Potential Therapeutic Uses of Novel Antimicrobial Peptides Isolated from the Rumen Microbiome Against *Pseudomonas aeruginosa*

Adam J. MULKERN[1], Linda B. OYAMA[1], Michael GRAZ[2], Gareth EVANS[2], Danielle WILLIAMS[2], Sharon A. HUWS[1]

[1] Institute of Biological, Environmental and Rural sciences, Aberystwyth University, Aberystwyth, SY23 3DA, Wales, UK.

[2] Neem Biotech, Units G+H Roseheyworth Business Park, Abertillery, Blaenau Gwent, NP13 1SX, Wales, UK.

Background

Within the European Union, broadly one in 2500 babies are born with cystic fibrosis (CF). Sufferers of CF are inadvertently more susceptible to opportunistic pathogens such as *Pseudomonas aeruginosa*. Persistent colonisation of the lungs with *P. aeruginosa* can lead to the formation of drug resistant biofilms, facilitating the development of life-threatening infection and limiting the effectiveness of antibiotics. Despite the wealth of knowledge on CF, there is still no known cure and developing alternative biofilm control agents is hugely important. This study will examine the potential of three novel, semi-synthetic antimicrobial peptides (AMPs), identified from the rumen as agents for treatment of *P. aeruginosa* infections in CF patients.

Methodology

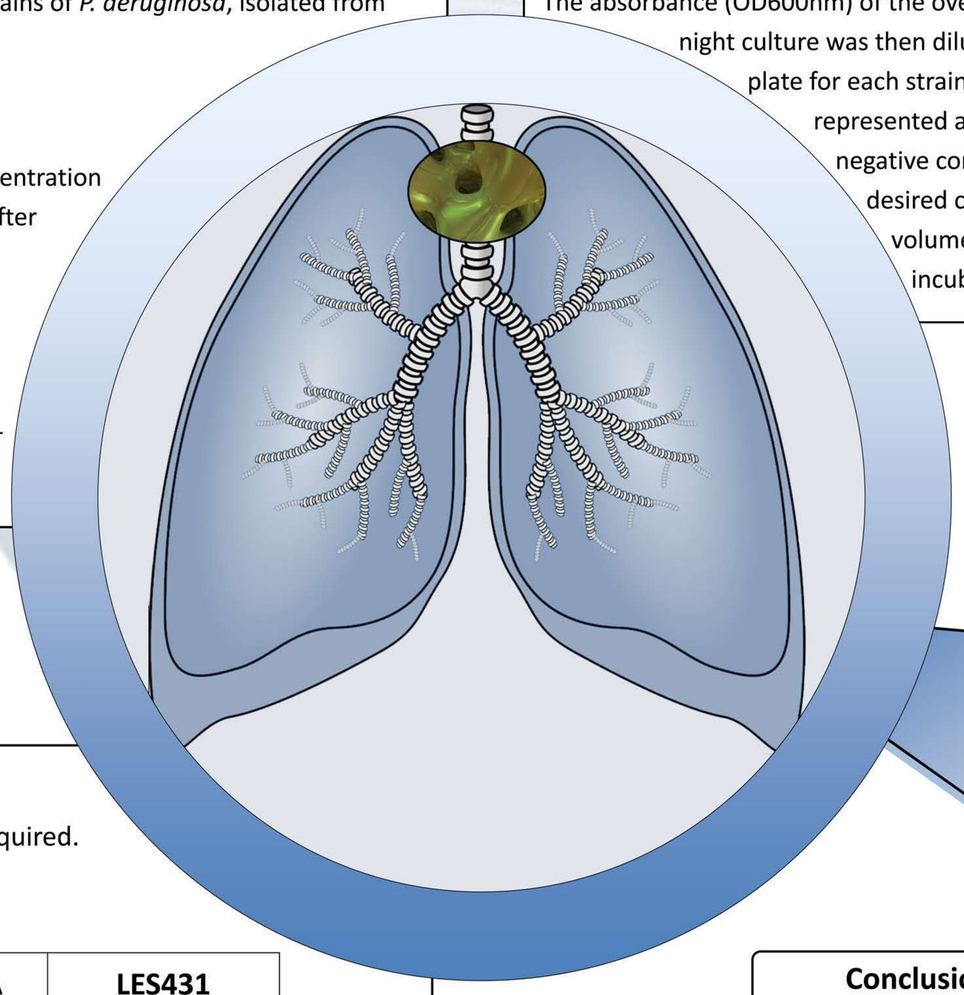
These AMPs have been previously shown to have activity against other Gram-negative bacterial strains. The efficacy of these AMPs is being tested against 62 strains of *P. aeruginosa*, isolated from CF patients including the Liverpool Epidemic strains (LES).

Minimum Inhibitory Concentration (MIC)

assays have been utilised in order to determine the lowest concentration of a particular AMP to inhibit the visible growth of each strain after overnight incubation in Mueller-Hinton broth.

Minimum Bactericidal Concentration (MBC)

assays have also been utilised to determine the lowest concentration of antimicrobial required to prevent the growth of an organism after subculture on to Mueller-Hinton agar.

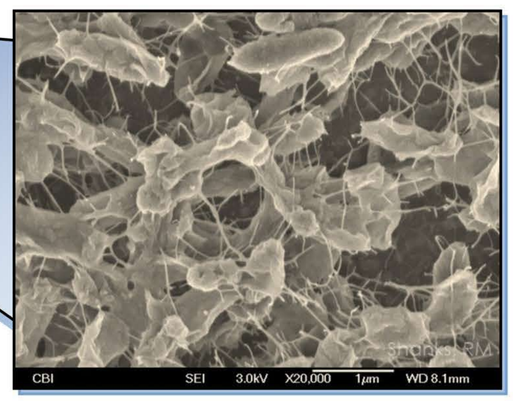


MIC Determination: Broth Microdilution for AMPs

The absorbance (OD600nm) of the overnight culture was checked to ensure the value is ≤1. The overnight culture was then diluted (1:100) in MH broth to form a 'starter culture'. A 96-well plate for each strain was filled with the corresponding culture, excluding row H that represented a growth control and columns 11 and 12 that were used as a negative control with MH broth only. The peptides were added at 10X the desired concentration to the first column and a serial dilution of 50µl volumes was performed from column 1 to 10. The plates were then incubated at 37°C overnight.

MBC Determination

Following MICs, all of the wells were then plated on MH agar. The visible colonies were then counted and the concentration at which 90% of the bacteria are dead was noted.



Results

Table 1. MIC and MBC data showing the µg/ml of peptide required.

	AES-1R		NH57388A		LES431	
	MIC	MBC	MIC	MBC	MIC	MBC
Petpide 2	16	32	16-32	64	8	16
Peptide 3	256	256-512	64	128	32-64	64-128
Peptide 7	128-256	256	64	128	64	128
Peptide 15sec	64	128	64	128	32-64	64
Polymyxin B	0.5	1	0.5	1	0.2	>0.5
Levofloxacin	4	>4	2	4	2	4

Conclusion

Following several repeats, minimum inhibitory concentrations of most strains were between 8-32 µg/ml. Biofilm and time kill kinetic assays are ongoing in an attempt to show that AMPs are capable of rapidly acting to decrease large numbers of bacterial cells within the first 2 hours of exposure. Further characterisation and mode of action studies are also ongoing. These promising antimicrobial peptides derived from the cow rumen may form new alternatives for the effective control of *P. aeruginosa* infections and hopefully improve the lives of cystic fibrosis patients.

Ysgoloriaethau Sgiliau Economi Gwybodaeth
Knowledge Economy Skills Scholarships

IBERS
Athrofa y Gwyddorau Biolegol, Amgylcheddol a Gwledig
Institute of Biological, Environmental and Rural Sciences

Ewrop & Chymru: Buddsoddi yn eich dyfodol
Cronfa Gymdeithasol Ewrop
Europe & Wales: Investing in your future
European Social Fund