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Ajoene: First in Class Quorum Sensing Inhibitor that Prevents Biofilm Formation, the Production of Virulence Factors and Modulates Established Biofilms

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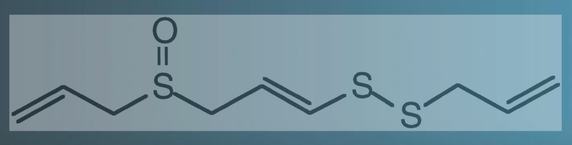
Background

In chronically-infected wounds, the prevention of biofilm formation, the disruption of mature biofilms, the reduction of virulence factors and thus the spreading of infection remains clinically elusive. Quorum sensing (QS) pathways regulate microbial motility, virulence factor production and the formation and maturation of biofilms and thus present a potential mode of therapeutic intervention.

Here we present the activity of ajoene, a QS Inhibitor (QSI), and its effects on motility, biofilm formation, virulence factor production and spreading infection, including activity on *ex-vivo* wound specific mature biofilm models.



Methods

Quorum sensing inhibition: A gene reporter assay was used with *rhlA*-gfp and *lasB*-gfp modified PAO1. SA 8325 derived spa::lacZ fusion strain was exposed to Ajoene (3-0.9mM) in an agar plate diffusion assay.

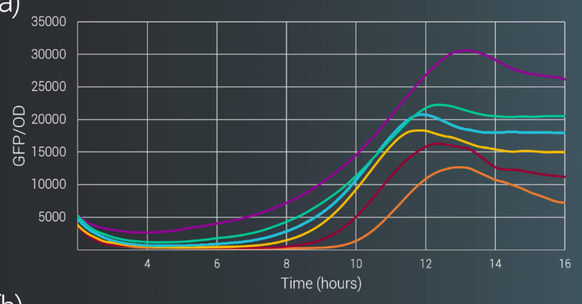
Inhibition and disruption of biofilms: P. Aeruginosa (PA01) and *S.aureus* (NCTC 8325) were grown for 24 h in the presence of compound at concentrations between 0.001 and 96 µM depending on strain. Biomass was stained with crystal violet and compared to vehicle control. *S.aureus* (NCTC 8325) was grown for 24 h in 96 well glass bottom plates and treated with 2 µM Ajoene and/or 0.15% Iodine and stained with ABCM live cell stain for confocal imaging (Leica DMi8).

Virulence assays: Isolates were inoculated (OD_{595} 0.7) on 0.4% agar plates containing increasing concentrations of ajoene or vehicle and incubated at 30°C for 24 h. Swarming motility was imaged and quantified using ImageJ analysis software. Bacteria were grown for 48 h in TSB with vehicle or Ajoene (125 µM). Exudates were collected and elastase and protease activity were measured and rhamnolipid and pyocyanin quantified using the orcinol method and spectrophotometry respectively. HaCaT cells were grown to 80-90% confluence, scratched and exposed to bacterial exudates for 20 h. Re-epithelialisation were compared between condition.

Ex vivo Lubbock model: chlorine sterilised *ex vivo* porcine skin, inoculated with a mature multispecies biofilm (*P. aeruginosa* and *S. aureus*). Treatment 24 h of 50 µg cm^{-2} of 1% ajoene hydrogel, with or without 20 min iodine wash, repeated over 5 days in comparison to a blank control.

Inhibition of Quorum Sensing mechanisms

(a)



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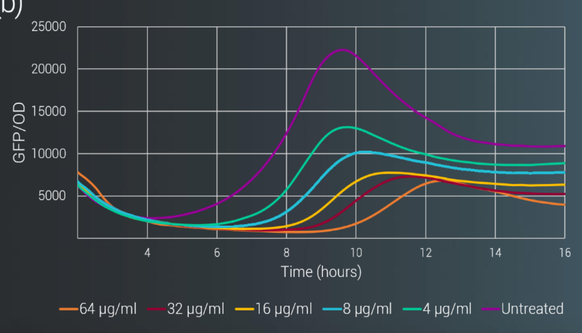
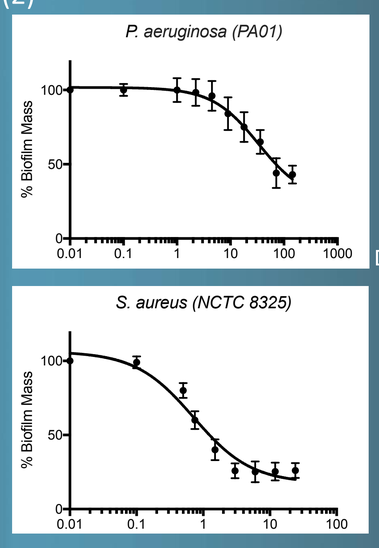


Addition of Ajoene to cultures of modified PA01 resulted in dose-dependent inhibition of *rhlA* (Figure 1a) and *lasB* (Figure 1b). GFP expression, normalised to growth was significantly reduced at 16 hrs ($F(5,37) = 2.79$, $P = 0.031$ and $F(5,37) = 52.26$, $P = 9.41E-16$ respectively).

Ajoene further showed dose-dependant effect on SA QSI in the *spa::lacZ* fusion strain, with zone diameter between 30-32mm (data not shown).

Inhibition and disruption of biofilms

(2)



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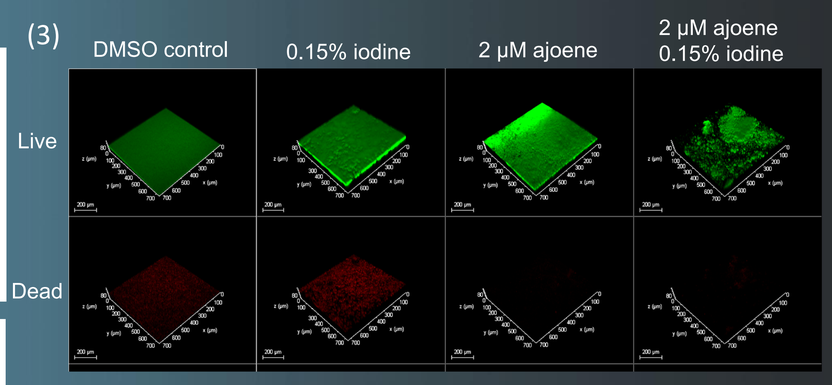
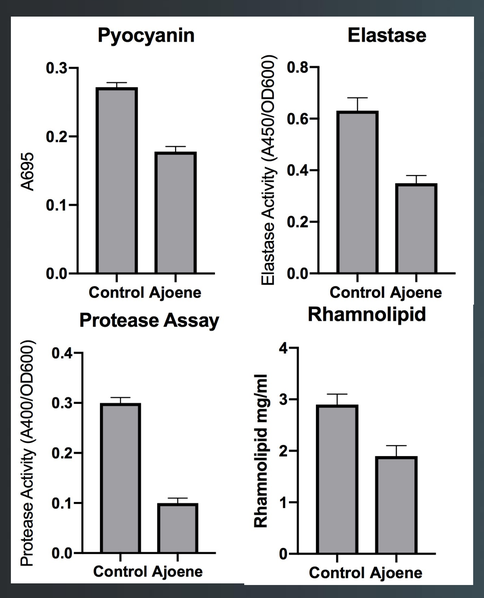


Ajoene dose dependently inhibits biofilm formation of *P. aeruginosa* and *S.aureus* (MBIC 39 µM and 0.3 µM respectively) (Figure 2).

Preformed *S.aureus* biofilms treated with Ajoene and 0.15% iodine show a disrupted structure and enhanced clearance. (Figure 3)

Inhibition of virulence of *P. aeruginosa*

(4)



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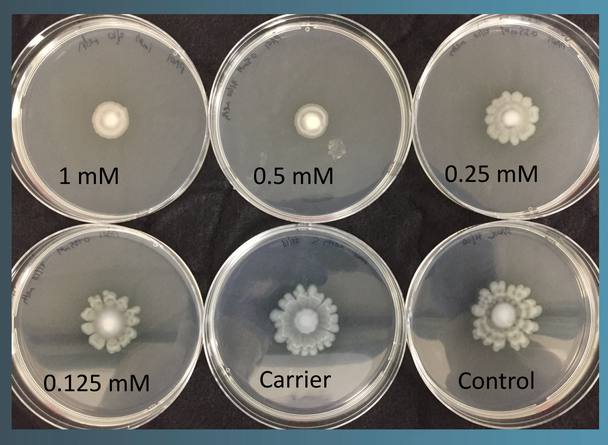
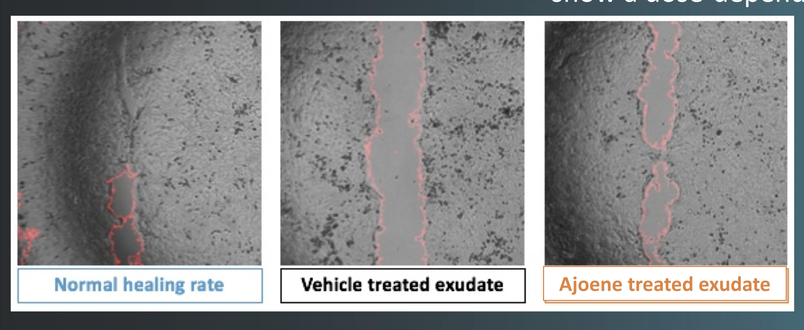


P. aeruginosa cultured in the presence of Ajoene for 48 h show reduction in protease and elastase activity, and the amount of secreted pyocyanin and rhamnolipid compared to vehicle control (Figure 4). *P. aeruginosa* grown on Ajoene containing agar plates show a dose-dependent inhibition of swarming motility, a key phenotype of virulence required for spreading infection (Figure 5).

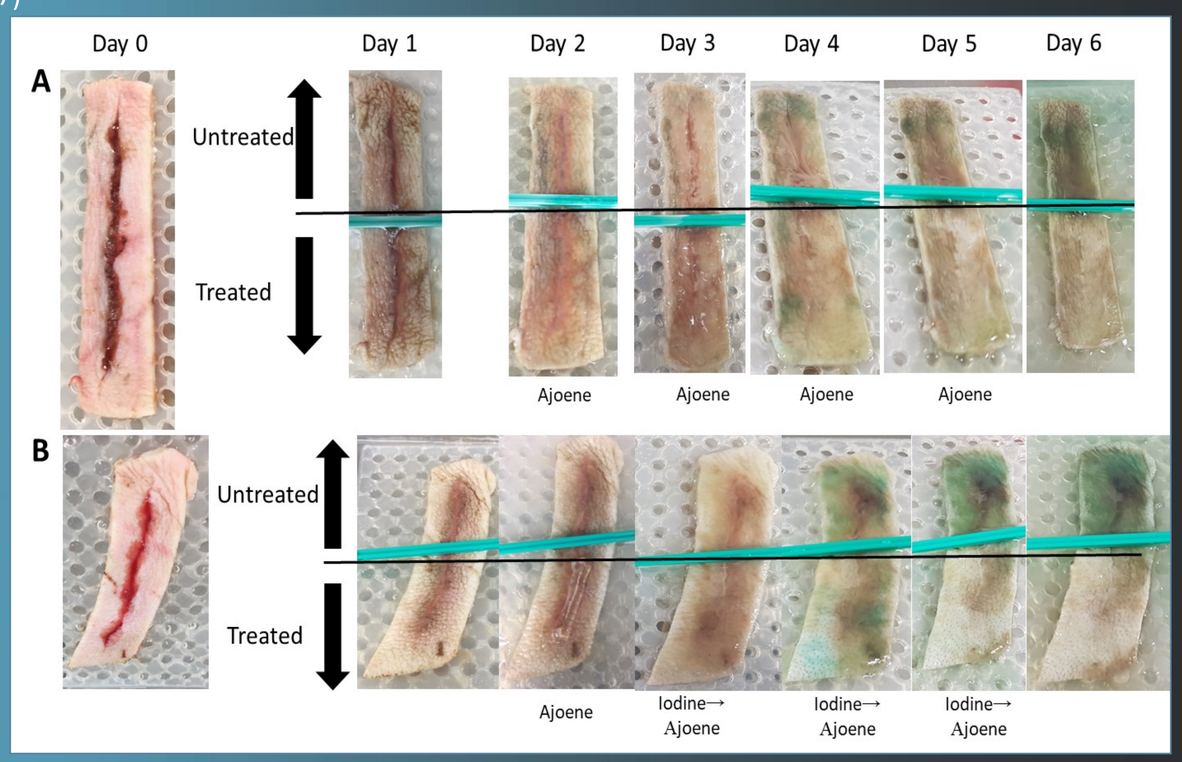
(6)



Ajoene treatment reduces the impact of bacterial exudate on HaCat cells, showing a rescued rate of re-epithelisation and improved healing (Figure 6).

Ex vivo efficacy against mixed species biofilm infection

(7)



Application of Ajoene within a clinically relevant hydrogel formulation for 24 h conferred a 4.8 and 5.5 log reduction of Pa and Sa cm^{-2} respectively.

When Ajoene was combined with an iodine wash at 10%, a 5.4 and 6.8 Log reduction of Pa and Sa cm^{-2} was observed.

SUMMARY

Ajoene is a quorum sensing inhibitor that prevents and disrupts the formation of biofilms and reduces virulence across bacterial strains.

Efficacy translates to an *ex vivo* wound model to enhance the eradication of mixed species biofilms.

Neem Biotech has established a screening cascade for virulence and biofilms. Novel proprietary analogues are now being compared for selection of a clinical development candidate.

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WELSH WOUND INNOVATION

Innovate UK