

SASP: Activity Against *Staphylococcus aureus* Cells Grown Under Varied Growth Conditions

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ABSTRACT

Background: SASP are antimicrobial proteins that act by binding to and inactivating bacterial DNA in a non-sequence specific manner. SASPject technology consists of utilising specifically modified vectors (bacteriophage) to deliver a gene encoding SASP to selected target bacteria. Exposure of targeted cells to SASP occurs following vector-cell binding, transcription and translation of the SASP gene. The interaction of bacteriophage with bacterial cells, as well as the expression pattern of many genes, is affected by the growth environment of the bacteria. The activity of SASP, delivered by vector PT1.2 to *Staphylococcus aureus* cells grown under the following range of conditions, was assessed: micro-aerobic growth, pH (5-8), temperature (32-40 °C) and calcium concentration (1-10 mM calcium). PT1.2 will be used initially for intranasal decolonisation of *S. aureus* and SASP activity in the presence of mucin was also assessed.

Methods: Time kill curves were performed using *S. aureus* USA300 strain 43484 and EMRSA-16 strain 252 grown in Luria-Bertani broth, pH 7 at 37 °C (except where stated otherwise) in the presence of PT1.2 (10^7 - 10^8 pfu/ml):

Microaerobic conditions, with control cultures grown aerobically.
pH values adjusted to range between 5 and 8
Cultures grown at 32, 37 and 40°C.
Mueller Hinton broth was used at 1-10 mM calcium ion concentrations.
Cultures supplemented with porcine mucin (2 %).

Results: SASP showed rapid bactericidal action, causing a 5-log drop in viable cells within 1 hour for 10^7 cfu/ml cultures. PT1.2 remained active under all the growth conditions tested. Mucin did not affect the delivery of SASP genes by PT1.2.

Conclusions:

- SASP is rapidly bactericidal against *S. aureus* in a wide range of growth conditions
- Delivery of SASP genes to *S. aureus* and SASP activity are unaffected by mucin, which could have relevance for an intranasal indication.

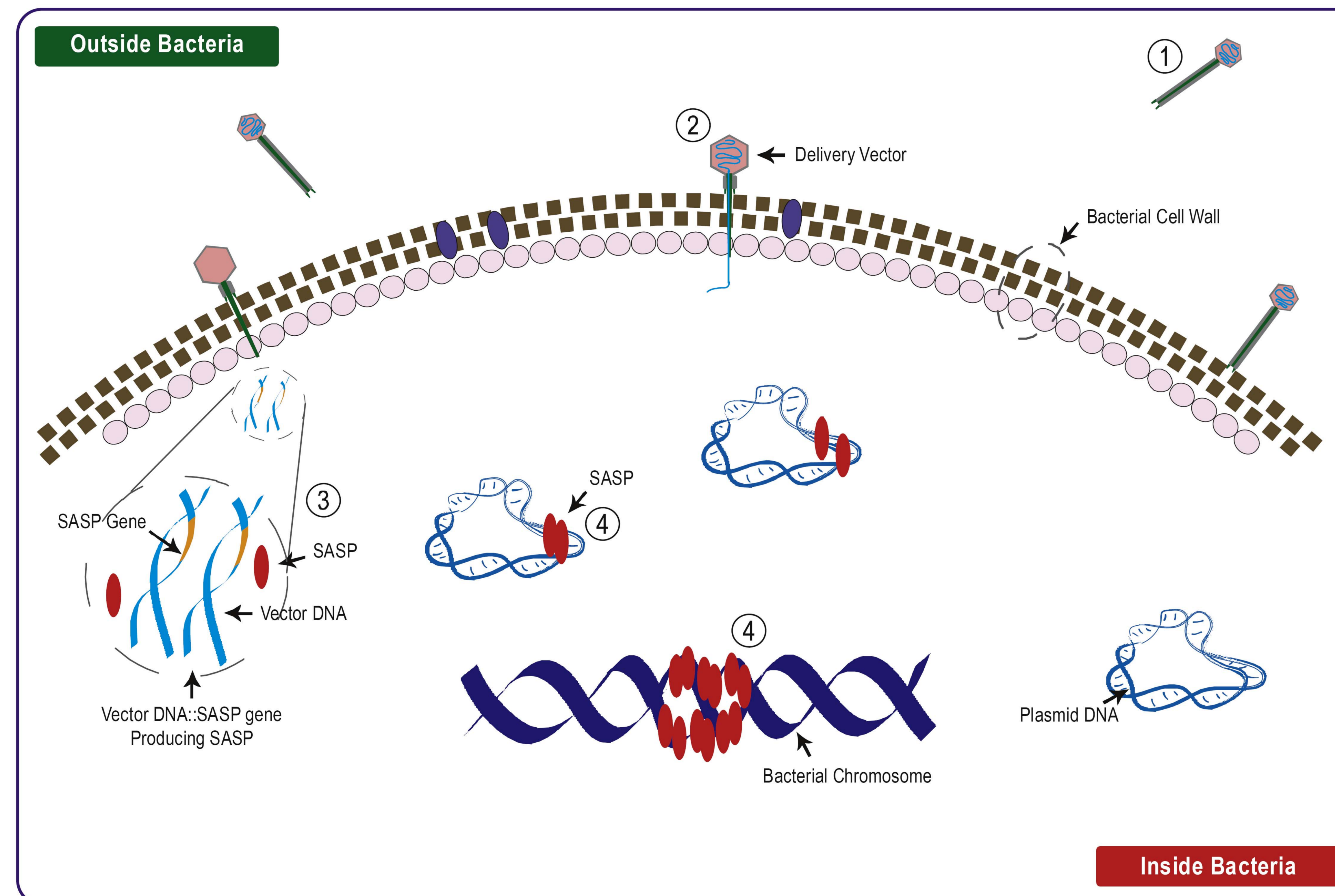
INTRODUCTION

A new class of antibiotic has been developed, called SASPject. The SASPject platform technology comprises modified, disabled bacteriophages delivering a gene encoding an anti-bacterial protein, SASP, into target bacteria (Figure 1). SASP are small proteins that inactivate bacterial DNA in a non sequence-specific manner. SASP, or small acid-soluble spore proteins, are produced only by spore forming bacteria during sporulation. SASP act to protect the DNA of dormant spores by saturating it and changing the conformation of the DNA. In vegetative bacteria which are actively metabolising, replicating DNA and transcribing genes, SASP are lethal. The SASP are uniquely broad spectrum in activity, but the use of bacteriophages as delivery vectors allows selected pathogens, or groups of pathogens, to be targeted, leaving the normal human bacterial flora intact.

A SASPject has been developed which is specific to *S. aureus*, PT1.2. Rapid bactericidal activity against a geographically diverse range of MRSA and MSSA, together with specificity to *S. aureus*, have previously been shown for PT1.2 (1, 2, 3). PT1.2 is currently in Phase I clinical trials for intra-nasal administration, and is intended initially for intra-nasal decolonisation of *S. aureus* (including MRSA).

PT1.2 is likely to encounter bacteria growing under a variety of conditions when administered onto skin. In this study the effects of growth under low oxygen and low pH conditions, as well as growth at varied temperature ranges and in the presence of low calcium concentrations was assessed. Mucin is secreted into the nasal cavity, and the affect of this upon PT1.2 activity was also assessed.

Figure 1. Mechanism of action of PT1.2



METHODS

In all studies, *S. aureus* EMRSA 16 strain 252 or *S. aureus* USA300 strain 43484 was used. Generally, the following was performed: overnight cultures were diluted down to 10^5 - 10^7 cfu/ml in Luria Bertani broth containing 10 mM calcium chloride (LBC broth). Cultures were incubated with PT1.2 at 1 to 1.5×10^8 pfu/ml final concentration, and samples were taken at 0, 1, 2, 4, 6 and 24 hour time points for viable cell counts. Untreated controls, where PT1.2 was replaced with buffer, were also used.

For the microaerobic study, cultures were grown in a microaerobic environment using Atmosphere Generation Pouches (Oxoid).

For the temperature study, cultures were grown at temperatures of 32, 37 and 40°C.

For the mucin study, sterilized porcine mucin was added to a final concentration of 2%. Samples were taken for viable cell counts over 6 hours.

For the low calcium ion concentration study, Mueller Hinton broth was also used containing calcium chloride at final concentrations of 1 and 10 mM. PT1.2 was added to a final concentration of 4×10^7 pfu/ml.

For the pH study, LBC broth was used at pH 5, 5.5, 6, 6.5, 7, 7.5, and 8.

RESULTS

- PT1.2 is rapidly bactericidal under all conditions tested, causing a 5-log drop on 10^7 cfu/ml cultures within 1 hour.
- S. aureus* growth under microaerobic growth conditions, as often encountered by bacteria infecting or colonising a host organism, do not affect PT1.2 activity (Figure 2).
- Growth of *S. aureus* cells in the presence of mucin, which is secreted into the nasal cavity, did not affect SASP activity (Figure 3).
- PT1.2 remained active against *S. aureus* at temperatures spanning those likely to be found at application sites on human skin (Figure 4).
- Calcium concentrations down to physiological levels, did not affect the activity of PT1.2. Therefore PT1.2 can tolerate a "low calcium" environment and remain active (Figure 5).
- A wide range of pH conditions, including spanning that likely to be encountered topically, did not affect PT1.2 activity against *S. aureus* (Figure 6).

Figure 2. Kill curves on EMRSA 16 strain 252 under aerobic or microaerobic conditions. Open symbols for controls (no PT1.2)

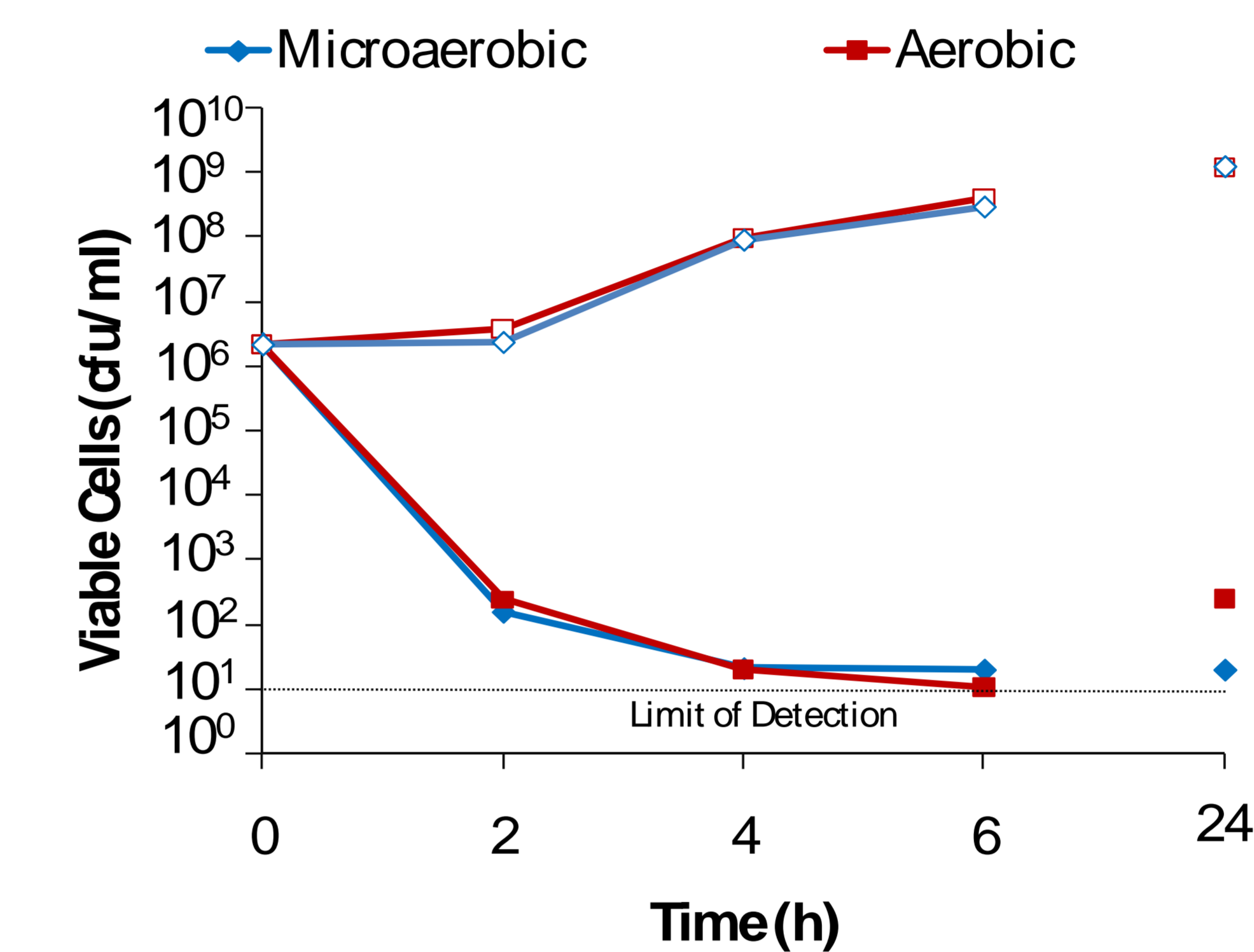


Figure 3. Kill curves on USA300 strain 43484 in the presence or absence of porcine mucin. Open symbols for controls (no PT1.2)

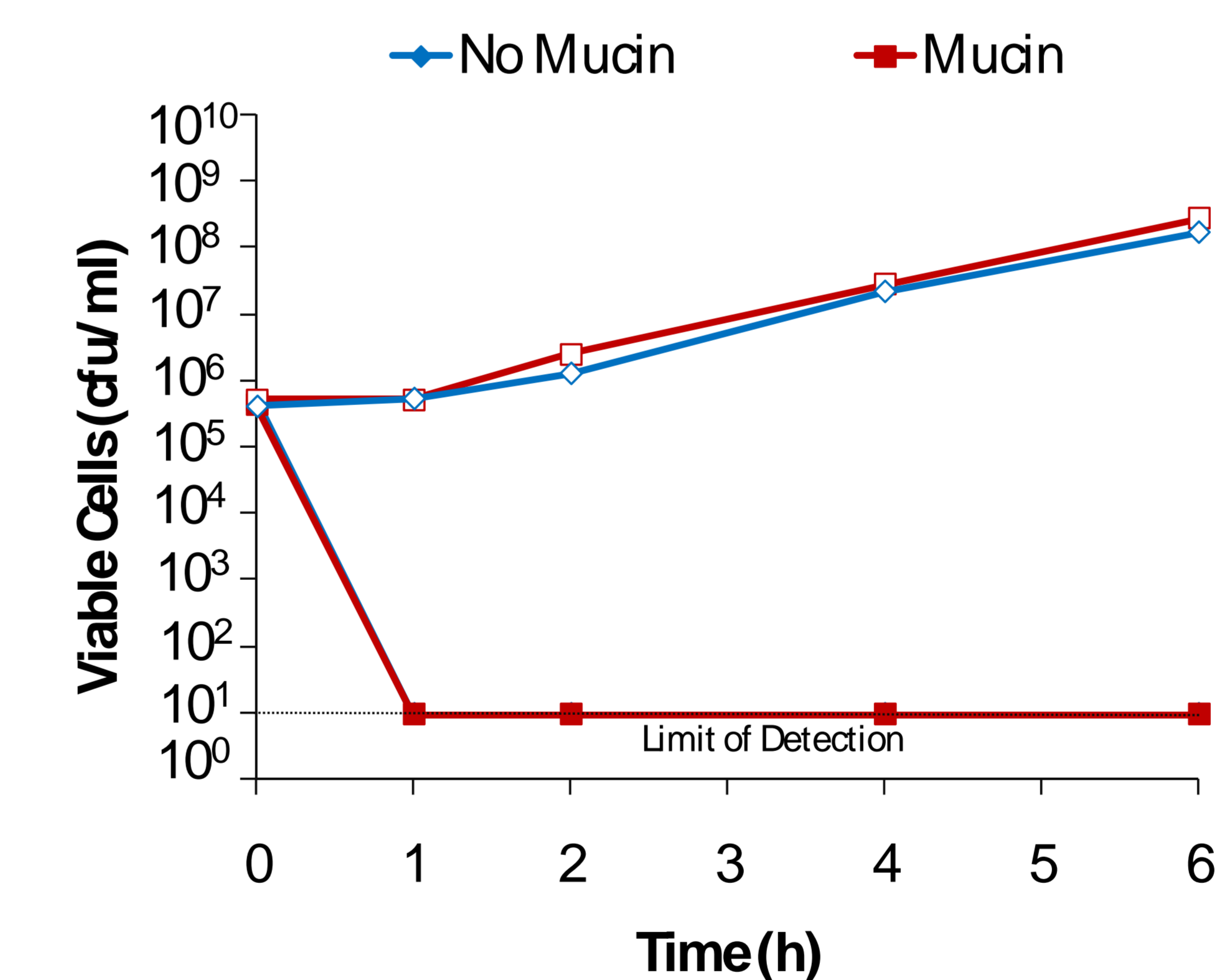


Figure 4. Kill curves on USA300 strain 43484 at 32, 37, and 40°C. Open symbols for controls (no PT1.2)

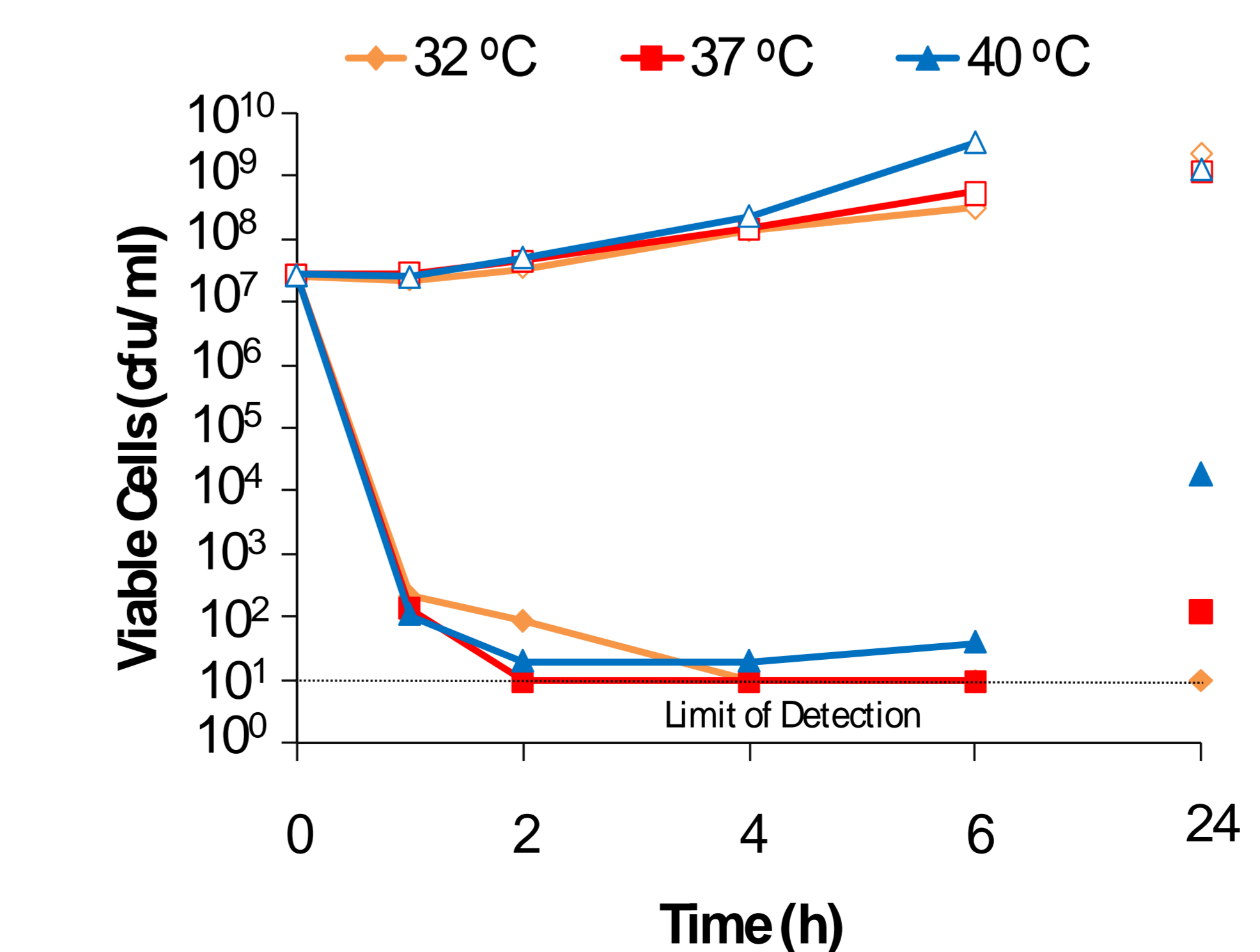


Figure 5. Kill curves on USA300 strain 43484 in different calcium concentrations and media. Open symbols for controls (no PT1.2)

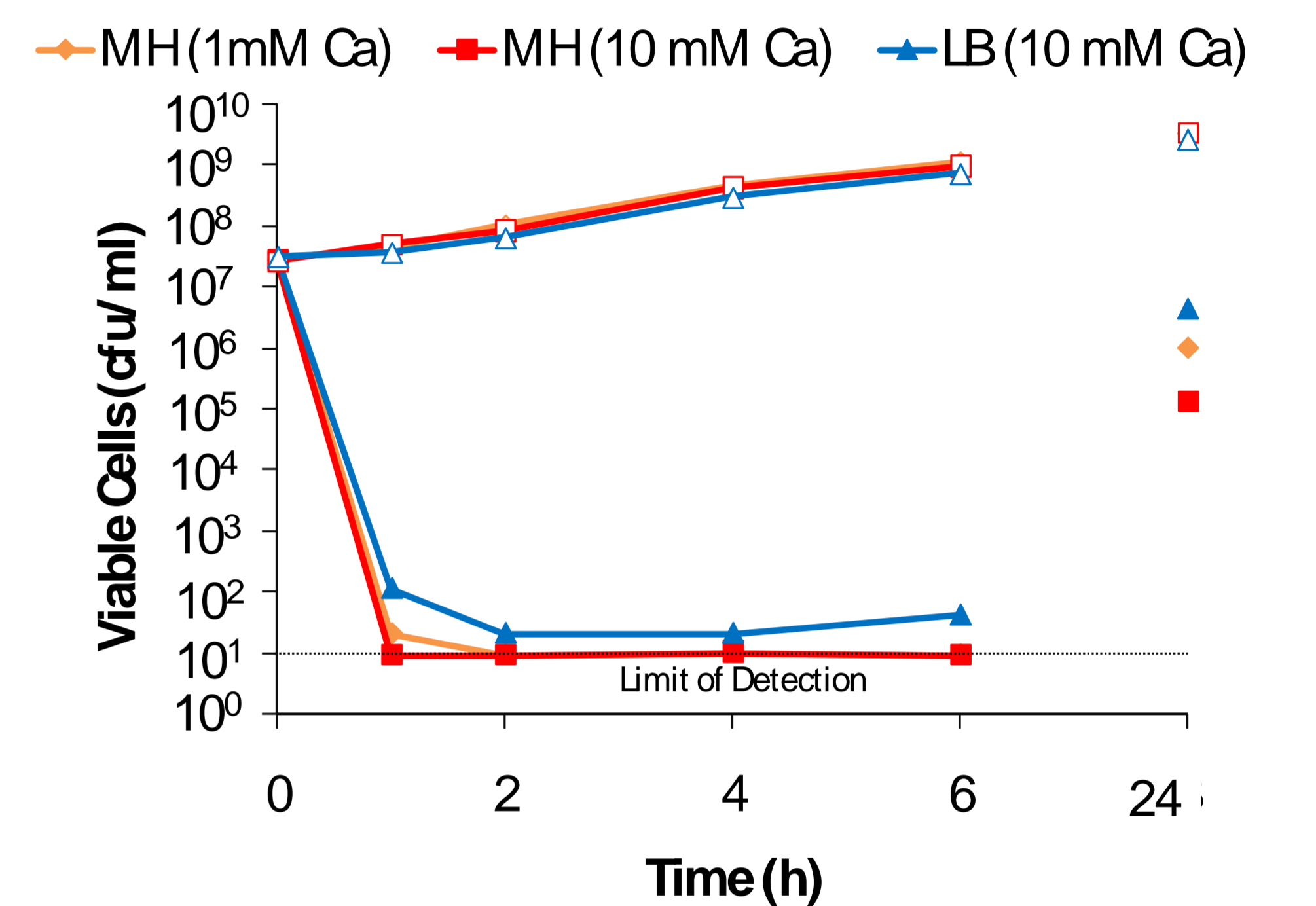
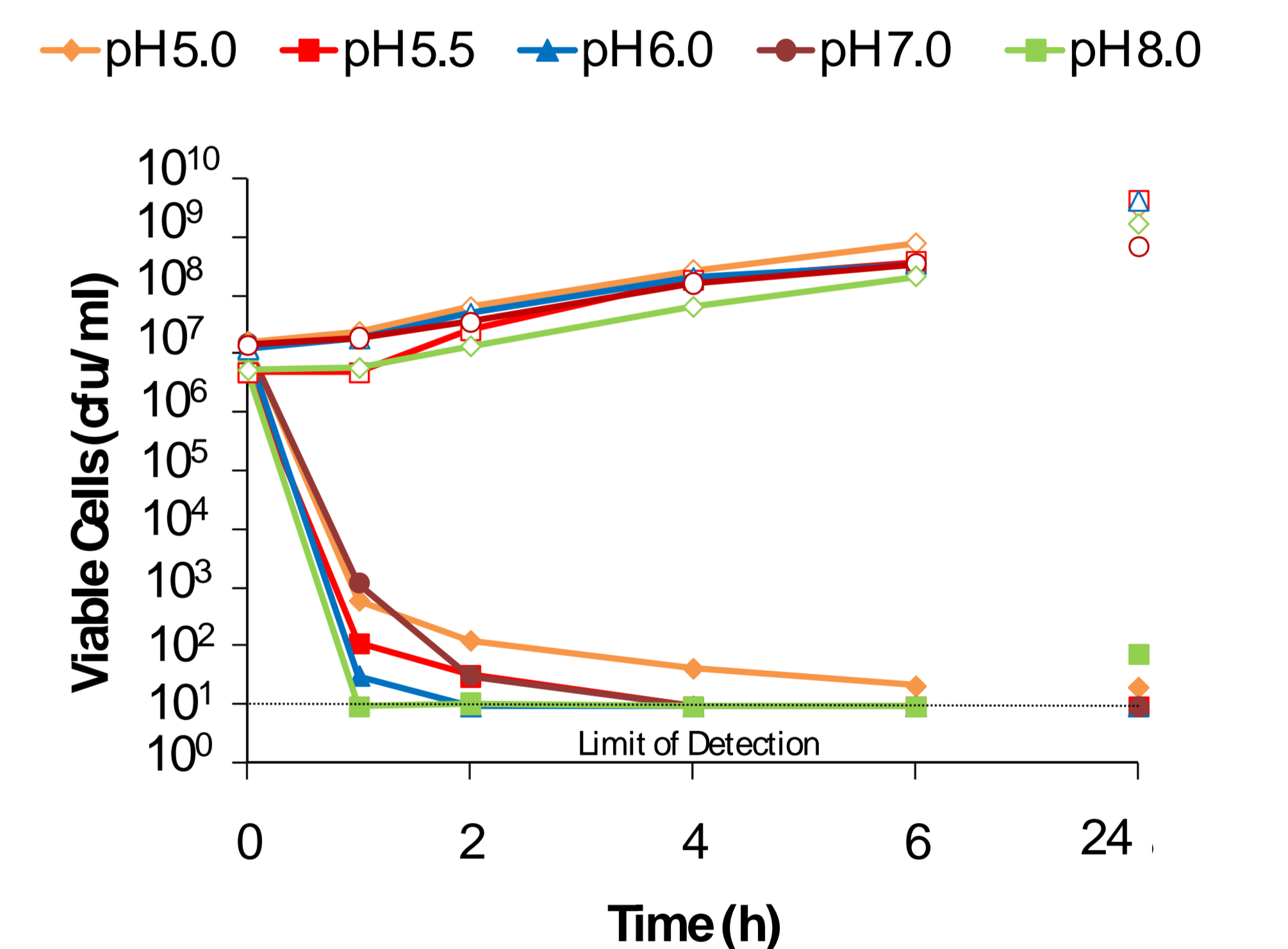


Figure 6. Kill curves on USA300 strain 43484 at pH 5-8 (data for pH 6.5 and 7.5 not shown). Open symbols for controls (no PT1.2)



CONCLUSIONS

- SASPject PT1.2 is unaffected by a diverse range of growth conditions including the presence of mucin, which has significance for the use of PT1.2 as an intranasal antibiotic.

REFERENCES

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