Investigation into bacterial siderophores and their uses as novel therapeutics

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INTRODUCTION

Iron is the fourth most prevalent metal in the Earth's crust and a micronutrient, which is typically inaccessible to microorganisms [1] and at very low concentrations which allows for healthy cellular functioning. Microorganisms produce and excrete powerful iron chelators to sequester Fe^{3+}, also known as siderophores (Fig.1) [2]. They are characterised and classified according to their chemical functional groups to chelate iron. These include catecholate-type (phenolate), hydroxamate, carboxylate, and mixed-type siderophores.

Siderophores can be conjugated to antibiotics, and using the iron pathway drugs can be delivered to otherwise resistant bacterial cells. This is a novel strategy that is gaining popularity [3]. Therefore, this study was conducted to identify potential bacteria from the environment which produce high levels of siderophores, as well as to characterise the siderophores and their uptake methods.

AIMS/OBJECTIVES

The current study was designed to: (i) identify and describe siderophores obtained from various bacterial cultures, isolated from Cambridge rivers; (ii) assess optimal growth conditions for siderophore synthesis utilising relevant physical-chemical factors; (iii) assessing siderophores as an antimicrobial agent against known laboratory strains.

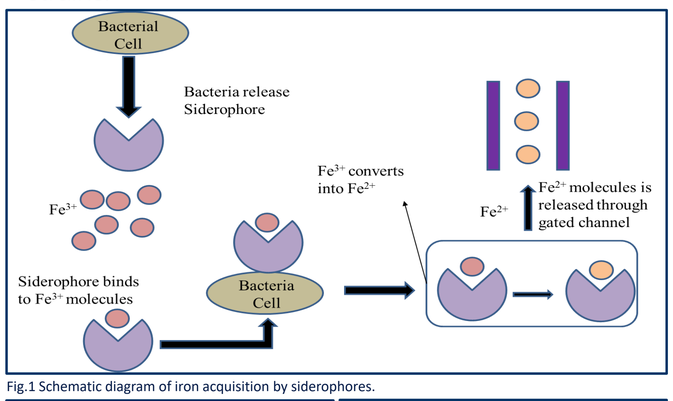
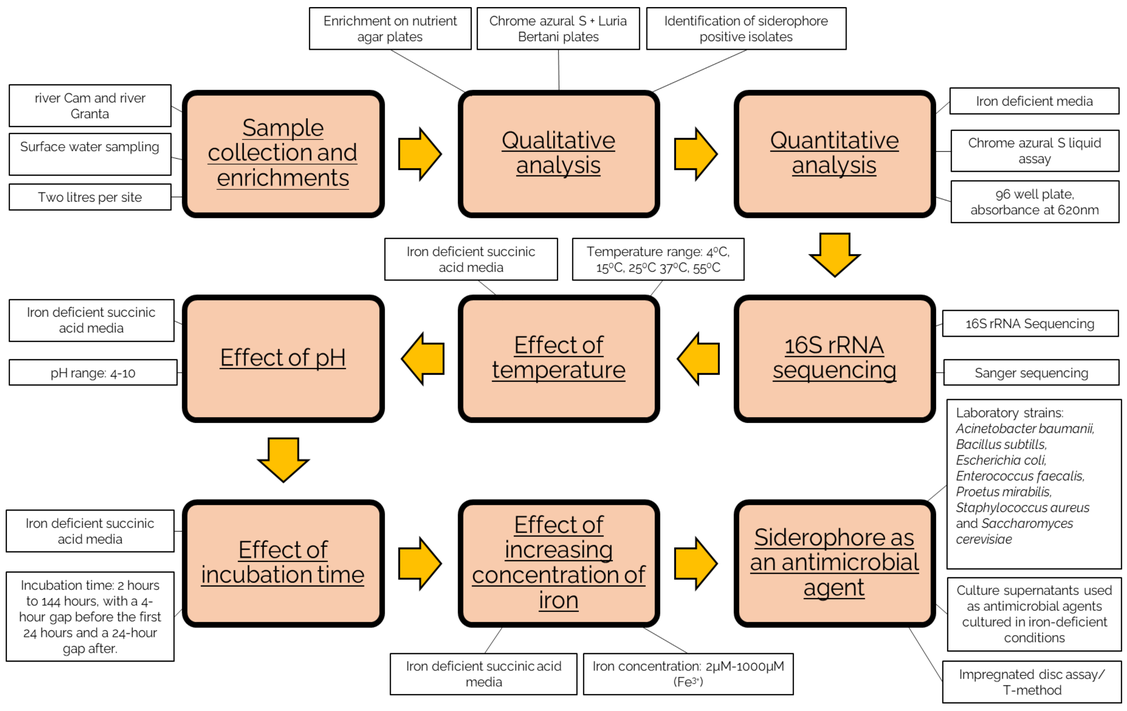


Fig.1 Schematic diagram of iron acquisition by siderophores.

MATERIALS & METHODS



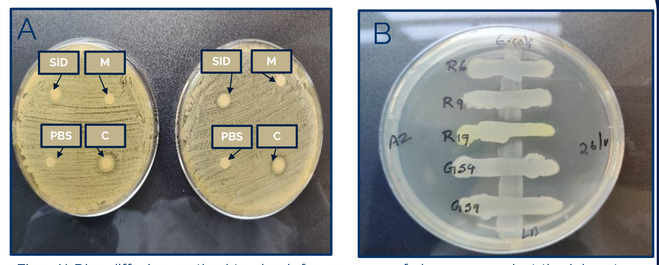


Fig.9 A) Disc diffusion method to check for any zone of clearance against the laboratory strains using disc impregnated with test samples. SID = culture supernatant grown in iron-deficient succinic acid media, M= succinic acid media, PBS= phosphate-buffered saline, C= target culture suspension. B) T technique, cultures spread across each other on Luria Bertani agar plates to see whether any secondary metabolites produced to function as an antimicrobial agent, reducing the proliferation of the other microorganisms.

RESULTS

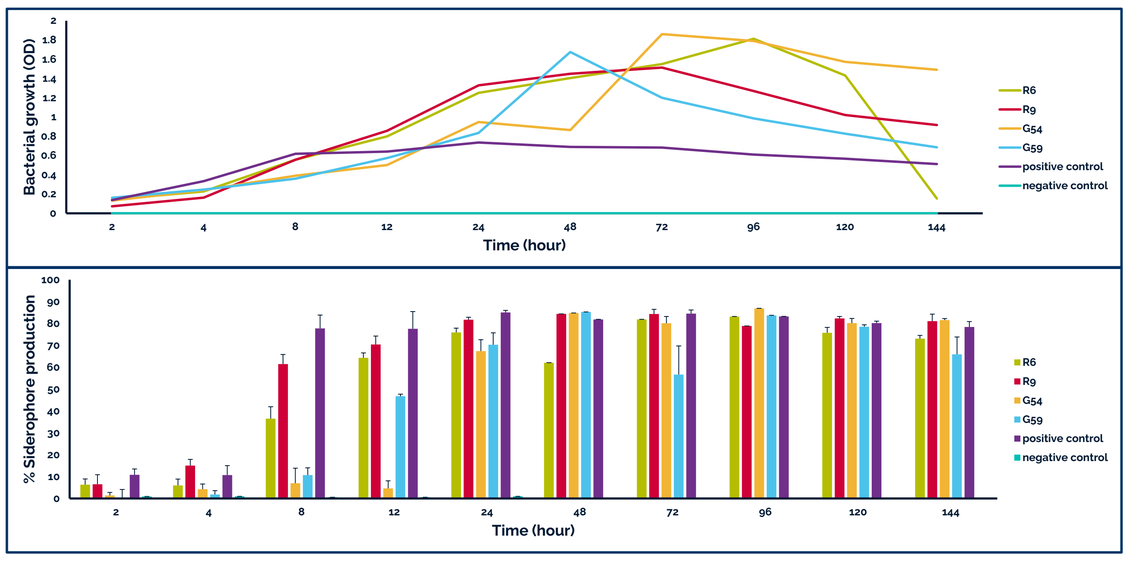


Fig.4 Bar graph of bacterial isolates G59, R6, G54, R9, and positive control represent siderophore production and line graph represents growth curve at different time points 4, 8, 12, 24, 24, 48, 72, 96, 120, and 144hrs. Abscissa shown at each point is the standard deviation of duplicate samples. % Relative to absorbance at λ = 620 nm to succinic acid media + CAS dye only, positive control= *Pseudomonas aeruginosa*, negative control= succinic acid media + CAS dye, control= cultures grown under optimized condition in succinic acid media. Results are expressed as mean ± SEM, n=3.

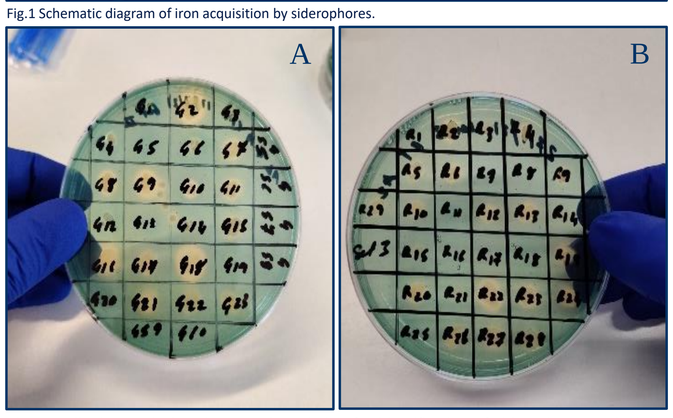


Fig.2 Qualitative analysis using spot inoculation for identification of siderophore producing bacteria on Luria Bertani+ Chrome Azurl S agar plates. A) isolates obtained from river Cam, B) isolates obtained from river Granta

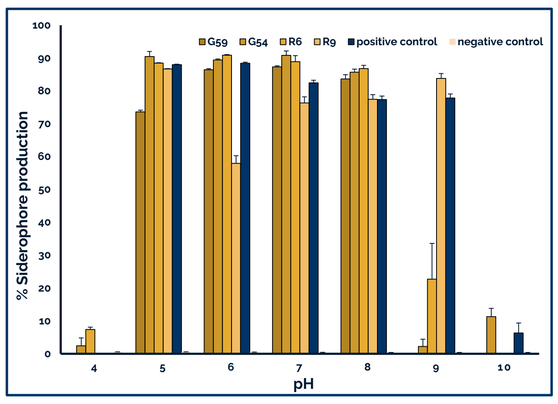


Fig.5 Effect of pH on siderophore production at different pH (4, 5, 6, 7, 8, 9, and 10). % Relative to absorbance at λ = 620 nm to succinic acid media + CAS dye only, positive control= *Pseudomonas aeruginosa*, negative control= Succinic acid media + CAS dye. Results are expressed as mean ± SEM, n=3.

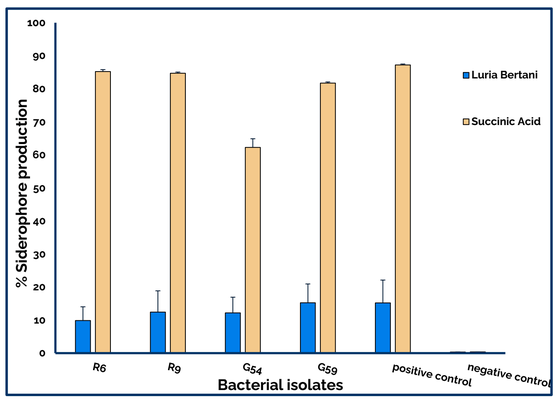


Fig.6 Effect of different media on siderophore production was estimated, to compare the effect of nutrient-rich media and nutrient and iron-deficient media. % Relative to absorbance at λ = 620 nm to succinic acid media + CAS dye only, positive control= *Pseudomonas aeruginosa*, negative control= succinic acid media + CAS dye. Results are expressed as mean ± SEM, n=3.

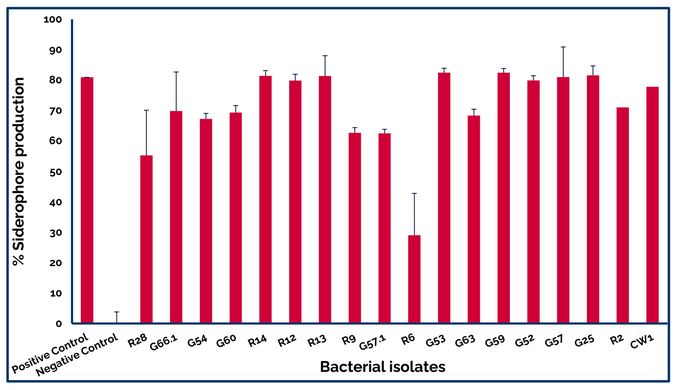


Fig. 3 Quantitative estimation of isolates showing highest siderophore producing microorganisms procured from qualitative analysis. % Relative to absorbance at λ = 620 nm to succinic acid media + CAS dye only, positive control = *Pseudomonas aeruginosa*, negative control = succinic acid media + CAS dye. Results are mean ± SEM, n=3.

16S rRNA Sequencing ↓

Laboratory ID	16S rRNA sequencing
(R9)	*Aeromonas autraliensis*
(R6)	*Aeromonas rivipollensis*
(G59)	*Kosakonia oryzendophytica*
(G54)	*Pentoea agglomerans*

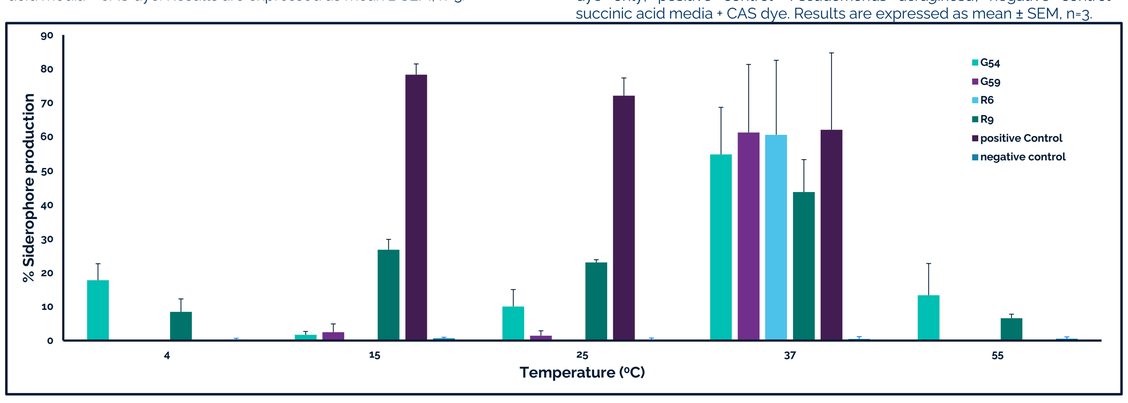


Fig.8 The effect of temperature on the formation of siderophores by isolates from the rivers Cam and Granta in an iron-deficient media. Bacterial isolates were cultivated overnight in nutrient broth without iron, washed, and reinoculated to a cell density of 0.6 OD 600 units in Succinic acid medium without $FeCl_3$. The cells were incubated at 37ºC with shaking, and siderophore production was calculated as a percentage relative to absorbance at λ = 620 nm to succinic acid medium + CAS dye, positive control= *Pseudomonas aeruginosa*, negative control= succinic acid media + CAS dye. The results are shown as mean ± SEM, n=3.

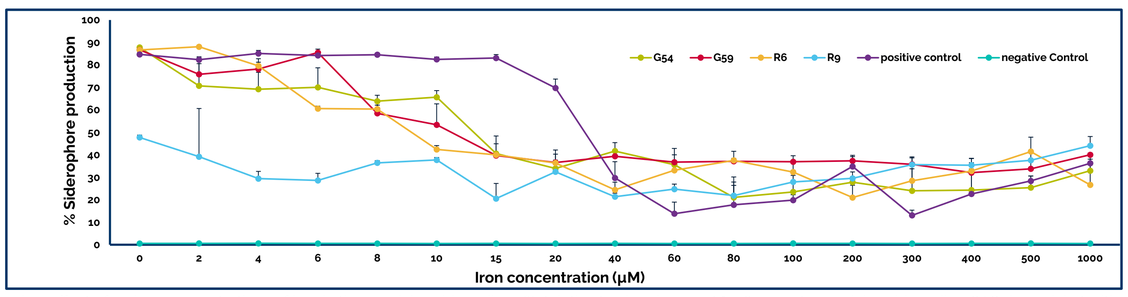


Fig.7 Effect of various concentrations of iron in the succinic acid medium. % Relative to absorbance at λ = 620 nm to succinic acid media + CAS dye only), Positive Control= *Pseudomonas aeruginosa*, negative control= Succinic acid media + CAS dye. Results are expressed as mean ± SEM, n=3.

DISCUSSION

- Isolates from river water cultured initially on LB agar were subsequently streaked on CAS agar as a secondary screening technique for qualitative estimation (Fig 2). Orange halos indicated the production of siderophores .
- Overall, 68 bacterial colonies produced siderophores, of which 19 isolates produced siderophores in the range of 80-85% (Fig 3) and were sequenced to identify from which five were further optimised.
- During optimisation, the greatest siderophore production was in nutrient-deficient conditions containing no iron at pH ranges of 5-8 and at 37ºC (Fig 5, 6, 7, 8). These are deemed suitable candidates to further explore.
- The influence of incubation period indicated that siderophore synthesis increased during the lag phase and then plateaued during the stationary phase (Fig.4), demonstrating that siderophore production is at its maximum during active bacterial growth.
- When administered as an antibacterial agent, supernatant containing siderophores did not show inhibition against known laboratory strains (Fig 9 A, B)

FUTURE WORK

- The siderophore production was optimised to maximise their yield, these will be purified using HPLC and further characterised to explore their ways as therapeutic agents.
- Specific genes involved in siderophore biosynthesis and transport mechanisms will be identified for gene knock-out experiments to better understand the uptake mechanisms of recipient cells.

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REFERENCES

1. Machuca, A., & Milagres, A. M. F. (2003). Use of CAS-agar plate modified to study the effect of different variables on the siderophore production by Aspergillus. 1987, 177–181.
2. Schwyn, B., & Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, *160*(1), 47–56. https://doi.org/10.1016/0003-2697(87)90612-9
3. Chaudhary, D. Y., Gosavi, P., & Durve-Gupta, A. (2017). isolation and application of siderophore producing bacteria. *international Journal of Applied Research*, *3*(4), 246–250. www.allresearchjournal.com