The role of outer membrane proteins and lipopolysaccharides for the sensitivity of escherichia coli to antimicrobial peptides

Bacterial resistance to classical antibiotics is emerging worldwide. The number of infections caused by multidrug resistant bacteria is increasing and becoming a serious threat for human health globally. In particular, Gram-negative pathogens including multidrug resistant Escherichia coli are of serious concern being resistant to the currently available antibiotics. All Gram-negative bacteria are enclosed by an outer membrane which acts as an additional protection barrier preventing the entry of toxic compounds including antibiotics and antimicrobial peptides (AMPs). In this study we report that the outer membrane component lipopolysaccharide (LPS) plays a crucial role for the antimicrobial susceptibility of E. coli BW25113 against the cationic AMPs Cap18, Cap11, Cap11-1-18m², melittin, indolicidin, cecropin P1, cecropin B, and the polypeptide antibiotic colistin, whereas the outer membrane protease OmpT and the lipoprotein Lpp only play a minor role for the susceptibility against cationic AMPs. Increased susceptibility toward cationic AMPs was found for LPS deficient mutants of E. coli BW25113 harboring deletions in any of the genes required for the inner part of core-oligosaccharide of the LPS, waaC, waaE, waaF, yaaG, and gmhA. In addition, our study demonstrates that the antimicrobial activity of Cap18, Cap11, Cap11-1-18m², cecropin B, and cecropin P1 is not only dependent on the inner part of the core oligosaccharide, but also on the outer part and its sugar composition. Finally, we demonstrated that the antimicrobial activity of selected Cap18 derivatives harboring amino acid substitutions in the hydrophobic interface, are non-active against wild-type E. coli ATCC29522. By deleting waaC, waaE, waaF, or waaG the antimicrobial activity of the non-active derivatives can be partially or fully restored, suggesting a very close interplay between the LPS core oligosaccharide and the specific Cap18 derivative. Summarizing, this study implicates that the nature of the outer membrane component LPS has a big impact on the antimicrobial activity of cationic AMPs against E. coli. In particular, the inner as well as the outer part of the core oligosaccharide are important elements determining the antimicrobial susceptibility of E. coli against cationic AMPs.
Dissection of the antimicrobial and hemolytic activity of Cap18: Generation of Cap18 derivatives with enhanced specificity

Due to the rapid emergence of resistance to classical antibiotics, novel antimicrobial compounds are needed. It is desirable to selectively kill pathogenic bacteria without targeting other beneficial bacteria in order to prevent the negative clinical consequences caused by many broad-spectrum antibiotics as well as reducing the development of antibiotic resistance. Antimicrobial peptides (AMPs) represent an alternative to classical antibiotics and it has been previously demonstrated that Cap18 has high antimicrobial activity against a broad range of bacterial species. In this study we report the design of a positional scanning library consisting of 696 Cap18 derivatives and the subsequent screening for antimicrobial activity against Y. ruckeri, A. salmonicida, S. Typhimurium and L. lactis as well as for hemolytic activity measuring the hemoglobin release of horse erythrocytes. We show that the hydrophobic face of Cap18, in particular I13, L17 and I24, is essential for its antimicrobial activity against S. Typhimurium, Y. ruckeri, A. salmonicida, E. coli, P. aeruginosa, L. lactis, L. monocytogenes and E. faecalis. In particular, Cap18 derivatives harboring a I13D, L17D, L17P, I24D or I24N substitution lost their antimicrobial activity against any of the tested bacterial strains. In addition, we were able to generate species-specific Cap18 derivatives by particular amino acid substitutions either in the hydrophobic face at positions L6, L17, I20, and I27, or in the hydrophilic face at positions K16 and K18. Finally, our data showed the proline residue at position 29 to be essential for the inherent low hemolytic activity of Cap18 and that substitution of the residues K16, K23, or G21 by any hydrophobic residues enhances the hemolytic activity. This study demonstrates the potential of generating species-specific AMPs for the selective elimination of bacterial pathogens.

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Antimicrobial peptide CAP18 and its effect on *Yersinia ruckeri* infections in rainbow trout *Oncorhynchus mykiss* (Walbaum): comparing administration by injection and oral routes

The antimicrobial peptide CAP18 has been demonstrated to have a strong in vitro bactericidal effect on *Yersinia ruckeri*, but its activity in vivo has not been described. In this work, we investigated whether CAP18 protects rainbow trout *Oncorhynchus mykiss* (Walbaum) against enteric red mouth disease caused by this pathogen either following i.p. injection or by oral administration (in feed). It was found that injection of CAP18 into juvenile rainbow trout before exposure to *Y. ruckeri* was associated with lowered mortality compared to non-medicated fish although it was less effective than the conventional antibiotic oxolinic acid. Oral administration of CAP18 to trout did not prevent infection. The proteolytic effect of secretions on the peptide CAP18 in the fish gastrointestinal tract is suggested to account for the inferior effect of oral administration.

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**Authors:** Chettri, J. K. (Intern), Mehrdana, F. (Ekstern), Hansen, E. B. (Intern), Ebbensgaard, A. E. (Intern), Overgaard, M. T. (Ekstern), Lauritsen, A. H. (Ekstern), Dalsgaard, I. (Intern), Buchmann, K. (Ekstern)  
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Comparative Evaluation of the Antimicrobial Activity of Different Antimicrobial Peptides against a Range of Pathogenic Bacteria

The rapid emergence of resistance to classical antibiotics has increased the interest in novel antimicrobial compounds. Antimicrobial peptides (AMPs) represent an attractive alternative to classical antibiotics and a number of different studies have reported antimicrobial activity data of various AMPs, but there is only limited comparative data available. The mode of action for many AMPs is largely unknown even though several models have suggested that the lipopolysaccharides (LPS) play a crucial role in the attraction and attachment of the AMP to the bacterial membrane in Gram-negative bacteria. We compared the potency of Cap18, Cap11, Cap11-1-18m2, Cecropin P1, Cecropin B, Bac2A, Bac2A-NH2, Sub5-NH2, Indolicidin, Melittin, Myxinidin, Myxinidin-NH2, Pyrrolocoricin, Apidaecin and Metalnikowin I towards Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Escherichia coli, Aeromonas salmonicida, Listeria monocytogenes, Campylobacter jejuni, Flavobacterium psychrophilum, Salmonella typhimurium and Yersinia ruckeri by minimal inhibitory concentration (MIC) determinations. Additional characteristics such as cytotoxicity, thermo and protease stability were measured and compared among the different peptides. Further, the antimicrobial activity of a selection of cationic AMPs was investigated in various E. coli LPS mutants.

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Activation and polar sequestration of PopA, a c-di-GMP effector protein involved in Caulobacter crescentus cell cycle control

When Caulobacter crescentus enters S-phase the replication initiation inhibitor CtrA dynamically positions to the old cell pole to be degraded by the polar ClpXP protease. Polar delivery of CtrA requires PopA and the diguanylate cyclase PleD that positions to the same pole. Here we present evidence that PopA originated through gene duplication from its parologue response regulator PleD and subsequent co-option as c-di-GMP effector protein. While the C-terminal catalytic domain (GGDEF) of PleD is activated by phosphorylation of the N-terminal receiver domain, functional adaptation has reversed signal transduction in PopA with the GGDEF domain adopting input function and the receiver domain serving as regulatory output. We show that the N-terminal receiver domain of PopA specifically interacts with RcdA, a component required for CtrA degradation. In contrast, the GGDEF domain serves to target PopA to the cell pole in response to c-di-GMP binding. In agreement with the divergent activation and targeting mechanisms, distinct markers sequester PleD and PopA to the old cell pole upon S-phase entry. Together these data indicate that PopA adopted a novel role as topology specificity factor to help recruit components of the CtrA degradation pathway to the protease specific old cell pole of C. crescentus.

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Chemoinformatics-assisted development of new anti-biofilm compounds

Bacterial biofilms are associated with a large number of infections. Biofilm-dwelling bacteria are particularly resistant to antibiotics, making it hard to eradicate biofilm-associated infections. Here, we use a novel cross-disciplinary approach combining microbiology and chemoinformatics to identify new and efficient anti-biofilm drugs. We found that ellagic acid (present in green tea) significantly inhibited biofilm formation of Streptococcus dysgalactiae. Based on ellagic acid, we performed in silico screening of the Chinese Natural Product Database to predict a 2nd-generation list of compounds with similar characteristics. One of these, esculetin, proved to be more efficient in preventing biofilm formation by Staphylococcus aureus. From esculetin a 3rd-generation list of compounds was predicted. One of them, fisetin, was even better to abolish biofilm formation than the two parent compounds. Fisetin dramatically inhibited biofilm formation of both S. aureus and S. dysgalactiae. The compounds did not affect planktonic growth in concentrations where they affected biofilm formation and appeared to be specific antagonists of biofilms. Arguably, since all three compounds are natural ingredients of dietary plants, they should be well-tolerated by humans. Our results indicate that such small plant components, with bacterial lifestyle altering properties are promising candidates for novel generations of antimicrobial drugs. The study underlines the potential in combining chemoinformatics and biofilm research.
Projects:

APUA: Animal Production without Antibiotics


National Food Institute
Research Group for Genomic Epidemiology
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University of Copenhagen
ISI Food Protection APS
BioMar A/S
Period: 01/01/2011 → 30/06/2015
Number of participants: 3
APUA, Animal Production, without Antibiotics
Project participant:
Aarestrup, Frank Møller (Intern)
Ebbensgaard, Anna Elisabeth (Intern)
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