activity than the others, and they work against inflammation by suppressing pro-inflammatory cytokines. With YL-36, 70–80% biofilm antifouling activity was observed with both *P. aeruginosa* and Methicillin-resistant *Staphylococcus epidermidis* (MRSE), although these results are inconclusive since the concentrations tested were over the MIC. In any case, the potential for antibiofilm activity could be due to the presence of lipid tails which can retard biofilm formation. The structures that are globally amphipathic probably line up to form micelles when there is an interaction with the polyanionic exopolysaccharide matrix, ultimately disrupting it. Note that YL-36 is not hemolytic, meaning that this compound is highly selective.

Peptoids as a Peptidomimetic Strategy

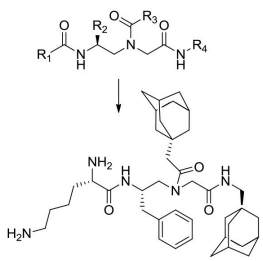
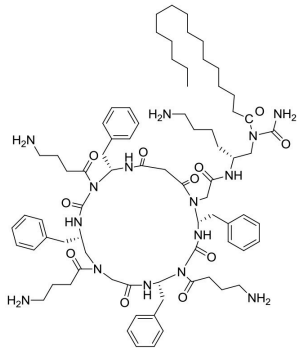
Peptoids are oligomers of *N*-substituted glycine units (**Figure 2**). Their side chains extend from the main-chain nitrogen rather than from the α-carbon, thus yielding secondary structures including helices, loops, and turns. They are achiral foldamer molecules, and retain the functionalities and backbone polarity of peptides (Yoo and Kirshenbaum, 2008; Zuckermann and Kodadek, 2009; Mándity and Fülöp, 2015).

Antibiofilm Peptoids

Hoque et al. (2015) explored a series of small acyclic amphiphilic peptoids based on AMP structures. They did this by inserting two non-amino acid positive charges, two lipophilic alkyl moieties, and two non-peptidic amide groups (**Table 1**, ID 3). They demonstrated that antimicrobial activity and hemolytic action correlate to the lipophilic alkyl chain/spacer and increases in chain length, which changes selectivity. The most promising molecule, "Compound 2d" (**Table 2**, ID 3), shows optimum amphiphilicity, and is able to disperse both *S. aureus* and *E. coli* mature biofilms at the solid-liquid and liquid-air interfaces, with complete eradication at 32 μg/mL even while biofilm is already formed on the cover slips. In addition, 2d also decreases bacterial viability inside the biofilm, whereas the cell viability of non-treated biofilm increases. Although the study made no mention of an antibiofilm structure-activity relationship (SAR), 2d was non-toxic to human erythrocytes and human kidney cells.

Kapoor et al. (2011) selected several peptoid analogs to an acyclic amphipathic and cationic dodecamer peptoid (**Table 2**, ID 4) based on AMP structures. The alkylated peptoids were

TABLE 1 | Summary of chemical and biological information on AApeptides.

	AA peptides				Concentration* (μg/mL)/action range (%)		Mechanism*				References
ID	Peptidomimetic	Chemical structure	Molecular weight (*M*)	Model*	Antifouling	Eradication	Biofilm	Antimicrobial	MIC (μg/mL)	Cytotoxicity (μg/mL)	
1	Acyclic model and the main compound 13	 Compound 13 ($C_{40}H_{62}N_5O_3$) R1, cationic and R2,3,4 hydrophobic	660.4855	*Escherichia coli* (ATCC 25922), *Acinetobacter baumannii*	0.6–2/50–~75		Not shown	Membrane disruption	3.12 (*E.coli*)	(Hemolysis) 85; (HK-2) 86; (K562) 83	Teng et al., 2016
	SAR: antibacterial activity was enhanced by increasing R4's hydrophobicity. Antibacterial and hemolytic activities were decreased through the introduction of cationic charges (*K*) at R1. No SAR or other correlation to antibiofilm activity was shown.										
2	The main compound **YL-36**	 Lipid tails added outside the cyclic rings through an amphipathic ϒ-AApeptide building block	Not shown	*Pseudomonas aeruginosa* and Methicillin resistant *Staphylococcus epidermidis*	<5–50/40–80 (YL-36: 6.25–12.56/70–80)	<5–50/10–70 (YL-36: <5)	Surfactant-like (micelles)	Membrane disruption	1- >25 (YL-36: 1–5)	(Hemolysis) 100–250 (YL-36: 100)	Padhee et al., 2015
	SAR: Cyclization reduces structure motility and facilitates bacterial membrane disruption, while lipidation encourages their interactions with membranes. Lipid tails may retard the growth of biofilms and form cationic micelles upon interaction with the matrix.										

*Columns: compound ID for the purposes of this paper; peptidomimetic, molecule, or chemical class mimicked by the AApeptide, chemical structure, and molecular weight of the main compound in the studied model; concentration and percentage of action range tested for the antifouling and/or eradication model; antibiofilm and antimicrobial mechanisms of action; minimal inhibitory concentration (MIC); and cytotoxicity. A brief structure activity relationship (SAR) is presented below each AApeptide. *Based on antibiofilm evaluation.*

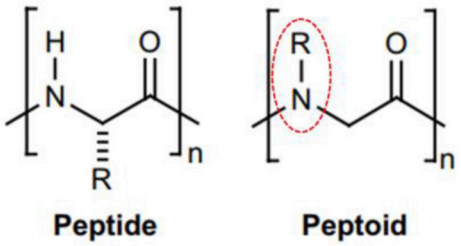


FIGURE 2 | Peptide and peptoid monomer structures differ. (**Left)** Illustration of a classic glycine peptide unit, which has a chiral carbon linked to amino, carboxyl, and radical groups. **(Right)** An *N*-substituted R = H for glycine amino acids residues. This has a radical group linked to the amino group instead of the chiral carbon, identified by dotted circles in red.

active against planktonic cells, while the unalkylated ones were not. The 1-C13$_{4mer}$ peptoid (H-*N*tridec-*N*Lys-*N*spe-*N*spe-*N*Lys-NH_2) is the main compound, preventing about 70% of biomass formation in *P. aeruginosa*. In addition, peptoids 1 and 1-C13$_{4mer}$ impaired preformed biofilms by 60 and 40%, and reduced cell viability by about 1.5 and 3 logs, respectively. Antibiofilm activity against *P. aeruginosa* was measured at 12.5 μg/mL (MIC) and the biomass was assessed with crystal violet staining. Still, the authors discuss the possibility of using peptoids to bind DNA and facilitate biofilm detachment and/or disruption. Indeed, their activity could be due to their inherent oligomerization via aromatic side-chain interactions. The hydrophobic tails might bestow their efficiency in reducing cell viability, as these confer a surfactant-like nature which causes micelles to form. Micelles may strongly interact with and disrupt the hydrophobic exopolysaccharide matrix, facilitating deeper peptoid penetration into the matrix.

Konai and Haldar (2015) went in the opposite direction. They started with spermidine and norspermidine polyamine structures, both known to have antibiofilm properties. Looking for significant antibacterial activity at minimum biofilm inhibitory concentrations (MBICs), they synthesized a series of acyclic amphipathic conjugate molecules (**Table 2**, ID 5). These contained a cationic moiety made of various fatty acids acting as lipophilic tails and the amino acid lysine (L-lysine and D-lysine), a known trigger for biofilm disassembly. Crystal violet staining, confocal imaging, and killing curve determination showed that "Compound 8" reduces *S. aureus* viability in preformed biofilm in a concentration-dependent manner. However, the best MBIC value (116 μM) was found in derivatives "4" (L,L configuration, $R = C_{15}H_{31}$) and "9" (D,D configuration, $R = C_{15}H_{31}$). The mechanism of biofilm disruption is still being investigated, but improved electrostatic and hydrogen bonding interactions with the biofilm's extracellular matrix components may play a major role. The compounds were non-toxic to erythrocytes.

Yang Liu et al. (2013) previously described a synthetic approach to designing acyclic oligomers based on AMP structures with alternating repeats of α-amino acids and β-peptoid residues. The representative compounds for two subclasses are shown in **Table 2** (ID 6). These have chain lengths of 4–16 residues, and longer chain lengths often correlated with increased antimicrobial activity within the subclass. This tendency was more pronounced in the lysine-containing groups (1 and 4) than in the homoarginine-rich ones (2 and 3). The hybrid oligomers also inhibited *S. epidermidis* biofilm formation and displayed antibiofilm activity against preformed *S. epidermidis* biofilm. In comparison with their lysine-containing counterparts (such as 1d and 4c), the fully guanidinylated (e.g., hArg-rich) oligomers (e.g., "2b") killed slow-growing cells faster, and had more antibiofilm capacity. Chirality appears to be essential for the efficient killing of both slow-growing planktonic cells and biofilms in all the studied oligomers. They were not toxic to erythrocytes, but are toxic to HeLa cells in a concentration-dependent manner. To keep cytotoxicity at acceptable levels, a promising strategy may be to design alternating oligomers that display only a 1:1 ratio of amino or guanidino/amino functional groups.

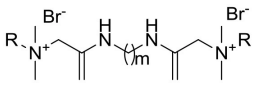
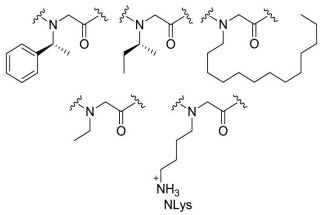
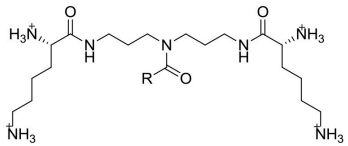
Finally, since the antibiotic bioactivity that has been explored is usually due to membrane disruption, cationic molecules and bacterial membrane structure-activity relationships have been thoroughly investigated, and amphiphilic molecules probably act in the same way since they act as partial cationics. Moreover, the evaluation of cytotoxicity levels shows that both peptidomimetics and their expected amphiphilicities have good potentials. Finally, both AApeptides and peptoids have been shown to act as effective antibiofilm agents, although their bioactivity and selectivity depend on optimal amphiphilicity. Therefore, we highlight that acyclic conformation and lipid tails, neutral aromatic compounds, and ornithine substituents should be the most advantageous peptidomimetic structural improvements in order to obtain antibiofilm molecules.

CONCLUSION

In microorganisms, biofilm lifestyle is a significant virulence factor that results in enhanced resistance to medical treatment (Otto, 2014). This means that antibiotics are less effective, and clearly biofilms have a considerable clinical impact (Del Pozo, 2018). Methods for combatting biofilms using natural peptides seem promising, but their therapeutic relevance is limited by inherently low stability and availability (Kang et al., 2014). Therefore, antibiofilm peptidomimetics are being studied as way to mimic natural peptides while avoiding their drawbacks (Mizuno et al., 2017).

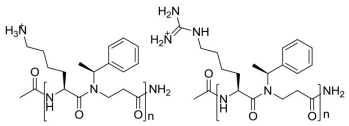
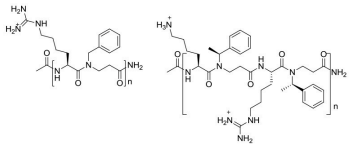
In short, the active structures we discuss tend to mimic naturally occurring antimicrobial molecules such as host defense peptides (HDPs) and antimicrobial peptides (AMPs). These are both endogenous polypeptides produced by multicellular organisms, and they act as an evolutionarily conserved mechanism of innate immune defense (Huang et al., 2014). Thousands of these peptides have been identified in bacteria, plants, insects, birds, fish, and mammals. Family members are highly diverse in their sequences, but generally of small size, made up of 12–50 amino acids. They have similar overall cationic charges of +2 to +9, and are amphipathic,

TABLE 2 | Summary of chemical and biological information for peptoids.

	Peptoids				Concentration* (μg/mL) / action range (%)		Mechanism*				References
ID	Peptidomimetic	Chemical structure	Molecular weight (M)	Model*	Antifouling	Eradication	Biofilm	Antimicrobial	MIC (μg/mL)	Cytotoxicity (μg/mL)	
3	Acyclic model and the main compound **2d**	 (Compound **2d**: $m = 6$ and $R = C_8H_{17}$) Two positive charges, two lipophilic moieties, and two non-peptidic amide groups	Not shown	*Staphylococcus aureus* and *Escherichia coli*		4–64 ∼100 (*S. aureus*)	Not shown	Membrane disruption	1.9 (*E. coli*) and 3.9 (*S. aureus*)	(Hemolysis) 780; (HEK293) 220	Hoque et al., 2015
	SAR: Varying the nature of the lipophilic alkyl chain and spacer chain length emphasizes the role of optimum amphiphilicity in the development of non-toxic yet potent membrane-active antibacterials.										
4	Submonomers structures of 1, 1-11_{mer}, 1-Pro_9, 1-achiral, **1-C13**$_{4mer}$, 1_{4mer}, 1-*N*ssb	 Peptoid submonomers: Alkylated and unalkylated analogs of an amphipathic and cationic dodecamer peptoid	Not shown	*Pseudomonas aeruginosa* (PA14)	<5–100 μM/40–70	<5–100 μM/40–60	eDNA, cell-cell detachment/surfactant-like (micelles)	Not shown	12.5- >100 μM	Not shown	Kapoor et al., 2011
	SAR: Peptoids can bind extracellular (eDNA) and may facilitate detachment or disruption of otherwise-stable biofilm structures. Oligomerization via interactions with aromatic side chains would increase the concentration of peptoids near the cell membrane, increasing peptoid activity and perhaps also contributing to biofilm detachment. The hydrophobic tail confers a surfactant-like nature that may aide in micelle formation, which could interact with and disrupt the hydrophobic matrix.										
5	Lysine–norspermidine conjugates model	 Configuration L,L and $R = C_9H_{29}$ (**1**), $C_{11}H_{23}$ (**2**), $C_{13}H_{27}$ (**3**), $C_{15}H_{31}$ (**4**), $C_{17}H_{35}$ (**5**), $C_{17}H_{33}$ (**6**), $C_{17}H_{31}$ (**7**) Configuration D,D and $R = C_{13}H_{27}$ (**8**), $C_{15}H_{31}$ (**9**) Structures of lipophilic lysine–norspermidine conjugates with trifluoroacetate counterions	Not shown	*Staphylococcus aureus* (MTCC 737)		116–1000 μM/> 80	Electrostatic and hydrogen bonding interactions with the matrix components of the biofilm	Membrane disruption	6	(Hemolysis) 730	Konai and Haldar, 2015

(Continued)

TABLE 2 | Continued

ID	Peptoids: Peptidomimetic	Chemical structure	Molecular weight (M)	Model*	Concentration* (μg/mL) / action range (%): Antifouling	Concentration* (μg/mL) / action range (%): Eradication	Mechanism*: Biofilm	Mechanism*: Antimicrobial	MIC (μg/mL)	Cytotoxicity (μg/mL)	References
	SAR: D-amino acids such as D-Tyr, D-Leu, D-Trp, and D-Met were also shown to be natural triggers for biofilm disassembly, although none of these possessed significant antibacterial activity. The introduction of four positive charges and hydrogen bond-forming units into a norspermidine backbone would yield greater electrostatic and hydrogen-bonding interactions with the matrix components. In addition, the lipophilic moiety should enhance interaction with the bacterial membrane.										
6	β-peptoid–peptide hybrid oligomers (i.e., 1a–3d) and the mixed amino/guanidino subtype (i.e., 4a–4d)	 **1 2** 1a ($n = 5$), 1b ($n = 6$), 1c ($n = 7$), 1d ($n = 8$) 2a ($n = 5$), 2b ($n = 6$), 2c ($n = 7$), 2d ($n = 8$)  **3 4** 3a ($n = 5$), 3b ($n = 6$), 3c ($n = 7$), 3d ($n = 8$) 4a ($n = 1$), 4b ($n = 2$), 4c ($n = 3$), 4d ($n = 4$) Incorporation of chiral hydrophobic β-peptoids and guanidinylated amino acid side chains while keeping the length relatively short	935–3734 (2b: 2815.84)	Methicillin resistant *Staphylococcus epidermidis* RP62A (ATCC 35984)	1–16 (2b: 4)/40–100 (2b: 100)	8–16 (2b: 8)/∼85 (2b: ∼85)	Multi mechanisms	Bactericidal	1–4	(Hemolysis) > 500; (HeLa) 46- > 1000 (2b: > 500; 90)	Liu et al., 2013
	SAR: Longer chain length was correlated with increased antimicrobial activity. This tendency was more pronounced in the lysine-containing subclasses (1 and 4) than in the homoarginine-rich ones (2 and 3). A design based on alternating oligomers with only amino or guanidino/amino functional groups in a 1:1 ratio may be a promising strategy to keep cytotoxicity at an acceptable level. Still, some guanidino side chain content is required for antibiofilm activity and chirality appears to be essential for efficient killing planktonic cells.										

*Columns: compound ID for the purposes of this paper; peptidomimetic, molecule or chemical class mimicked by the peptoids, chemical structure and molecular weight of the main compound in the studied model; concentration and percentage of action range tested for the antifouling and/or eradication model; antibiofilm and antimicrobial mechanisms of action; minimal inhibitory concentration (MIC); and cytotoxicity. A brief structure activity relationship (SAR) is presented below each peptoid. *Based on antibiofilm evaluation.*

with over 50% hydrophobic residues (Kindrachuk and Napper, 2010). Moreover, it is known that AMPs show broad-spectrum antimicrobial activities and have a low propensity for developing resistance (Seo et al., 2012; Lázár et al., 2018). Peptidomimetics are the same, even though they are all designed to be amphiphilic instead of cationic like AMPs. To confer the desired amphiphilicity, the molecules are linked to different substituents, which are charged and non-charged radicals. The most relevant of those are lipid tails, neutral aromatic compounds, and charged ornithine amino acids.

Although there is an incomplete understanding of the mechanisms of action of antibiofilm peptidomimetics, it seems that their amphiphilicity improves their hydrogen and electrostatic interactions with matrix components, such as those with surfactants.

In the past 40 years, more than 30,000 articles have been published about microbial biofilms (source: PubMed database). This mass of research has been dedicated to understanding biofilms dynamics and to decreasing its effects, but so far no effective treatment has been developed (Bjarnsholt et al., 2013). Antibiofilm AApeptides and peptoids are two very promising families of peptidomimetics for the development and refining of new antibiofilm agents to be used in the fight against resistant microorganisms.

AUTHOR CONTRIBUTIONS

RG wrote the manuscript with support from SG, AM, and RGB. All authors provided critical feedback and helped shape the manuscript.

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REFERENCES

Ahn, M., Gunasekaran, P., Rajasekaran, G., Kim, E. Y., Lee, S. J., and Bang, G. (2017). Pyrazole derived ultra-short antimicrobial peptidomimetics with potent anti-biofilm activity. *Eur. J. Med. Chem.* 125, 551–564. doi: 10.1016/j.ejmech.2016.09.071

Andrea, A., Molchanova, N., and Jenssen, H. (2018). Antibiofilm peptides and peptidomimetics with focus on surface immobilization. *Biomolecules* 8:E27. doi: 10.3390/biom8020027

Avan, I., Hall, C. D., and Katritzky, A. R. (2014). Peptidomimetics via modifications of amino acids and peptide bonds. *Chem. Soc. Rev.* 43, 3575–3594. doi: 10.1039/c3cs60384a

Baquero, F., and Coque, T. M. (2011). Multilevel population genetics in antibiotic resistance. *FEMS Microbiol. Rev.* 35, 705–706. doi: 10.1111/j.1574-6976.2011.00293.x

Beloin, C., Renard, S., Ghigo, J. M., and Lebeaux, D. (2014). Novel approaches to combat bacterial biofilms. *Curr. Opin. Pharmacol.* 18, 61–68. doi: 10.1016/j.coph.2014.09.005

Bjarnsholt, T., Ciofu, O., Molin, S., Givskov, M., and Høiby, N. (2013). Applying insights from biofilm biology to drug development - can a new approach be developed? *Nat Rev. Drug Discov.* 12, 791–808. doi: 10.1038/nrd4000

Cegelski, L., Marshall, G. R., Eldridge, G. R., and Hultgren, S. J. (2008). The biology and future prospects of antivirulence therapies. *Nat. Rev. Microbiol.* 6, 17–27. doi: 10.1038/nrmicro1818

Chen, P., Seth, A. K., Abercrombie, J. J., Mustoe, T. A., and Leung, K. P. (2014). Activity of imipenem against Klebsiella pneumoniae biofilms *in vitro* and *in vivo*. *Antimicrob. Agents Chemother.* 58, 1208–1213. doi: 10.1128/AAC.01353-13

Costerton, J. W., Stewart, P. S., and Greenberg, E. P. (1999). Bacterial biofilms: a common cause of persistent infections. *Science* 284, 1318–1322. doi: 10.1126/science.284.5418.1318

Croft, N. P., and Purcell, A. W. (2011). Peptidomimetics: modifying peptides in the pursuit of better vaccines. *Expert Rev. Vaccines* 10, 211–226. doi: 10.1586/erv.10.161

Culotti, A., and Packman, A. I. (2014). *Pseudomonas aeruginosa* promotes *Escherichia coli* biofilm formation in nutrient-limited medium. *PLoS ONE* 9:e107186. doi: 10.1371/journal.pone.0107186

Davies, D. (2003). Understanding biofilm resistance to antibacterial agents. *Nat. Rev. Drug Discov.* 2, 114–122. doi: 10.1038/nrd1008

de la Fuente-Núñez, C., Reffuveille, F., Fernández, L., and Hancock, R. E. (2013). Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. *Curr. Opin. Microbiol.* 16, 580–589. doi: 10.1016/j.mib.2013.06.013

Del Pozo, J. L. (2018). Biofilm-related disease. *Expert Rev. Anti Infect. Ther.* 16, 51–65. doi: 10.1080/14787210.2018.1417036

Flemming, H.-C., and Wingender, J. (2010). The biofilm matrix. *Nat. Rev. Microbiol.* 8, 623–633. doi: 10.1038/nrmicro2415

Grauer, A., and König, B. (2009). Peptidomimetics – a versatile route to biologically active compounds. *Eur. J. Org. Chem.* 30, 5099–5111. doi: 10.1002/ejoc.200900599

Hall, C. W., and Mah, T. F. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiol. Rev.* 41, 276–301. doi: 10.1093/femsre/fux010

Høiby, N., Bjarnsholt, T., Givskov, M., Molin, S., and Ciofu, O. (2010). Antibiotic resistance of bacterial biofilms. *Int. J. Antimicrob. Agents* 35, 322–332. doi: 10.1016/j.ijantimicag.2009.12.011

Hoque, J., Konai, M. M., Samaddar, S., Gonuguntala, S., Manjunath, G. B., and Ghosh, C., et al. (2015). Selective and broad spectrum amphiphilic small molecules to combat bacterial resistance and eradicate biofilms. *Chem. Commun.* 51, 13670–13673. doi: 10.1039/C5CC05159B

Huang, W., Seo, J., Willingham, S. B., Czyzewski, A. M., Gonzalgo, M. L., and Weissman, I. L. (2014). Learning from host-defense peptides: cationic, amphipathic peptoids with potent anticancer activity. *PLoS ONE* 9:e90397. doi: 10.1371/journal.pone.0090397

Jabbouri, S., and Sadovskaya, I. (2010). Characteristics of the biofilm matrix and its role as a possible target for the detection and eradication of *Staphylococcus epidermidis* associated with medical implant infections. *FEMS Immunol. Med. Microbiol.* 59, 280–291. doi: 10.1111/j.1574-695X.2010.00695.x

Kang, S. J., Park, S. J., Mishig-Ochir, T., and Lee, B. J. (2014). Antimicrobial peptides: therapeutic potentials. *Expert Rev. Anti Infect. Ther*. 12, 1477–1486. doi: 10.1586/14787210.2014.976613

Kapoor, R., Wadman, M. W., Dohm, M. T., Czyzewski, A. M., Spormann, A. M., and Barron, A. E. (2011). Antimicrobial peptoids are effective against *Pseudomonas aeruginosa* biofilms. *Antimicrob. Agents Chemother*. 55, 3054–3057. doi: 10.1128/AAC.01516-10

Kindrachuk, J., and Napper, S. (2010). Structure-activity relationships of multifunctional host defence peptides. *Mini Rev. Med. Chem.* 10, 596–614. doi: 10.2174/138955710791383983

Konai, M. M., and Haldar, J. (2015). Lysine-based small molecules that disrupt biofilms and kill both actively growing planktonic and nondividing stationary phase bacteria. *ACS Infect. Dis*. 1, 469–478. doi: 10.1021/acsinfecdis.5b00056

Kuppusamy, R., Yasir, M., Berry, T., Cranfield, C. G., Nizalapur, S., and Yee, E. (2018). Design and synthesis of short amphiphilic cationic peptidomimetics based on biphenyl backbone as antibacterial agents. *Eur. J. Med. Chem*. 143, 1702–1722. doi: 10.1016/j.ejmech.2017.10.066

Lázár, V., Martins, A., Spohn, R., Daruka, L., Grézal, G., and Fekete, G. (2018). Antibiotic-resistant bacteria show widespread collateral sensitivity to antimicrobial peptides. *Nat. Microbiol*. 3, 718–731. doi: 10.1038/s41564-018-0164-0

Liu, Y., Knapp, K. M., Yang, L., Molin, S., Franzyk, H., and Folkesson, A. (2013). High *in vitro* antimicrobial activity of beta-peptoid-peptide hybrid oligomers against planktonic and biofilm cultures of Staphylococcus epidermidis. *Int. J. Antimicrob. Agents* 41, 20–27. doi: 10.1016/j.ijantimicag.2012.09.014

Longo, F., Vuotto, C., and Donelli, G. (2014). Biofilm formation in *Acinetobacter baumannii*. *New Microbiol*. 37, 119–127.

Maki, D. G., Kluger, D. M., and Crnich, C. J. (2006). The risk of bloodstream infection in adults with different intravascular devices: a systematic review of 200 published prospective studies. *Mayo Clin. Proc*. 81, 1159–1171. doi: 10.4065/81.9.1159

Mándity, I. M., and Fülöp, F. (2015). An overview of peptide and peptoid foldamers in medicinal chemistry. *Expert Opin. Drug Discov*. 10, 1163–1177. doi: 10.1517/17460441.2015.1076790

Miller, S. M., Simon, R. J., Zuckermann, R. J., Kerr, J. M., Moos, W. H., et al. (1995). Comparison of the proteolytic susceptibilities of homologous L-amino acid, D-amino acid, and N-substituted glycine peptide and peptoid oligomers. *Drug Dev. Res*. 35, 20–32.

Miquel, S., Lagrafeuille, R., Souweine, B., and Forestier, C. (2016). Anti-biofilm activity as a health issue. *Front. Microbiol*. 7:592. doi: 10.3389/fmicb.2016.00592

Mizuno, A., Matsui, K., and Shuto, S. (2017). From peptides to peptidomimetics: a strategy based on the structural features of cyclopropane. *Chemistry* 23, 14394–14409. doi: 10.1002/chem.201702119

Niu, Y., Wu, H., Li, Y., Hu, Y., Padhee, S., and Li, Q., et al. (2013). AApeptides as a new class of antimicrobial agents. *Org. Biomol. Chem*. 11, 4283–4290. doi: 10.1039/c3ob40444g

Otto, M. (2014). Physical stress and bacterial colonization. *FEMS Microbiol Rev*. 38, 1250–1270. doi: 10.1111/1574-6976.12088

Padhee, S., Li, Y. Q., and Cai, J. F. (2015). Activity of lipo-cyclic gamma-AApeptides against biofilms of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. *Bioorg. Med. Chem. Lett*. 25, 2565–2569. doi: 10.1016/j.bmcl.2015.04.039

Riool, M., de Breij, A., Drijfhout, J. W., Nibbering, P. H., and Zaat, S. A. (2017). Antimicrobial peptides in biomedical device manufacturing. *Front. Chem*. 5:63. doi: 10.3389/fchem.2017.00063

Sang, P., Shi, Y., Teng, P., Cao, A., Xu, H., and Li, Q.,et al. (2017). Antimicrobial AApeptides. *Curr. Top. Med. Chem*. 17, 1266–1279. doi: 10.2174/1568026616666161018145945

Savage, V. J., Chopra, I., and O'neill, A. J. (2013). *Staphylococcus aureus* biofilms promote horizontal transfer of antibiotic resistance. *Antimicrob. Agents Chemother*. 57, 1968–1970. doi: 10.1128/AAC.02008-12

Seo, M. D., Won, H. S., Kim, J. H., Mishig-Ochir, T., and Lee, B. J.,et al. (2012). Antimicrobial peptides for therapeutic applications. *Rev. Mol*. 17, 12276–12286. doi: 10.3390/molecules171012276

Shi, Y., Teng, P., Sang, P., She, F., Wei, L., and Cai, J. (2016). gamma-AApeptides: design, structure, and applications. *Acc. Chem. Res*. 49, 428–441. doi: 10.1021/acs.accounts.5b00492

Strempel, N., Strehmel, J., and Overhage, J. (2015). Potential application of antimicrobial peptides in the treatment of bacterial biofilm infections. *Curr. Pharma. Design* 21, 67–84. doi: 10.2174/1381612820666140905124312

Teng, P., Huo, D., Nimmagadda, A., Wu, J., She, F., and Su, M. (2016). Small antimicrobial agents based on acylated reduced amide scaffold. *J. Med. Chem*. 59, 7877–7887. doi: 10.1021/acs.jmedchem.6b00640

Von Borowski, R. G., Macedo, A. J., and Gnoatto, S. C. B. (2017). Peptides as a strategy against biofilm-forming microorganisms: Structure-activity relationship perspectives. *Eur. J. Pharm. Sci*. 114, 114–137. doi: 10.1016/j.ejps.2017.11.008

Yoo, B., and Kirshenbaum, K. (2008). Peptoid architectures: elaboration, actuation, and application. *Curr. Opin. Chem. Biol*. 12, 714–721. doi: 10.1016/j.cbpa.2008.08.015

Zuckermann, R. N., and Kodadek, T. (2009). Peptoids as potential therapeutics. *Curr. Opin. Mol. Therap*. 11, 299–307.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6.6 Peptidomimetics design

The following molecules are proposed and based on experimental results of the lead peptide (RSCQQQIQQAQQLSSCQQYLKQ, named capsicumicine) as well as based, discussed and justify on the review article “Promising Antibiofilm Activity of Peptidomimetics” (Gomes Von Borowski *et al.*, 2018).

The synthesis and biological evaluation of these derivatives are in progress as part of the current CAPES-COFECUB project but are not part of the specific objectives of this work.

Peptide-peptoid (or *beta*-peptoid) hybrids:

1) RSCQQQIQQAQ**X**QLSSCQQYLKQ

X = Ornithine

X = *N*-Lipid tail-glycine 1a

X = *N*-Lipid tail-glycine 1b

X = *N*-Lipid tail-glycine 1c

X = *N*-Lipid tail-glycine 1d

X = *N*-Neutral aromatic compound -glycine 1e

X = *N*-Neutral aromatic compound -glycine 1f

X = *N*-Neutral aromatic compound -glycine 1g

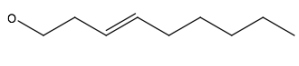
X = *N*-Acid aromatic compound -glycine 1h

X = *N*-Acid aromatic compound -glycine 1i

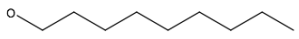
X = *N*-Acid aromatic compound -glycine 1j

Lipid tails :

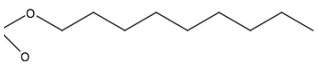
1a)



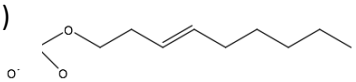
1b)



1c)

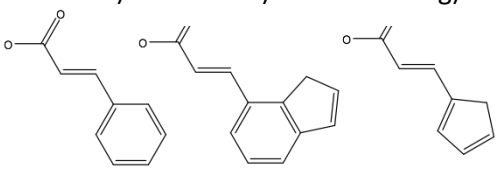


1d)



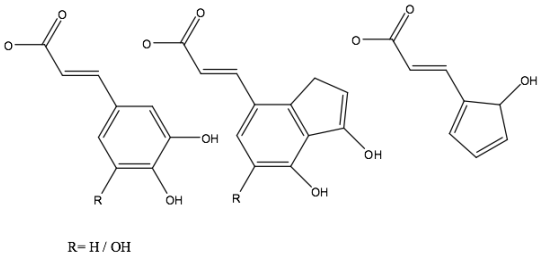
Neutral aromatic compounds :

1e) 1f) 1g)



Acid aromatic compounds :

1h) 1i) 1j)



R= H / OH

2) X_1RSCQQQIQQAQX_2QLSSCQQYLKQX_3

X1 = Ornithine, or *N*-Lipid tail-glycine or *N*-Neutral aromatic compound -glycine or *N*-Acid aromatic compound -glycine

X2 = Ornithine, or *N*-Lipid tail-glycine or *N*-Neutral aromatic compound -glycine or *N*-Acid aromatic compound -glycine

X3 = Ornithine, or *N*-Lipid tail-glycine or *N*-Neutral aromatic compound -glycine or *N*-Acid aromatic compound –glycine

3) X_1RSCQQQIQQAQQLSSCQQYLKQX_2

X1 = Ornithine, or *N*-Lipid tail-glycine or *N*-Neutral aromatic compound -glycine or *N*-Acid aromatic compound -glycine

X2 = Ornithine, or *N*-Lipid tail-glycine or *N*-Neutral aromatic compound -glycine or *N*-Acid aromatic compound –glycine

4) *N*Arg-SCQQQ-*N*Ile-QQ-*N*Ala-QQ-*N*Leu-SSCQQY-*N*Leu-*N*Lys-Q

5) *N*Arg-SCQQQ-*N*Ile-QQAQQ-*N*Leu-SSCQQYL-*N*Lys-Q

6) *N*Arg-SCQQQIQQ-*N*Ala-QQLSSCQQYL-*N*Lys-Q

7) *N*Arg-SCQQQIQQAQQLSSCQQYL-*N*Lys-Q

8) *N*Arg-SCQQQIQQAQQLSSCQQY-*N*Leu-*N*Lys-Q

9) *N*Arg-SCQQQIQQ-*N*Ala-QQLSSCQQYLKQ

10) RSCQQQIQQ-*N*Ala-QQLSSCQQYL-*N*Lys-Q

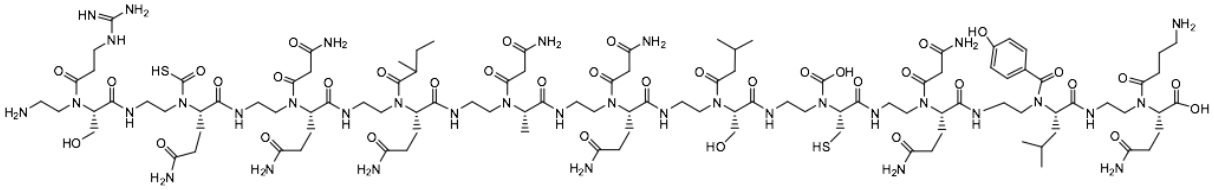
11) RSCQQQIQQ-*N*Ala-QQLSSCQQYLKQ

*N*Arg : *N*-(3-guanidinopropyl)glycine; *N*Ile : *N*-(*sec*-butyl)glycine; *N*Ala : *N*-methylglycine; *N*Leu : *N*-isobutylglycine ; *N*Lys : *N*-(4-aminobutyl)glycine

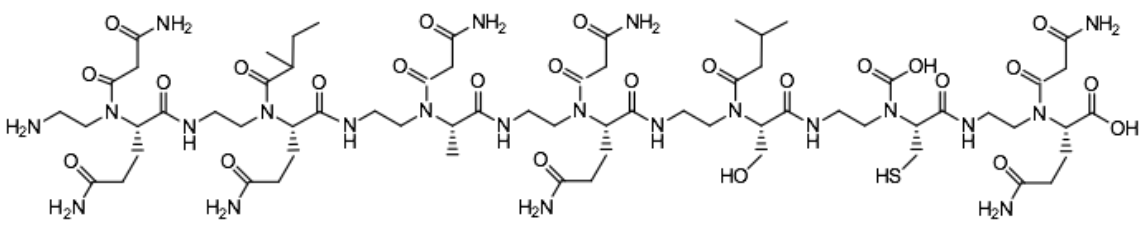
AA-peptides

- *alfa*-AA-peptides:

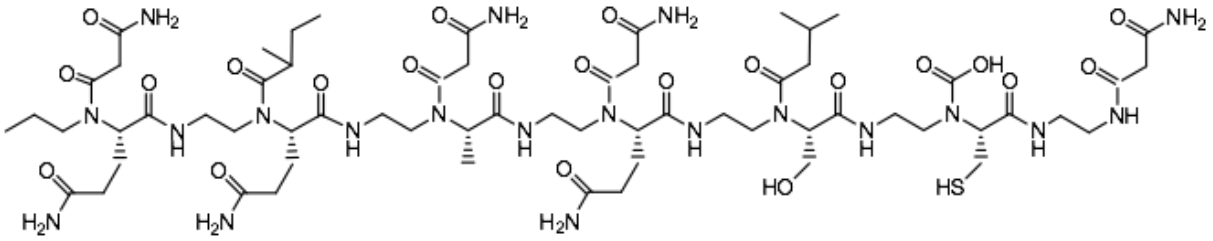
1) α-AA-RSCQQQIQQAQQLSSCQQYLKQ



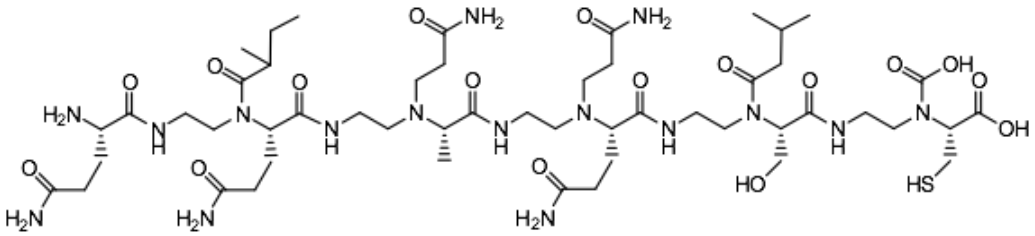
2) α-AA-QQIQQAQQLSSCQQ



3) α-AA-QIQQAQQLSSCQ

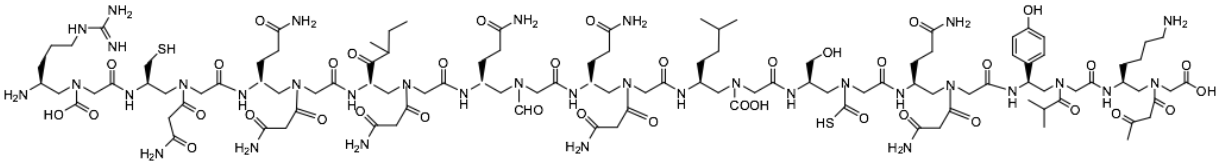


4) α-AA-IQQAQQLSSC

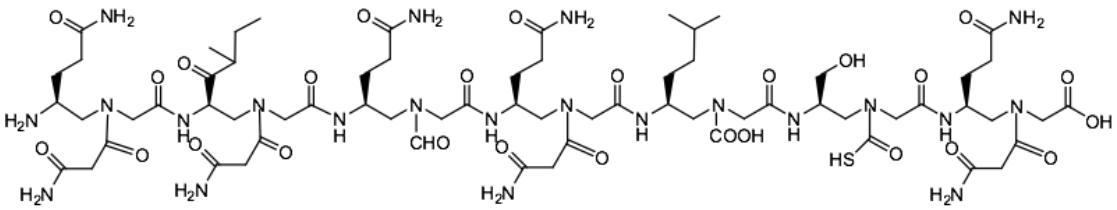


- *gama*-AA-peptides:

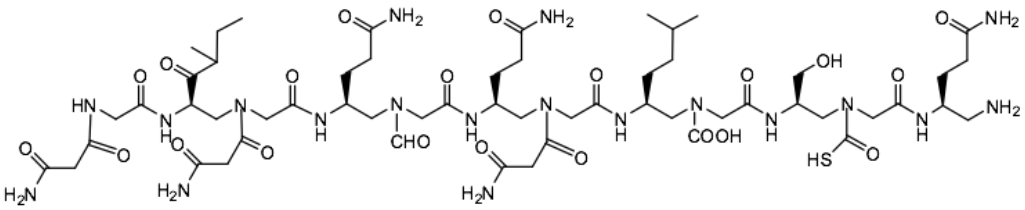
5) γ-AA-RSCQQQIQQAQQLSSCQQYLKQ



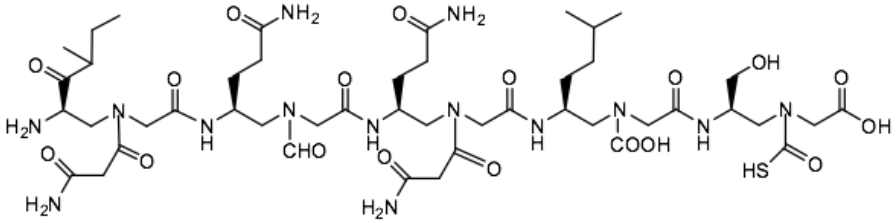
6) γ-AA-QQIQQAQQLSSCQ



7) γ-AA-QIQQAQQLSSC



8) γ-AA-IQQAQQLSS



6.7 Molecular simplification

To determine antibiofilm minor peptide we proposed a molecular simplification of the lead peptide, capsicumicine (P3). Brief, we synthesized and screened two new peptides (P3a and P3b) from the P3 central cleavage as illustrated in figure 1.

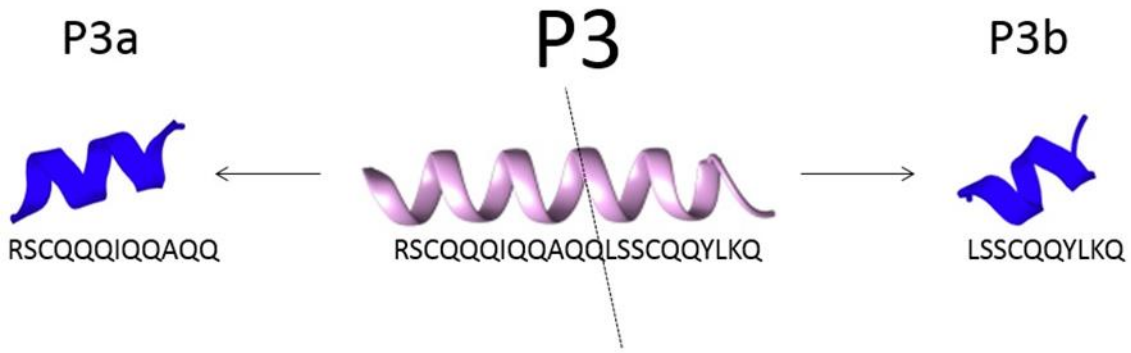
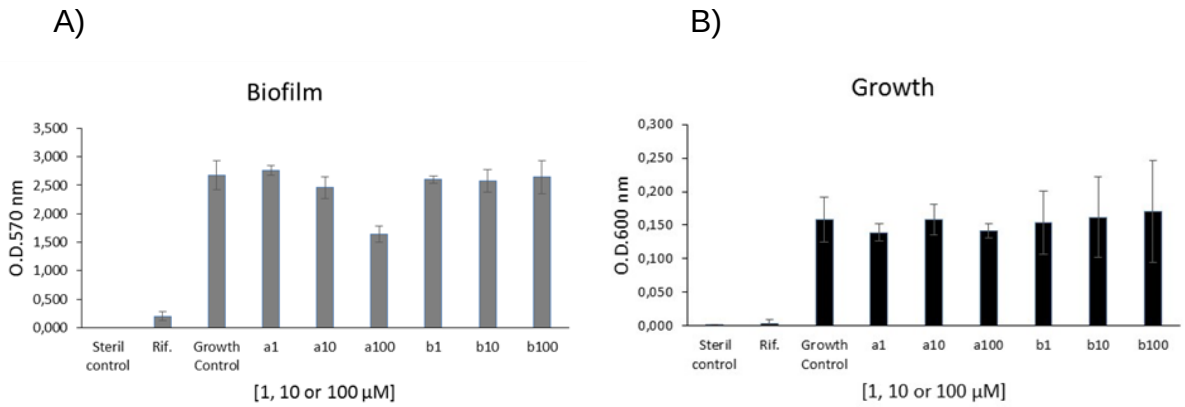


Figure 1) Structural prediction of the minor peptides P3a on the left and peptide P3b on the right. These peptides were designed based on the lead peptide P3, illustrated on the center.

For both P3a, P3b and P3a+b the antibiofilm activity were reduced when compared to P3 at all tested concentrations in bacterial model (Figure 2). However, the absence of antibiotic activity was remained in both situation. This may indicate that the P3 sequence and conformation are essential for antibiofilm activity in bacterial model or that its central portion is indispensable.



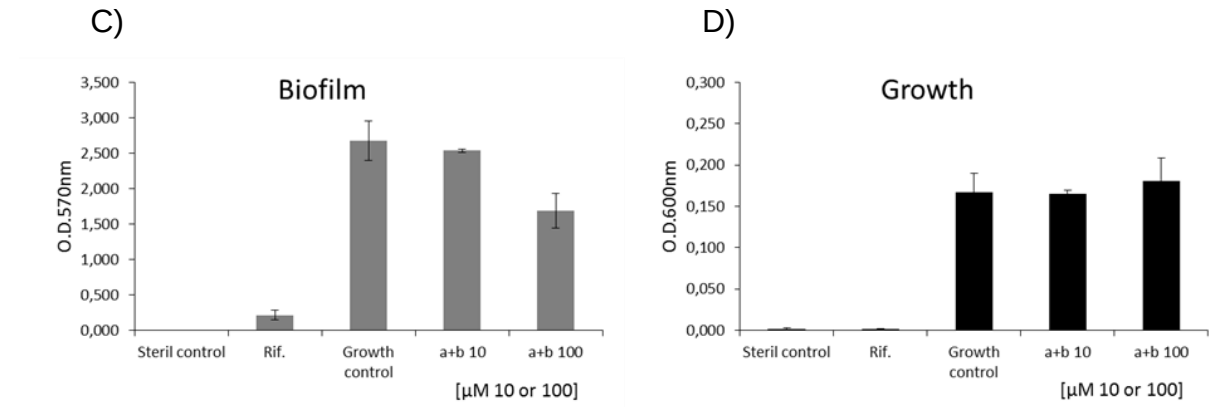


Figure 2) Staphylococcus epidermidis antibiofilm and antimicrobial activities of peptides P3a, b and a+b (1-100µM), after 24 hours. A-B) Peptides P3a and P3b tested separately. C-D) Peptides P3a and P3b tested together. The black bars show bacterial growth (Optical density at 600 nm) and the grey bars show biofilm quantification (Optical density at 570 nm). Rif., represent the antibiotic control (rifampicin) and Growth control, the positive control of growth and biofilm without peptides exposition.

Then, to test the matrix assembly interaction in the absence of bacteria regulation, we screened P3, P3b and P3a+b in Molecular Self-assembly Real-time (MSART), (Figure 3). These results show an important relationship between the shorter peptides and the synthetic matrix molecular self-assembly. P3b at low concentration (10µM) was more effective then P3 in he same concentration, enhancing the reaction kinetic in MSART (Figure 3, A). In other hand, P3a+b at high concentration (100µM) were as effective as P3 to enhance the reaction kinetic in MSART (Figure 3, B). These results show that maybe the shorter peptides are more susceptible to bacteria degradation then P3 however; they are probably a good model of molecular simplification to peptidomimetics development.

These results are preliminary and were performed once during the standardization of the test. It is necessary to test P3a alone, P3a and b in different concentrations.The complete MSART standard test protocol is described in materials and methods of the main article (see chapter 2).

A) B)

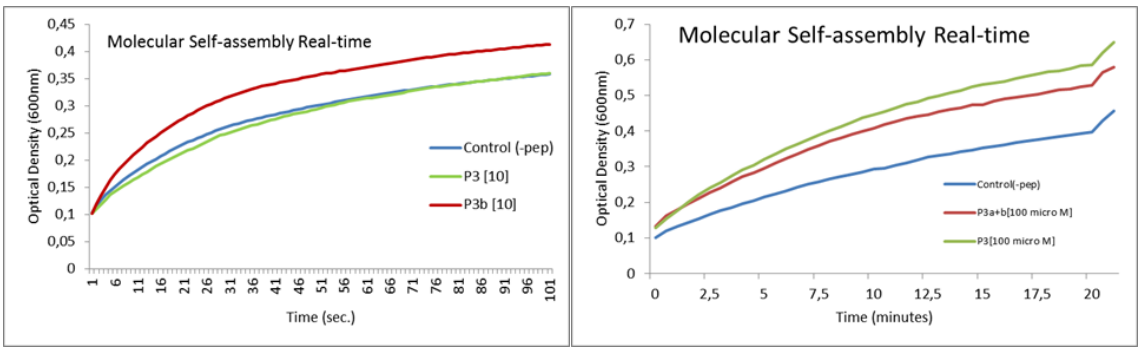


Figure 3) Synthetic matrix interaction test: Molecular Self-assembly Real-time (MSART). A) MSART screening using automatic UVIKON XS spectrophotometer. Peptides P3 and P3b tested at 10µM. B) MSART screening using manual spectrophotometer. Peptides P3 and P3a+b tested at 100µM. Control (-pep) represents no peptides exposition.

7. General Discussion

In the context of antibiotic resistant bacteria, natural peptides have been increasingly prominent as antimicrobial and antibiofilm agents (Feuillie *et al.*, 2017; Grassi *et al.*, 2017; Von Borowski *et al.*, 2017). During my master degree we identified some natural peptides from *C. bacattum* pepper with promising antibiofilm activity (Gomes Von Borowski, 2015). Those results allowed us to select three antibiofilm peptides to synthesis and pharmacology studies, the mains of this work.

Plants are in constant presence of a variety of pathogens and to defend themselves synthesize protective factors such as defense peptides and proteins (Castro e Fontes, 2005). These peptides are common chemical components in seeds of the genus *Capsicum*, as well as antimicrobial peptides (AMPs) (Lee *et al.*, 2004; Ribeiro *et al.*, 2007; Ribeiro *et al.*, 2012; Dias *et al.*, 2013; Ribeiro *et al.*, 2013). Some studies suggest that these plant-derived proteins may exhibit anti-adhesive activities (Lengsfeld *et al.*, 2004; Wittschier *et al.*, 2007; Bensch *et al.*, 2011). However, reports on the chemical composition of the species are still scarce, making it necessary to expand studies in this area (Zimmer *et al.*, 2012a).

In this study we identified and characterized capsicumicine, a special antibiofilm peptide (patent registration), non-antibiotic, able to prevent the establishment and maintenance of biofilm architecture. It decreases adhesion and cellular aggregation of methicillin resistant *S. epidermidis*.

Our findings regarding the non-antibiotic effect of capsicumicine are in accordance with another study that point to the absence of antibacterial activity of *Capsicum* extracts (Kappel *et al.*, 2008). Likewise, some studies correlate peptide-derived compounds with antiadhesive activity, among them, one that studied extracts of *Capsicum annum* fruits against *Campylobacter jejuni* (Bensch *et al.*, 2011) but none for *C. baccatum*.

In contribution, non-bactericidal peptides are encouraging to find new applications as antivirulence therapy and biomaterials coating, alone or in combination with other drugs to fight against bacteria multiresistance. Therefore, bacterial biofilms are promising targets for combatting this problem. Thus, the successful development of antibiofilm compounds will therefore be an important tool for controlling human infections (Miquel et al., 2016).

We demonstrate the mechanism of action of this peptide, interacting with the biofilm matrix in initial phase (Figure 3), modifying matrix self-assembly (Stewart *et al.*, 2015) and consequently producing loss of functionality. Notably, this mechanism is independently of cell regulation. Importantly, the peptide shows no cytotoxicity to mammalian cells, displaying an important selectivity.

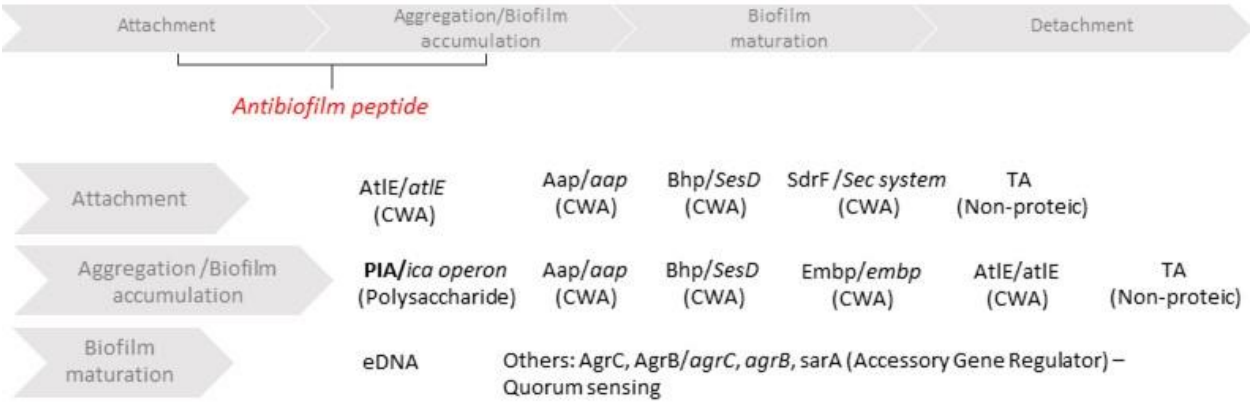


Figure 3. Scheme showing the main stages of biofilm formation in *S. epidermidis*. At each stage, the main compounds/genes involved are briefly described. Highlight in red, the steps involved in the activity of the lead antibiofilm peptide. CWA – Cell wall ancored proteins.

Finally, the reduction in bacterial adhesion and biofilm formation by a pathway that does not involve cell death is a hallmark and contemplates a new concept in antivirulence therapy, aiming to do the microorganisms more susceptible to antimicrobial agents and to the immune system (Brancatisano *et al.*, 2014). Several non-biocidal strategies have been proposed such as biofilm inactivation as molecular target (Batoni *et al.*, 2016).

8. General Conclusion

Thereby, this study reports the discovery of a new antibiofilm peptide (Capsicumicine, patent registration) that (a) significantly prevents biofilm establishment and maintenance. Expressively, it decreases adhesion and cellular aggregation of methicillin resistant *S. epidermidis* (b) without antibiotic activity. We discuss a very promising mechanism of action, less susceptible to the development of tolerance (c). Moreover, the complete absence of cytotoxicity of this peptide (d) with its excellent antibiofilm activity encourages us to keep going the studies through molecular improvements using peptidemimetic strategy (e).

a) the amino acid sequence that forms the composition of the selected natural peptide was identified and we determined the minor lead peptide by molecular simplification (in progress);

b) the bacteria adhesion and aggregation profiles were defined through topographic structural analysis of plastic coupons exposed to the lead peptide by scanning electron microscopy;

c) It was evidenced that the target of the lead peptide is the extracellular matrix through a set of microscopies (mainly microscopy of fluorescence and ultrastructural analysis of exposed bacteria through transmission electron microscopy). Furthermore, a synthetic model of matrix self-assembly was applied to demonstrate it. Gene expression variation was also performed by qRT-PCR in the presence/absence of the lead peptide.

d) the cytotoxicity of the lead peptide was evaluated using different mammalian lines and multiparameter high-throughput image analysis;

e) It was possible to propose several peptidomimetic designs based on the natural lead peptide after literature review and the currently molecular simplification results (perspectives).

9. References

ABAD, C. L.; HALEEM, A. Prosthetic Joint Infections: an Update. **Curr Infect Dis Rep,** v. 20, n. 7, p. 15, May 2018. ISSN 1523-3847. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/29789958 >.

ARCIOLA, C. R. et al. Biofilm formation in Staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. **Biomaterials,** v. 33, n. 26, p. 5967-5982, Sep 2012. ISSN 0142-9612. Disponível em: < <Go to ISI>://WOS:000306720400001 >.

ARMBRUSTER, C. R.; PARSEK, M. R. New insight into the early stages of biofilm formation. **Proc Natl Acad Sci U S A,** v. 115, n. 17, p. 4317-4319, 04 2018. ISSN 1091-6490. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/29632199 >.

AUMILLER, W. M.; DAVIS, B. W.; KEATING, C. D. Phase separation as a possible means of nuclear compartmentalization. **Int Rev Cell Mol Biol,** v. 307, p. 109-49, 2014. ISSN 1937-6448. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/24380594 >.

BANANI, S. F. et al. Biomolecular condensates: organizers of cellular biochemistry. **Nat Rev Mol Cell Biol,** v. 18, n. 5, p. 285-298, 05 2017. ISSN 1471-0080. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28225081 >.

BATONI, G.; MAISETTA, G.; ESIN, S. Antimicrobial peptides and their interaction with biofilms of medically relevant bacteria. **Biochimica Et Biophysica Acta-Biomembranes,** v. 1858, n. 5, p. 1044-1060, May 2016. ISSN 0005-2736. Disponível em: < <Go to ISI>://WOS:000374603600015 >.

BELOIN, C. et al. Novel approaches to combat bacterial biofilms. **Curr Opin Pharmacol,** v. 18C, p. 61-68, Sep 2014. ISSN 1471-4973. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25254624 >.

BENSCH, K. et al. Investigations into the antiadhesive activity of herbal extracts against Campylobacter jejuni. **Phytother Res,** v. 25, n. 8, p. 1125-32, Aug 2011. ISSN 1099-1573. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21280113 >.

BETEMPS, C.; ELOI PINTO, L. Pimenta: diversidade e usos. **Agricultura familiar Biodiversidade Transferência de Tecnologia**, 2015. Disponível em: < https://www.embrapa.br/web/portal/busca-de-noticias/-/noticia/2675520/pimenta-diversidade-e-usos >. Acesso em: 09/21.

BISWAS, S. et al. Mixed Macromolecular Crowding: A Protein and Solvent Perspective. **ACS Omega,** v. 3, n. 4, p. 4316-4330, Apr 2018. ISSN 2470-1343. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/30023892 >.

BJARNSHOLT, T. et al. Applying insights from biofilm biology to drug development - can a new approach be developed? **Nature Reviews Drug Discovery,** v. 12, n. 10, p. 791-808, Oct 2013. ISSN 1474-1776. Disponível em: < <Go to ISI>://WOS:000325149700016 >.

BRANCATISANO, F. L. et al. Inhibitory effect of the human liver-derived antimicrobial peptide hepcidin 20 on biofilms of polysaccharide intercellular adhesin (PIA)-positive and PIA-negative strains of Staphylococcus epidermidis. **Biofouling,** v. 30, n. 4, p. 435-46, 2014. ISSN 1029-2454. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/24645694 >.

BRAUNER, A. et al. Distinguishing between resistance, tolerance and persistence to antibiotic treatment. **Nat Rev Microbiol,** v. 14, n. 5, p. 320-30, 04 2016. ISSN 1740-1534. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/27080241 >.

CASTRO, M. S.; FONTES, W. Plant defense and antimicrobial peptides. **Protein and Peptide Letters,** v. 12, n. 1, p. 13-18, Jan 2005. ISSN 0929-8665. Disponível em: < <Go to ISI>://WOS:000226057400004 >.

CDCP. Center for Disease Control and Prevention. Vital signs: central line-associated blood stream infections -United States, 2001, 2008, and 2009. **MMWR Morb Mortal Wkly Rep,** v. 60, n. 8, p. 243-8, Mar 2011. ISSN 1545-861X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/21368740 >.

CERI, H. et al. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. **J Clin Microbiol,** v. 37, n. 6, p. 1771-6, Jun 1999. ISSN 0095-1137. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/10325322 >.

CHAN, C.; BURROWS, L. L.; DEBER, C. M. Alginate as an auxiliary bacterial membrane: binding of membrane-active peptides by polysaccharides. **J Pept Res,** v. 65, n. 3, p. 343-51, Mar 2005. ISSN 1397-002X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/15787964 >.

CONIBEAR, T. C.; COLLINS, S. L.; WEBB, J. S. Role of mutation in Pseudomonas aeruginosa biofilm development. **PLoS One,** v. 4, n. 7, p. e6289, Jul 2009. ISSN 1932-6203. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/19606212 >.

DAVIES, D. Understanding biofilm resistance to antibacterial agents. **Nat Rev Drug Discov,** v. 2, n. 2, p. 114-22, Feb 2003. ISSN 1474-1776. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12563302 >.

DE LA FUENTE-NUNEZ, C. et al. D-Enantiomeric Peptides that Eradicate Wild-Type and Multidrug-Resistant Biofilms and Protect against Lethal Pseudomonas aeruginosa Infections (vol 22, pg 196, 2015). **Chemistry & Biology,** v. 22, n. 9, p. 1280-1282, Sep 17 2015. ISSN 1074-5521. Disponível em: < <Go to ISI>://WOS:000364012100013 >.

DEFRAINE, V.; FAUVART, M.; MICHIELS, J. Fighting bacterial persistence: Current and emerging anti-persister strategies and therapeutics. **Drug Resist Updat,** v. 38, p. 12-26, 05 2018. ISSN 1532-2084. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/29857815 >.

DIAS, G. B. et al. Isolation, Characterization and Antifungal Activity of Proteinase Inhibitors from Capsicum chinense Jacq. Seeds. **Protein Journal,** v. 32, n. 1, p. 15-26, Jan 2013. ISSN 1572-3887. Disponível em: < <Go to ISI>://WOS:000321624200003 >.

DORKEN, G. et al. Aggregation by depletion attraction in cultures of bacteria producing exopolysaccharide. **J R Soc Interface,** v. 9, n. 77, p. 3490-502, Dec 2012. ISSN 1742-5662. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/22896568 >.

ECOCROP. **Ecocrop database**: FAO - Food and Agriculture Organization of the UN 2013.

EMBRAPA, H. Capsicum: Pimentas e pimentões no Brasil., Web Page, 2002. Disponível em: < http://www.cnph.embrapa.br/capsicum/historia.htm >. Acesso em: 11 de junho.

EVEN, C. et al. Recent advances in studying single bacteria and biofilm mechanics. **Adv Colloid Interface Sci,** v. 247, p. 573-588, Sep 2017. ISSN 1873-3727. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28754382 >.

FANTONI, R.; SANTOS, A. Depletion force in the infinite-dilution limit in a solvent of nonadditive hard spheres. **J Chem Phys,** v. 140, n. 24, p. 244513, Jun 2014. ISSN 1089-7690. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/24985660 >.

FAOSTAT 2001: FAO Statistical Databases. Food & Agriculture Organization of the United Nations (FAO) 2003. ISBN ISBN 10: 9250045352 / ISBN 13: 9789250045351.

FEUILLIE, C. et al. Molecular interactions and inhibition of the staphylococcal biofilm-forming protein SdrC. **Proc Natl Acad Sci U S A,** v. 114, n. 14, p. 3738-3743, 04 2017. ISSN 1091-6490. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28320940 >.

FEY, P. D.; OLSON, M. E. Current concepts in biofilm formation of Staphylococcus epidermidis. **Future Microbiol,** v. 5, n. 6, p. 917-33, Jun 2010. ISSN 1746-0921. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/20521936 >.

FLAMM, R. K. et al. Linezolid Surveillance Results for the United States (LEADER Surveillance Program 2014). **Antimicrob Agents Chemother,** v. 60, n. 4, p. 2273-80, Apr 2016. ISSN 1098-6596. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/26833165 >.

FLEMING, D.; RUMBAUGH, K. P. Approaches to Dispersing Medical Biofilms. **Microorganisms,** v. 5, n. 2, Apr 2017. ISSN 2076-2607. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28368320 >.

FLEMMING, H.-C.; WINGENDER, J. The biofilm matrix. **Nature Reviews Microbiology,** v. 8, n. 9, p. 623-633, Sep 2010. ISSN 1740-1526. Disponível em: < <Go to ISI>://WOS:000280855500009 >.

FORDE, É. et al. Differential In Vitro and In Vivo Toxicities of Antimicrobial Peptide Prodrugs for Potential Use in Cystic Fibrosis. **Antimicrob Agents Chemother,** v. 60, n. 5, p. 2813-21, 05 2016. ISSN 1098-6596. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/26902766 >.

GOMES VON BOROWSKI, R. **Avaliação da atividade antibiofilme de Capsicum baccatum var. pendulum (Solanaceae)**. 2015. 98 (Master). Programa de Pós

Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, LUME, Digital Repository.

GOMES VON BOROWSKI, R. et al. Promising Antibiofilm Activity of Peptidomimetics. **Front Microbiol,** v. 9, p. 2157, 2018. ISSN 1664-302X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/30271394 >.

_____. Red pepper Capsicum baccatum : source of antiadhesive and antibiofilm compounds against nosocomial bacteria. Industrial Crops and Products**,** v. 127, p. 148-157, 2019. ISSN 0926-6690.

GOVINDARAJAN, V. S. CAPSICUM - PRODUCTION, TECHNOLOGY, CHEMISTRY, AND QUALITY .3. CHEMISTRY OF THE COLOR, AROMA, AND PUNGENCY STIMULI. **Crc Critical Reviews in Food Science and Nutrition,** v. 24, n. 3, p. 245-355, 1986 1986. ISSN 0099-0248. Disponível em: < <Go to ISI>://WOS:A1986D865100001 >.

GRASSI, L. et al. Combination Strategies to Enhance the Efficacy of Antimicrobial Peptides against Bacterial Biofilms. **Front Microbiol,** v. 8, p. 2409, 2017. ISSN 1664-302X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/29375486 >.

HAUSSLER, S.; FUQUA, C. Biofilms 2012: New Discoveries and Significant Wrinkles in a Dynamic Field. **Journal of Bacteriology,** v. 195, n. 13, p. 2947-2958, Jul 2013. ISSN 0021-9193. Disponível em: < <Go to ISI>://WOS:000320109000001 >.

HOIBY, N. et al. Antibiotic resistance of bacterial biofilms. **International Journal of Antimicrobial Agents,** v. 35, n. 4, p. 322-332, Apr 2010. ISSN 0924-8579. Disponível em: < <Go to ISI>://WOS:000274869800003 >.

HOPE, R. et al. Non-susceptibility trends among staphylococci from bacteraemias in the UK and Ireland, 2001-06. **J Antimicrob Chemother,** v. 62 Suppl 2, p. ii65-74, Nov 2008. ISSN 1460-2091. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/18819981 >.

JABBOURI, S.; SADOVSKAYA, I. Characteristics of the biofilm matrix and its role as a possible target for the detection and eradication of Staphylococcus epidermidis associated with medical implant infections. **FEMS Immunol Med Microbiol,** v. 59, n. 3, p. 280-91, Aug 2010. ISSN 1574-695X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/20528930 >.

KAPPEL, V. D. et al. Phenolic content and antioxidant and antimicrobial properties of fruits of Capsicum baccatum L. var. pendulum at different maturity stages. **Journal of Medicinal Food,** v. 11, n. 2, p. 267-274, Jun 2008. ISSN 1096-620X. Disponível em: < <Go to ISI>://WOS:000257633800010 >.

KIM, S. et al. Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species. **Nature Genetics,** v. 46, n. 3, p. 270-+, Mar 2014. ISSN 1061-4036. Disponível em: < <Go to ISI>://WOS:000332036700011 >.

KRACHLER, A. M.; ORTH, K. Targeting the bacteria-host interface: strategies in anti-adhesion therapy. **Virulence,** v. 4, n. 4, p. 284-94, May 2013. ISSN 2150-5608. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/23799663 >.

KUDLAY, A.; CHEUNG, M. S.; THIRUMALAI, D. Influence of the shape of crowding particles on the structural transitions in a polymer. **J Phys Chem B,** v. 116, n. 29, p. 8513-22, Jul 2012. ISSN 1520-5207. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/22616622 >.

LALANI, T. et al. Prosthetic valve endocarditis due to coagulase-negative staphylococci: findings from the International Collaboration on Endocarditis Merged Database. **Eur J Clin Microbiol Infect Dis,** v. 25, n. 6, p. 365-8, Jun 2006. ISSN 0934-9723. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/16767483 >.

LAMIABLE, A. et al. PEP-FOLD3: faster de novo structure prediction for linear peptides in solution and in complex. **Nucleic Acids Res,** v. 44, n. W1, p. W449-54, Jul 2016. ISSN 1362-4962. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/27131374 >.

LAVERTY, G.; GORMAN, S. P.; GILMORE, B. F. Biomolecular mechanisms of staphylococcal biofilm formation. **Future Microbiology,** v. 8, n. 4, p. 509-524, Apr 2013. ISSN 1746-0913. Disponível em: < <Go to ISI>://WOS:000316799400012 >.

LAZARIS, A. et al. Novel multiresistance cfr plasmids in linezolid-resistant methicillin-resistant Staphylococcus epidermidis and vancomycin-resistant Enterococcus faecium (VRE) from a hospital outbreak: co-location of cfr and optrA in VRE. **J Antimicrob Chemother,** v. 72, n. 12, p. 3252-3257, Dec 2017. ISSN 1460-2091. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28961986 >.

LE, K. Y.; OTTO, M. Quorum-sensing regulation in staphylococci-an overview. **Front Microbiol,** v. 6, p. 1174, 2015. ISSN 1664-302X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/26579084 >.

LEE, J. Y. H. et al. Global spread of three multidrug-resistant lineages of Staphylococcus epidermidis. **Nat Microbiol**, Sep 2018. ISSN 2058-5276. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/30177740 >.

LEE, Y. M. et al. Molecular characterization of a cDNA for a cysteine-rich antifungal protein from Capsicum annuum. **Journal of Plant Biology,** v. 47, n. 4, p. 375-382, Dec 31 2004. ISSN 1226-9239. Disponível em: < <Go to ISI>://WOS:000226290800012 >.

LENGSFELD, C. et al. Glycosylated compounds from okra inhibit adhesion of Helicobacter pylori to human gastric mucosa. **Journal of Agricultural and Food Chemistry,** v. 52, n. 6, p. 1495-1503, Mar 24 2004. ISSN 0021-8561. Disponível em: < <Go to ISI>://WOS:000220285600014 >.

LEONARDI, B. F. **Avaliação farmacológica de Capsicum baccatum var. pendulum L. em um protocolo experimental patronizado de síndrome metabólica in vivo**. 2017. 137 (Master). Programa de Pós Graduação em Ciências Farmacêuticas, Universidade Federal do Rio grande do Sul, LUME, Digital Repository.

LEVIN-REISMAN, I. et al. Antibiotic tolerance facilitates the evolution of resistance. **Science,** v. 355, n. 6327, p. 826-830, 02 2017. ISSN 1095-9203. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28183996 >.

LEWIS, K. Riddle of biofilm resistance. **Antimicrob Agents Chemother,** v. 45, n. 4, p. 999-1007, Apr 2001. ISSN 0066-4804. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/11257008 >.

LIU, P. et al. Genetic Selection of Peptide Aptamers That Interact and Inhibit Both Small Protein B and Alternative Ribosome-Rescue Factor A of Aeromonas veronii C4. **Front Microbiol,** v. 7, p. 1228, 2016. ISSN 1664-302X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/27588015 >.

_____. Targeting Inhibition of SmpB by Peptide Aptamer Attenuates the Virulence to Protect Zebrafish against. **Front Microbiol,** v. 8, p. 1766, 2017. ISSN 1664-302X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28955325 >.

LIVAK, K. J.; SCHMITTGEN, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. **Methods,** v. 25, n. 4, p. 402-8, Dec 2001. ISSN 1046-2023. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/11846609 >.

MADEO, J.; FRIERI, M. Bacterial biofilms and chronic rhinosinusitis. **Allergy and Asthma Proceedings,** v. 34, n. 4, p. 335-341, Jul-Aug 2013. ISSN 1088-5412. Disponível em: < <Go to ISI>://WOS:000321808200004 >.

MAKI, D. G.; KLUGER, D. M.; CRNICH, C. J. The risk of bloodstream infection in adults with different intravascular devices: a systematic review of 200 published prospective studies. **Mayo Clin Proc,** v. 81, n. 9, p. 1159-71, Sep 2006. ISSN 0025-6196. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/16970212 >.

MANDAVA, S. H. et al. Infection retardant coated inflatable penile prostheses decrease the incidence of infection: a systematic review and meta-analysis. **J Urol,** v. 188, n. 5, p. 1855-60, Nov 2012. ISSN 1527-3792. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/22999690 >.

MARENDUZZO, D.; FINAN, K.; COOK, P. R. The depletion attraction: an underappreciated force driving cellular organization. **J Cell Biol,** v. 175, n. 5, p. 681-6, Dec 2006. ISSN 0021-9525. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/17145959 >.

MENICHINI, F. et al. The influence of fruit ripening on the phytochemical content and biological activity of Capsicum chinense Jacq. cv Habanero. **Food Chemistry,** v. 114, n. 2, p. 553-560, May 15 2009. ISSN 0308-8146. Disponível em: < <Go to ISI>://WOS:000263662400027 >.

MOLON, D. F. **Isolamento e elucidação estrutural de compostos com potencial anti-inflamatório na pimenta dedo-de-moça (Capsicum baccatum var. pendulum)**. 2016. 73 (M). Programa de Pós Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, LUME, Digital Repository.

MÉRIC, G. et al. Disease-associated genotypes of the commensal skin bacterium Staphylococcus epidermidis. **Nat Commun,** v. 9, n. 1, p. 5034, 11 2018. ISSN 2041-1723. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/30487573 >.

NEELY, A. N.; MALEY, M. P. Survival of enterococci and staphylococci on hospital fabrics and plastic. **J Clin Microbiol,** v. 38, n. 2, p. 724-6, Feb 2000. ISSN 0095-1137. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/10655374 >.

NISHIZAKI, Y. et al. Japanese features of native valve endocarditis caused by coagulase-negative staphylococci: case reports and a literature review. **Intern Med,** v. 52, n. 5, p. 567-72, 2013. ISSN 1349-7235. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/23448766 >.

O'BRIEN, E. P. et al. Influence of Nanoparticle Size and Shape on Oligomer Formation of an Amyloidogenic Peptide. **J Phys Chem Lett,** v. 2, n. 10, p. 1171-1177, May 2011. ISSN 1948-7185. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/21691423 >.

OTTO, M. Staphylococcal biofilms. **Bacterial Biofilms,** v. 322, p. 207-228, 2008 2008. ISSN 0070-217X. Disponível em: < <Go to ISI>://WOS:000258523900010 >.

_____. Staphylococcal Infections: Mechanisms of Biofilm Maturation and Detachment as Critical Determinants of Pathogenicity. **Annual Review of Medicine, Vol 64,** v. 64, p. 175-188, 2013 2013. ISSN 0066-4219. Disponível em: < <Go to ISI>://WOS:000316384400013 >.

_____. Physical stress and bacterial colonization. **FEMS Microbiol Rev,** v. 38, n. 6, p. 1250-70, Nov 2014. ISSN 1574-6976. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25212723 >.

PAHARIK, A. E.; HORSWILL, A. R. The Staphylococcal Biofilm: Adhesins, Regulation, and Host Response. **Microbiol Spectr,** v. 4, n. 2, 04 2016. ISSN 2165-0497. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/27227309 >.

PENESYAN, A.; GILLINGS, M.; PAULSEN, I. T. Antibiotic discovery: combatting bacterial resistance in cells and in biofilm communities. **Molecules,** v. 20, n. 4, p. 5286-98, Mar 2015. ISSN 1420-3049. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/25812150 >.

POLSON, J. M.; KERRY, D. R. Segregation of polymers under cylindrical confinement: effects of polymer topology and crowding. **Soft Matter,** v. 14, n. 30, p. 6360-6373, Aug 2018. ISSN 1744-6848. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/30028460 >.

RASKO, D. A.; SPERANDIO, V. Anti-virulence strategies to combat bacteria-mediated disease. **Nat Rev Drug Discov,** v. 9, n. 2, p. 117-28, Feb 2010. ISSN 1474-1784. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/20081869 >.

RIBEIRO, S. F. F. et al. Isolation and characterization of novel peptides from chilli pepper seeds: Antimicrobial activities against pathogenic yeasts. **Toxicon,** v. 50, n. 5, p. 600-611, Oct 2007. ISSN 0041-0101. Disponível em: < <Go to ISI>://WOS:000250310300002 >.

_____. New Small Proteinase Inhibitors from Capsicum annuum Seeds: Characterization, Stability, Spectroscopic Analysis and a cDNA Cloning. **Biopolymers,**

v. 100, n. 2, p. 132-140, Apr 2013. ISSN 0006-3525. Disponível em: < <Go to ISI>://WOS:000336528200003 >.

_____. Capsicum annuum L. trypsin inhibitor as a template scaffold for new drug development against pathogenic yeast. **Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology,** v. 101, n. 3, p. 657-670, Mar 2012. ISSN 0003-6072. Disponível em: < <Go to ISI>://WOS:000300314200019 >.

ROGERS, K. L.; FEY, P. D.; RUPP, M. E. Coagulase-negative staphylococcal infections. **Infect Dis Clin North Am,** v. 23, n. 1, p. 73-98, Mar 2009. ISSN 1557-9824. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/19135917 >.

ROHDE, H. et al. Structure, function and contribution of polysaccharide intercellular adhesin (PIA) to Staphylococcus epidermidis biofilm formation and pathogenesis of biomaterial-associated infections. **Eur J Cell Biol,** v. 89, n. 1, p. 103-11, Jan 2010. ISSN 1618-1298. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/19913940 >.

ROY, R. et al. Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. **Virulence,** v. 9, n. 1, p. 522-554, 01 2018. ISSN 2150-5608. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28362216 >.

RUPP, M. E. Clinical characteristics of infections in humans due to Staphylococcus epidermidis. **Methods Mol Biol,** v. 1106, p. 1-16, 2014. ISSN 1940-6029. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/24222451 >.

SAKAGUCHI, A.; HIGASHIGUCHI, K.; MATSUDA, K. Bundle formation of supramolecular fibers of amphiphilic diarylethene by depletion force. **Chem Commun (Camb),** v. 54, n. 34, p. 4298-4301, Apr 2018. ISSN 1364-548X. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/29632919 >.

SCHWARZ-LINEK, J. et al. Phase separation and rotor self-assembly in active particle suspensions. **Proc Natl Acad Sci U S A,** v. 109, n. 11, p. 4052-7, Mar 2012. ISSN 1091-6490. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/22392986 >.

SCOPEL, M. et al. Dipeptide cis-cyclo(Leucyl-Tyrosyl) produced by sponge associated Penicillium sp F37 inhibits biofilm formation of the pathogenic Staphylococcus epidermidis. **Bioorganic & Medicinal Chemistry Letters,** v. 23, n. 3, p. 624-626, Feb 1 2013. ISSN 0960-894X. Disponível em: < <Go to ISI>://WOS:000313694200005 >.

SHARMA, A. et al. dPABBs: A Novel in silico Approach for Predicting and Designing Anti-biofilm Peptides. **Sci Rep,** v. 6, p. 21839, Feb 2016. ISSN 2045-2322. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/26912180 >.

SHEN, Y. et al. Improved PEP-FOLD Approach for Peptide and Miniprotein Structure Prediction. **J Chem Theory Comput,** v. 10, n. 10, p. 4745-58, Oct 2014. ISSN 1549-9626. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/26588162 >.

SIERADZKI, K. et al. Heterogeneously vancomycin-resistant Staphylococcus epidermidis strain causing recurrent peritonitis in a dialysis patient during vancomycin therapy. **J Clin Microbiol,** v. 37, n. 1, p. 39-44, Jan 1999. ISSN 0095-1137. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/9854061 >.

SILVA, L. N. E. A. Plant Natural Products Targeting Bacterial Virulence Factors. ***Chemical Reviews*****,** v. 116.16, p. 9162-9236, July 20, 2016 2016.

SPEZIALE, P. et al. Protein-based biofilm matrices in Staphylococci. **Front Cell Infect Microbiol,** v. 4, p. 171, 2014. ISSN 2235-2988. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/25540773 >.

STEWART, E. J. et al. Artificial biofilms establish the role of matrix interactions in staphylococcal biofilm assembly and disassembly. **Sci Rep,** v. 5, p. 13081, Aug 2015. ISSN 2045-2322. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/26272750 >.

STEWART, P. S. Prospects for Anti-Biofilm Pharmaceuticals. **Pharmaceuticals (Basel),** v. 8, n. 3, p. 504-11, Aug 2015. ISSN 1424-8247. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/26343685 >.

STREIT, J. M. et al. Assessment of pathogen occurrences and resistance profiles among infected patients in the intensive care unit: report from the SENTRY Antimicrobial Surveillance Program (North America, 2001). **Int J Antimicrob Agents,** v. 24, n. 2, p. 111-8, Aug 2004. ISSN 0924-8579. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/15288308 >.

STREMPEL, N.; STREHMEL, J.; OVERHAGE, J. Potential Application of Antimicrobial Peptides in the Treatment of Bacterial Biofilm Infections. **Current Pharmaceutical Design,** v. 21, n. 1, p. 67-84, 2015 2015. ISSN 1381-6128. Disponível em: < <Go to ISI>://WOS:000345434600008 >.

TAGLIALEGNA, A. et al. Staphylococcal Bap Proteins Build Amyloid Scaffold Biofilm Matrices in Response to Environmental Signals. **PLoS Pathog,** v. 12, n. 6, p. e1005711, 06 2016. ISSN 1553-7374. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/27327765 >.

TETERYCZ, D. et al. Outcome of orthopedic implant infections due to different staphylococci. **Int J Infect Dis,** v. 14, n. 10, p. e913-8, Oct 2010. ISSN 1878-3511. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/20729115 >.

THÉVENET, P. et al. PEP-FOLD: an updated de novo structure prediction server for both linear and disulfide bonded cyclic peptides. **Nucleic Acids Res,** v. 40, n. Web Server issue, p. W288-93, Jul 2012. ISSN 1362-4962. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/22581768 >.

TRAVIER, L. et al. Escherichia coli Resistance to Nonbiocidal Antibiofilm Polysaccharides Is Rare and Mediated by Multiple Mutations Leading to Surface Physicochemical Modifications. **Antimicrobial Agents and Chemotherapy,** v. 57, n. 8, p. 3960-3968, Aug 2013. ISSN 0066-4804. Disponível em: < <Go to ISI>://WOS:000321761800064 >.

UÇKAY, I. et al. Meticillin resistance in orthopaedic coagulase-negative staphylococcal infections. **J Hosp Infect,** v. 79, n. 3, p. 248-53, Nov 2011. ISSN 1532-2939. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/21955452 >.

VON BOROWSKI, R. G.; MACEDO, A. J.; GNOATTO, S. C. B. Peptides as a strategy against biofilm-forming microorganisms: Structure-activity relationship perspectives. **Eur J Pharm Sci,** v. 114, p. 114-137, Nov 2017. ISSN 1879-0720. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/29133243 >.

VUONG, C. et al. Polysaccharide intercellular adhesin (PIA) protects Staphylococcus epidermidis against major components of the human innate immune system. **Cell Microbiol,** v. 6, n. 3, p. 269-75, Mar 2004. ISSN 1462-5814. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/14764110 >.

WHO; ORGANIZATION, W. H. **WHO: Global action plan on antimicrobial resistance** 2015.

_____. **WHO: Antibiotic resistance** 2018.

WITTSCHIER, N. et al. Large molecules as anti-adhesive compounds against pathogens. **Journal of Pharmacy and Pharmacology,** v. 59, n. 6, p. 777-786, Jun 2007. ISSN 0022-3573. Disponível em: < <Go to ISI>://WOS:000247024000004 >.

WRIGHT, G. D. Antibiotic Adjuvants: Rescuing Antibiotics from Resistance. **Trends in Microbiology,** v. 24, n. 11, p. 862-871, Nov 2016. ISSN 0966-842X. Disponível em: < <Go to ISI>://WOS:000386644800004 >.

ZHOU, H. X. Effect of mixed macromolecular crowding agents on protein folding. **Proteins,** v. 72, n. 4, p. 1109-13, Sep 2008. ISSN 1097-0134. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/18506780 >.

ZIMMER, A. R. et al. Antioxidant and anti-inflammatory properties of Capsicum baccatum: From traditional use to scientific approach. **Journal of Ethnopharmacology,** v. 139, n. 1, p. 228-233, Jan 6 2012. ISSN 0378-8741. Disponível em: < <Go to ISI>://WOS:000299976900031 >.

_____. Antioxidant and anti-inflammatory properties of Capsicum baccatum: From traditional use to scientific approach. **Journal of Ethnopharmacology,** v. 139, n. 1, p. 228-233, Jan 6 2012a. ISSN 0378-8741. Disponível em: < <Go to ISI>://WOS:000299976900031 >.

_____. Long-Term Oral Administration of Capsicum baccatum Extracts Does Not Alter Behavioral, Hematological, and Metabolic Parameters in CF1 Mice. **Evid Based Complement Alternat Med,** v. 2012, p. 196358, 2012. ISSN 1741-4288. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23320023 >.

Titre: Obtention et évaluation de l'activité antibiofilm de peptides et peptidomimétiques issus de *Capsicum baccatum* var. *pendulum* (Solanaceae)

Mots clés : Peptide - peptidomimétique - biofilm - antimicrobien - capsicum - virulence

Résumé : Le biofilm confère aux bactéries de nombreux avantages en tant que matrice qui améliore leur résistance et tolérance aux antibiotiques. *Staphylococcus epidermidis* est l'une des bactéries cliniques les plus importantes en raison de sa capacité à former des biofilms sur des dispositifs médicaux, notamment les stimulateurs cardiaques, les cathéters urinaires et les prothèses. Dans ce contexte, les peptides ont été proposés comme une alternative importante, que ce soit en tant que traitement médicamenteux ou en tant qu'agents de surfaces anti-infectieux. Cette étude porte sur l'identification de nouveaux peptides naturels et synthetiques antibiofilm issus du piment *Capsicum baccatum* var. *pendulum*. Un peptide majeur responsable de l'activité antibiofilm contre *S. epidermidis* a été sélectionné et étudié de manière approfondie. Il agit par un nouveau mécanisme d'action que nous nommons « anti-assemblage de la matrice » (AAM). Dans le premier chapitre, nous décrivons le lien entre les peptides, les biofilms pathogènes et l'activité antibiofilm. Le chapitre 2 est consacré aux principaux résultats expérimentaux de cette thèse. Il intégre la caractérisation antibiofilm du peptide majeur, agissant par le nouveau mécanisme d'action AAM, indépendant de la régulation cellulaire. Des tests de cytotoxicité sont également présentés. Ces résultats nous ont permis de breveter le peptide en question, référencé au chapitre 3. Le dernier chapitre décrit la possible utilisation de peptidomimétiques antibiofilm en tant que perspective. La stratégie consiste à créer de petites molécules analogues à des peptides. Ces peptidomimétiques conservent les capacités inhérentes au peptide majeur, mais sont plus résistants aux protéases et/ou plus actifs.

Title: Peptides and peptidomimetics obtention from *Capsicum baccatum* var. *pendulum* (Solanaceae) aiming to the antibiofilm activity

Keywords : Peptide - peptidomimetic - biofilm - antimicrobial - capsicum – virulence

Abstract : Biofilm confers to bacteria many benefits due to the production of a matrix that improves their resistance and tolerance to antibiotics. *Staphylococcus epidermidis* is one of the most important clinical bacteria, able to form biofilm on medical devices such as pacemakers, urinary catheters and prostheses. In this context, peptides have been proposed as an important alternative as a treatment or as anti-infective surface agents. This study focuses on the identification of new antibiofilm natural and synthetic peptides from the *Capsicum baccatum* var. *pendulum* pepper. As a result, a lead peptide responsible for the antibiofilm activity against *S. epidermidis* was selected and extensively studied. It acts by a new mechanism of action that we call "matrix anti-assembly" (MAA). In the first chapter, we explore the link between peptides, pathogenic biofilms and the antibiofilm activity. Chapter 2 consists of the main experimental results of this thesis. It describes the antibiofilm characterization of the lead peptide acting by the MAA new mechanism of action, independent of cell regulation. Cytotoxicity tests are also presented. These results allowed us to patent this peptide, referenced in Chapter 3. The last chapter presents the possible use of antibiofilm peptidomimetics as a perspective. The strategy is to create small peptide-like molecules. These peptidomimetics retain the inherent capabilities of the lead peptide, but are more resistant to proteases and / or more active.