Inhibition of bacteriophages as a result of serum supplementation to the media

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Introduction

Bacterial infections are often **complicated** due to the **formation of biofilms**, especially in the setting of medical implantable devices such as **orthopedic implants**. When bacteria enter these surgical sites, they can **adhere** up to **10 000 times better** to the **implant material** than the native tissue surrounding the implant.

An estimated 80% of all clinical infections have an important biofilm component, thereby causing recurrent infections.

These (poly)microbial micro-communities, which are embedded in a self-produced polymeric matrix, are known to become **more antibiotic insensitive** when **compared to their planktonic counterparts** due essentially to the **metabolic shift** these bacteria undergo during biofilm maturation**.**

An important limitation is that current biofilm models used in laboratory practice are predominantly mono-species thereby not fully representing the clinical picture.

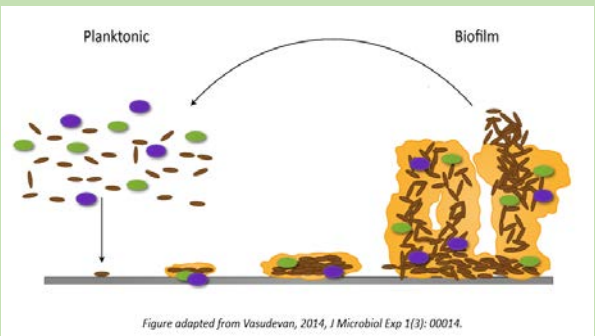


Figure adapted from Vasudevan, 2014, J Microbiol Exp 1(3): 00014.

Objectives

Creating a **multispecies biofilm model** incorporating ***P.aeruginosa***, ***S.aureus/ S.epidermidis*** and ***C.albicans,*** three well-know pathogens causing recurrent orthopedic infections, to eventually investigate phage-antibiotic synergies between routinely used antibiotics and de novo isolated bacteriophages on top of the effect of serum supplementation to the media

Methods

A **multispecies biofilm model** was first **developed in 96-well plates** incorporating the three microbial species mentioned above and grown in RPMI media supplemented with 1% glucose and 10% FBS. After stable biofilm formation and growth was obtained for several days, the **biofilm model was transferred to titanium coupons** to mimic the clinical picture found in orthopedic infections.

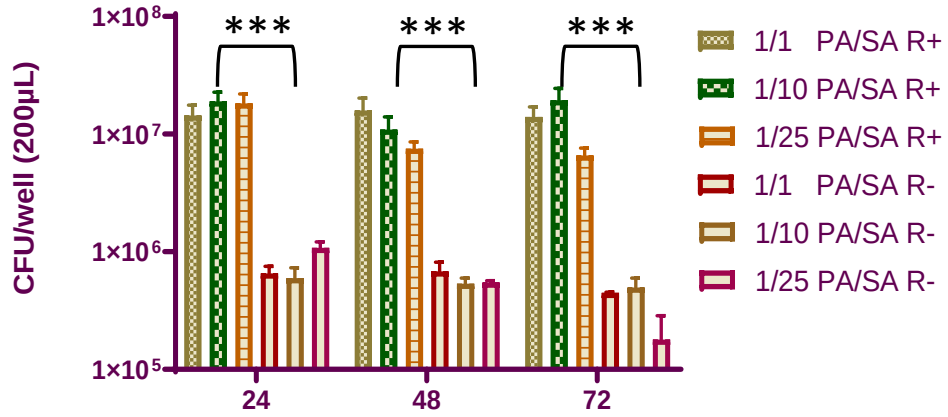
Phage-antibiotic synergy (PAS) assays were performed on mature biofilms, with routinely used antibiotics and **de novo isolated bacteriophages** (from a wide variety of environmental sources) targeting both *P.aeruginosa* and *S.aureus/epidermidis*.

After several unsuccessful PAS-assays on S.aureus/epidermidis, the **effect of serum supplementation to the media** was investigated.

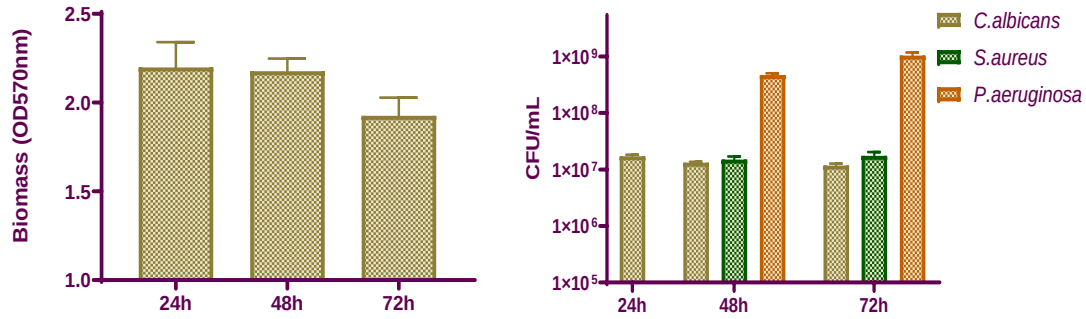
Biomass evaluations (1% crystal violet, OD570nm) and viable cell counts were performed for all treatment modalities.

Results

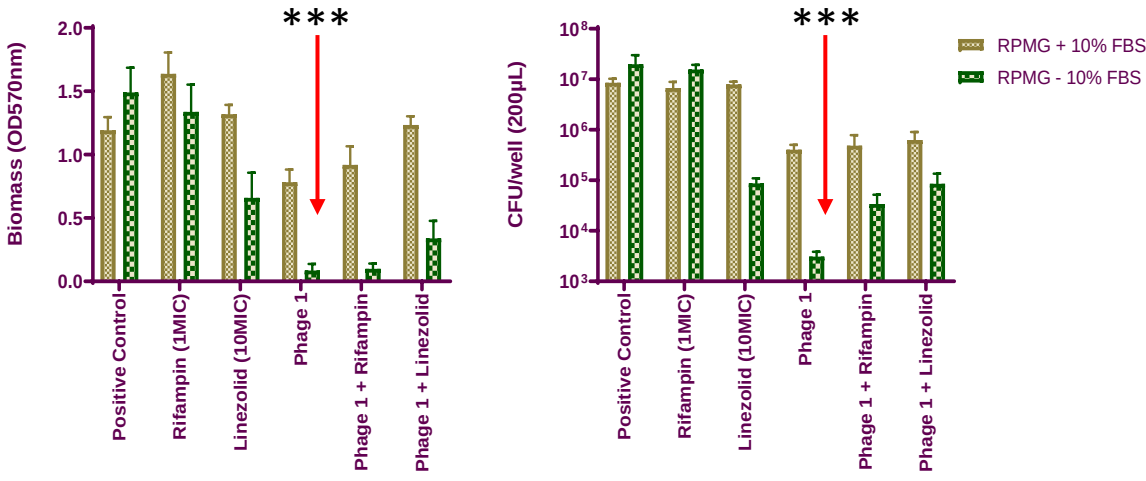
P.aeruginosa is known to quickly **overgrow and even push out *S.aureus*** in multi-species biofilm models. At the start of the project, different media were evaluated to try and balance these inhibitory effects as well as different inoculation ratios between both bacterial species. RPMI media supplemented with 1% glucose was shown to be the best option for a balanced biofilm model. However, ***S.aureus*** **was still strongly inhibited**. **Serum supplementation** was shown to **protect against** these inhibitory effects and resulted in **vastly increased staphylococcal populations** in a dual-species biofilm.



After a stable dual-species biofilm was obtained in 96-well plates, ***C.albicans*** was **introduced** into the biofilm model and **transferred to titanium coupons**. For this **C.albicans** was **grown individually for 24h**, to ensure hyphal formation, after which ***P.aeruginosa*** and ***S.aureus*** were added at a **1/10 ratio**.



First, **phage-antibiotic synergy** (PAS) assays were performed on **mono-species biofilm** models. Although this serum supplementation did not affect phages targeting *P. aeruginosa* in preliminary work, first experiments on staphylococcal biofilms were unsuccessful. Repeated assays clearly indicated that no phage-antibiotic synergies could be observed. However, **when serum was removed** from the media, **drastic reductions** were observed for **both biomass and viable cell counts**. Importantly, these drastic effects were observed for a de novo isolated phage active against *S.epidermidis* on biofilms formed by a **clinical orthopedic strain**.



Conclusion

Serum supplementation inhibits the effect of bacteriophages targeting S. aureus/epidermidis on biofilms. With clinical application in mind, the underlying mechanisms of this serum inhibition needs to be further investigated in great detail.

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