Summer Student Project Report

Student
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Title of Project

Investigating the potential for *C.albicans* infective endocarditis strain to induce immunothrombosis and the resulting consequences on antifungal resistance.

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Lay Summary

Candida albicans is a commensal fungus that resides on the skin, gastrointestinal tract, or mouth of up to 60% of healthy people (Kim and Sudbery, 2011; Pappas et al., 2018). It can enter the blood (Candidaemia) through different indwelling medical instruments such as cannulas and catheters (Richards et al., 2000). This fungus can become an issue if the host is immunocompromised, especially with a low neutrophil count meaning they cannot adequately fight off infection. Thrombus formation occurs secondary to immune response which together are known as immunothrombosis (Eberl et al., 2019). Amongst other components which make up blood, platelets, and neutrophils play an important role in fighting off Candidaemia and they work together to do this efficiently.

We performed a series of experiments on two strains of *Candida albicans* to understand how they interact with platelets, activate neutrophils and how these blood cells work together to reduce the pathogen spreading in circulation.

What was the aim of your project?

The aim was to compare the two strains of *Candida albicans* with different virulence in relation to immunothrombosis induction, with BS3 being more virulent than SC314.

How did you address the aim?

Hyphal morphology

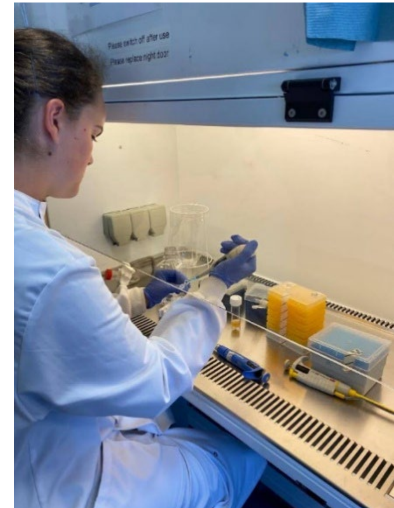
To grow the fungi to the stationary stage, a colony of the respective strain was incubated in YPD at 37°C overnight under shaking conditions. To make and visualise the hyphae, the yeast was stained with calcofluor white, resuspended in RPMI and incubated in static conditions at 37°C two hours. We used a fluorescent microscope to visualise the hyphae followed by ImageJ to measure hyphal length, then created bar graphs to compare hyphal length, number of yeast and number of hyphae in aggregates in the different strains.

Platelet aggregometry

We used a plate-based aggregation to investigate the effect of the different strains on platelet aggregation.

Platelet activation markers and Platelet-Neutrophil Complexes (PNCs)

To compare the two strains in relation to PNCs and platelet activation markers, we used flow cytometry.



NET formation

We used electron microscopy to investigate the impact of the two strains on NET formation with and without platelet presence.

Biofilm formation

We used spectrophotometry for both experiments on biofilm formation to investigate biofilm formation and response to two antifungal treatments (first experiment) and comparing the effects of neutrophils and platelets on the two strains (second experiment).

What did you find out?

Mycology (Figure 1A)

SC5314 and BS3 grew differently overnight (Ai); hyphae were a similar length statistically (Aii), however visually, SC5314 hyphae were longer than BS3 hyphae (Aii + Aiv). This could mean the BS3 strain has adapted to evade the immune system compared to the ancestral SC5314.

Platelet aggregation (Figure 1B)

BS3 increased platelet aggregation more than SC5314 which could be another adaptation of the BS3 strain compared to the SC5314 strain. BS3 tended to increase platelet aggregation more than SC5314, but this is not statistically significant. In panel Bi, the concentration response curve for the concentration of TRAP6 is reported; in Bii, a bar graph for 1µM TRAP6-induced platelet aggregation is shown.

Platelet activation markers and Platelet Neutrophil Complexes (PNCs) (Figure 1C)

BS3 and SC5314 both appeared to increase fibrinogen binding when compared to PRP alone, however there appeared to be no significant differences in fibrinogen binding when BS3 and SC5314 are compared to each other (Ci). SC5314 and BS3 appeared to increase P-selectin exposure compared to PRP, and there may be a quantitative difference between BS3 and SC5314 with the latter potentially stimulating more P-selectin exposure, however this was not statistically significant, potentially due to a small sample size (Cii).

Platelet Neutrophil Complexes (PNCs) (Figure 1C)

There is no statistically significant difference between strains and whole blood alone when PNCs are stimulated (Ciii) and unstimulated (Ciii), however studies have shown increases in PNCs in relation to *C albicans* hyphae which may be due to differences in experimental methodology. Further optimisation of experiments is needed.

Neutrophils and Neutrophil Extracellular Traps (NETs) (Figure 1D)

Compared to unstimulated neutrophils (Di), when TRAP6 was added to neutrophils, they formed NETs (Dii). When platelets were added to neutrophils (Diii and Div), we observe PNC formation only in cells

stimulated with TRAP6 (Div). Neutrophils appeared to interact with hyphae in the presence of platelets (Dv) but recruitment and defence of platelets and neutrophils against *C.albicans* is increased when TRAP6 Is added, with increased phagocytosis and NET formation observed (Dvi).

Biofilm formation (Figure 1E)

Two experiments were produced for this, one investigated biofilm formation and antifungal resistance between the two strains (Ei and Eii), and the other investigated biofilm formation when blood cells (Eiii and Eiv) were added to the strains.

SC5314 was reduced by Capsofungin and both strains were not cleared by fluconazole (Ei, Eii). Both strains were abolished in wells containing the neutrophils (Eiii, Eiv), corresponding with the behaviour of platelets and neutrophils in the NET formation experiment (Dv, Dvi).

In both biofilm experiments (Ei + Eii, Eiii + Eiv), BS3 did not grow well; in future experiments, BS3 would be allowed to grow for a longer period, or the concentration of yeast cells doubled.

What did you learn from participating in this project?

Overall, I enjoyed the project a lot, as it enabled me to learn more sophisticated techniques that I will find very useful for my final year project and other lab experiments including microscopy (fluorescent microscopy on different types of microscope including a smaller microscope to make sure the experiment had worked, followed by using a more technical microscope to visualise the samples in a higher definition), production and mounting of cover slips, reverse pipetting and flow cytometry, phlebotomy (including ethics, patient care and handling of blood), extraction of different blood components such as PRP, PPP and neutrophils and preparation for experiments including making Tyrodes solution, aseptic techniques, streak plates and general lab maintenance such as autoclaving.

I also had the opportunity to experience optimisation of a protocol as there was no adequate literature on how to create a biofilm assay for fungi, so my supervisor adapted a protocol used for bacteria and we used it for our experiments on *C albicans*. We ran two experiments, the first being a small scale experiment to see if the protocol worked, and the second was the actual experiment after a few modifications to the protocol from the previous week.

How has this project affected your long-term goals?

This project has made me really want to do a PhD especially in the field of platelet research as I love learning new things and it was it was so fascinating to understand how platelets interact with other cells and how this means someone is more likely to develop clots. The project has also inspired me to do a platelet related dissertation for my final year project in the upcoming year.

I have enjoyed passing my knowledge onto other people as I was posting a few photos of what I had been up to, on social media which sparked a lot of interest in the people who interacted with my posts which has made me think about wanting to do more relating to spreading awareness of thrombosis and formation of a thrombus and the different causes of it.

It has been wonderful to work as part of a wider thrombosis research team alongside other researchers who are also producing potentially groundbreaking research as well as PhD students which allowed me to ask them questions about what made them want to do a PhD and the project they were doing.

References

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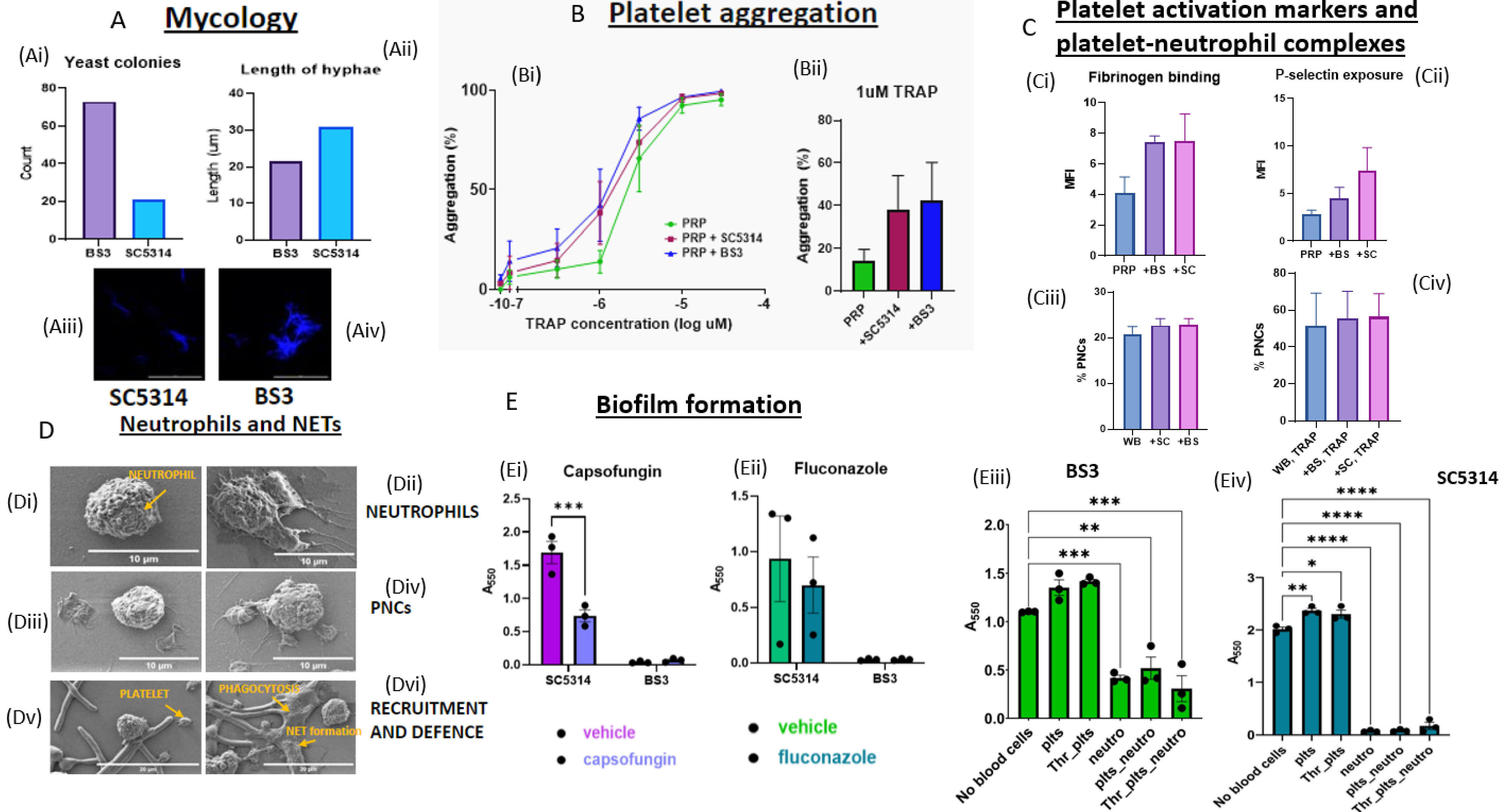

Figure 1: Visual summary compilation of the data from the project, including Mycology (A), Platelet aggregation (B) Platelet activation markers and platelet neutrophil complexes (C), Neutrophils and NETs (D) and Biofilm formation (E).