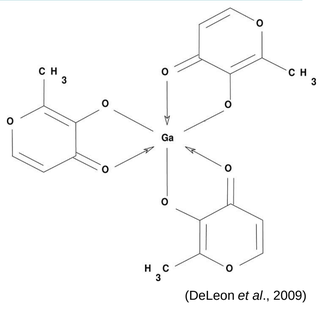
Characterisation of the Antimicrobial Mode of Action of Gallium Maltolate

Magdalena Piatek[a], Darren Griffith[b], Kevin Kavanagh[a]

a. SSPC Research Centre, Department of Biology, Maynooth University, Maynooth, Co. Kildare, Ireland.
b. SSPC Research Centre, RCSI, Dublin 2.

Introduction

The unconventional mechanism of action of gallium has made it an attractive novel therapeutic for the treatment of microbial infections. The structural resemblance of gallium and iron is accounted for by a similar ionic radius, however unlike iron, gallium cannot be reduced to a divalent form. Bacteria preferentially incorporate gallium and as a result irreversibly inhibit iron-dependent metabolic pathways that are vital for growth, proliferation and virulence in pathogenic bacteria [1].



(DeLeon *et al.*, 2009)

The development of gallium maltolate (GaM), a central trivalent gallium ion bound to three maltolate ligands, has reduced toxicity and improved stability and efficacy of the treatment [2].

The aim of this research was to investigate the antimicrobial potential of GaM against the opportunistic pathogen *P. aeruginosa* and gain insight into the intrinsic mechanisms of action of GaM via proteomic analysis.

Method

Label-free Quantitative Proteomics

P. aeruginosa supplemented with 0.5 mg/ml and 1.0 mg/ml of GaM was cultured until exponential growth was established. *P. aeruginosa* proteins were extracted, digested and purified and loaded onto a Q Exactive (ThermoFisher Scientific) LC-MS/MS system for protein identification and quantification.

Raw MS data was processed via MaxQuant software and coordinated with a UniProt database for *P. aeruginosa*. Further analysis of the data was performed using statistical and illustrative methods on Perseus v1.6.6.0.

Results Part A

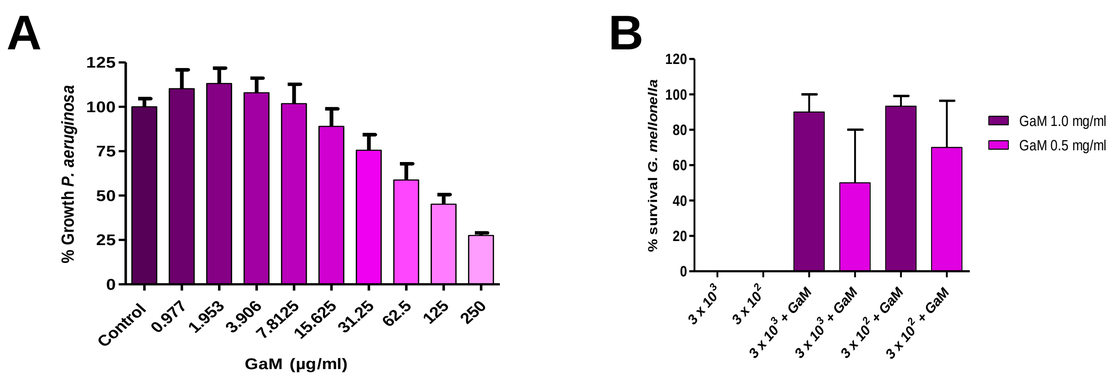
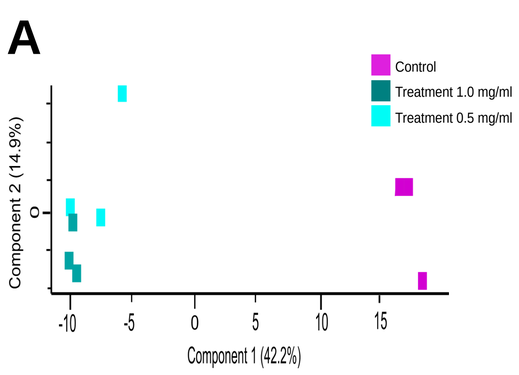


Fig 1. The effect of GaM on *P. aeruginosa* growth *in vitro* and *in vivo*. (A) The susceptibility of *P. aeruginosa* to a range of GaM concentrations (µg/ml). (B) Survival of infected *Galleria mellonella* larvae treated with GaM. Larvae injected with 300 and 3000 colony forming units (CFUs) *P. aeruginosa* and 20 µl GaM 0.5 and 1.0 mg/ml were observed after 24 hours at 37 °C.

Results Part B



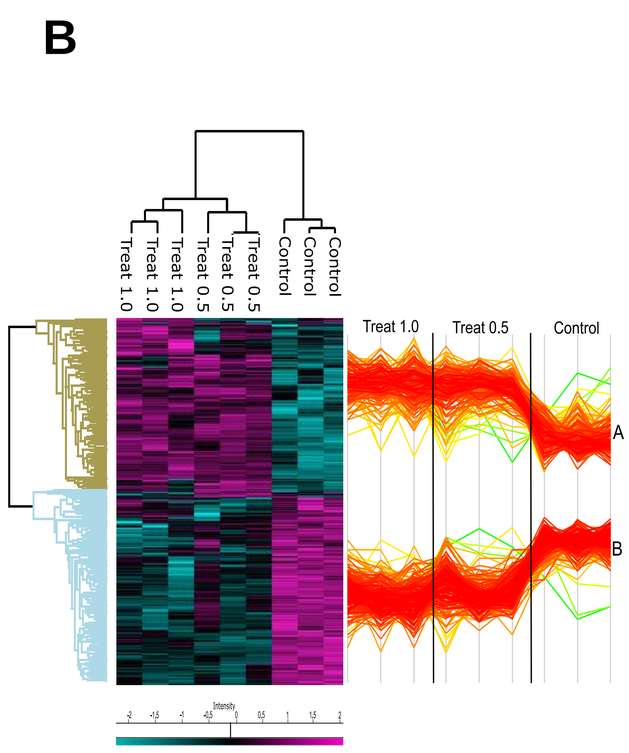


Fig 2. Label-free quantitative proteomic analysis of *P. aeruginosa* in response to GaM after 6 hours. (A) Principal component analysis (PCA) shows a distinct contrast between GaM treated samples versus the control. (B) Two-way unsupervised hierarchal clustering of the median expression levels of all statistically significant differentially abundant proteins (SSDA), (n = 351). On the basis of similar expression trends, three replicates are grouped together into three hierarchal clusters (columns), each representing two protein clusters (A and B).

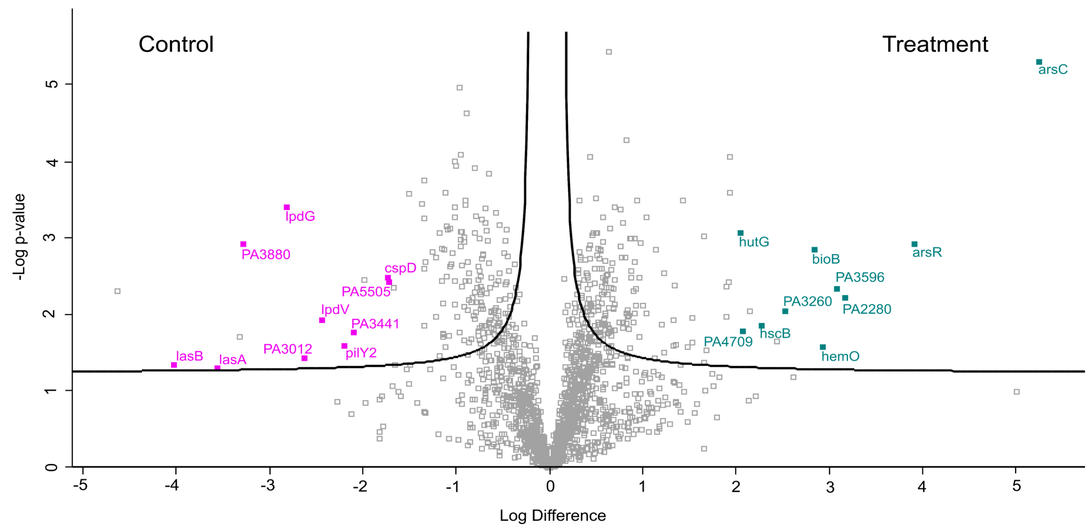


Fig 3. Volcano plot representing differentially expressed proteins in *P. aeruginosa* treated with 1.0 mg/ml GaM for 6 hours. The distribution of quantified proteins is based on significance ($-\log_{10}$ p-value) versus the fold change (Log_2 LFQ intensity difference). Statistically significant (p-value <0.05) proteins are located above the horizontal line. Expression transcripts with relative fold changes of >1.5 are shown to the right (Treatment) and left (Control) of the vertical lines. The top 20 characterised and differentially abundant proteins are annotated.

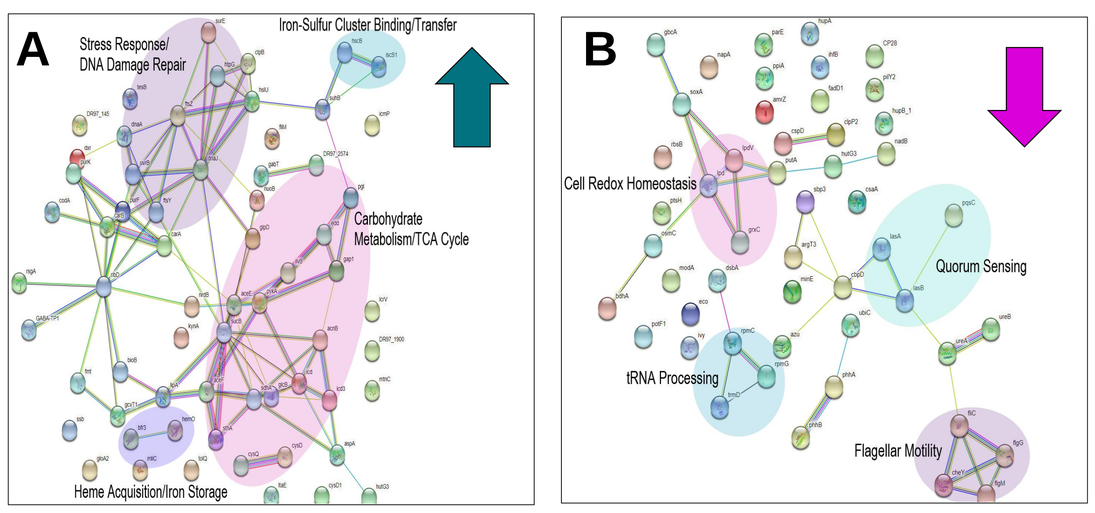


Fig 4. Network analysis of proteins increased and decreased in abundance in *P. aeruginosa* treated with 1.0 mg/ml GaM. Data obtained from the STRING database using gene lists from SSDA proteins from pair wise t-tests (p <0.05) shows interactions among individual proteins. (A) Protein pathways upregulated in GaM-treated *P. aeruginosa*. (B) Protein pathways downregulated in GaM-treated *P. aeruginosa*.

Discussion and Conclusion

Treatment of *P. aeruginosa in vitro* inhibited growth by up to 72.5% after 24 hours using a maximum concentration of 250 µg/ml GaM (Fig 1. (A)).
The non-toxic, bacteriostatic activity of GaM increased survival rates of infected *G. mellonella* larvae from 0 to 90% and 100% when treated with GaM post inoculation with 300 and 3000 CFUs of *P. aeruginosa*, respectively (Fig 1.(B)).

Proteomic analysis identified a distinct contrast between the proteomes of GaM-treated *P.aeruginosa* versus the controls (Fig. 2). Further investigation of protein pathways revealed increased expression of iron-storage protein Bacterioferritin B, the HemO component of the heme acquisition system and iron-sulfur clusters [3]. Upregulation of these proteins suggest a compensatory mechanism is adopted for the uptake of gallium into the cells in place of iron. Evidence of cell stress is shown through the upregulation of Chaperone Proteins ClpB, HtpG, and DnaJ [4,5]. Decreased abundance of proteins associated with flagellar motility, tRNA processing and quorum sensing indicate attenuation of virulence and hence growth inhibition [6,7] (Fig 4).
In conclusion, this proteomic approach has provided insight on the mechanisms of action of GaM, a promising novel therapeutic for the treatment of *P. aeruginosa*.

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Acknowledgements

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