Evaluation of the antibacterial activity of bacteriocins as a new therapeutic option for the treatment of *Mycobacterium abscessus* infections in patients with cystic fibrosis

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Purpose

This application note details a protocol using the oCelloScope to test the antimicrobial activity of bacteriocins, antimicrobial peptides, provided by the start-up Syngulon (http://syngulon.be) against *Mycobacterium abscessus* in an hospital environment and to monitor the growth kinetic analysis in real time.

Introduction

Mycobacterium abscessus is one of the most drug resistant bacteria among the rapidly growing mycobacteria (RGM). It is naturally resistant to the currently used antibiotics, including almost all anti-tuberculosis agents, which seriously complicates and limits treatment options. *M. abscessus* is considered as an important pathogen in cystic fibrosis patients. Bacteriocins could represent an alternative therapeutic option against this infection to overcome the problem of bacterial resistance. The oCelloScope offers the advantages of speed compared to the classical broth dilution assay. It provides images and video, and it can rapidly screen for antimicrobial agent activity. We can detect growth of *M. abscessus* in 6h instead of 3-5 days with the classical serial dilution test in broth medium.

Reagents and Materials

- 96 plate well
- Middlebrook 7H9 medium (supplemented with 0.5% glycerol and 10% OADC).

Methods

oCelloScope

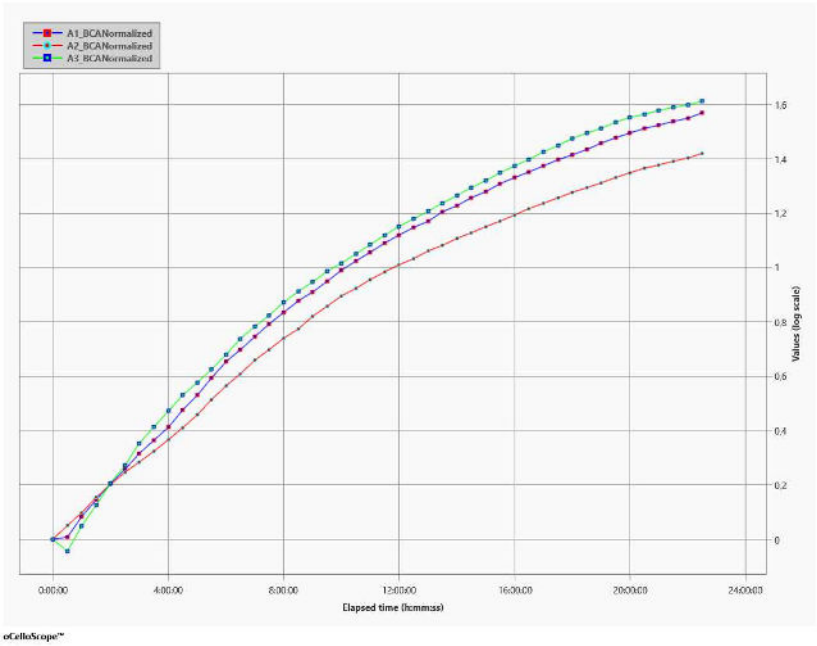
Protocol

1. Place the oCelloScope in a standard laboratory incubator at 37°C
2. Grow *M. abscessus* reference strain or clinical isolates in 7H10 agar medium for 3-5 days at 37°C
3. Prepare bacterial suspensions to McFarland 0.5 based on OD600 measurements. Dilute your suspension 1/10 in 7H9 medium (supplemented with 0.5% glycerol and 10% OADC).
4. Add bacterial suspensions to a 96-well plate containing the bacteriocin

5. Introduce the 96-well plate loaded with the bacterial suspensions in the oCelloScope plate holder, tighten firmly and close the instrument lid. The recommended total volume of bacterial suspension to add to each well is 200 µL.
6. Proceed with the AST analysis using the oCelloScope and the Growth Kinetics module.
7. Configure the UniExplorer software for time-lapse analysis by clicking the 'New job' button in the top left corner of the main window.
8. Give the new job a name, then choose the acquisition modules from the list to the left by double clicking (or dragging) the icons in the following order: (i) 'Acquire' (for recording new image data) and (ii) 'Growth Kinetics Analysis' (for monitoring bacterial growth/growth inhibition over time). Press 'Next'.
9. Let the UniExplorer software recognise the oCelloScope instrument by selecting the instrument to use from the instrument list with a left click. Press 'Next' to continue with experimental setup.
10. Select 96 well plate.
11. Adjust the time of analysis by selecting the number of acquired images ('Number of repetitions') and the time interval between two sequential images ('Repetition interval'). For instance, by selecting 'Number of repetitions' = 48 and 'Repetition interval' = 00:30:00, the oCelloScope will take the images every 30 minutes for 24 hours with the first image taken at t = 0.
12. Select and enable the wells that should be included in the analysis with the cursor. Enabled wells are shown as blue
13. Press 'Start' to start the experiment.
14. Curves for real-time growth/growth inhibition as well as image data and time-lapse videos can be inspected and compared during the experiment in the 'Current scan' tab.

Figures

Figure 1 – *M. abscessus* growth (in triplicate) measured with oCelloScope algorithms BCA normalized



Time (h)

Figure 2 - 7h30 *Mycobacterium abscessus (rough phenotype)* growth time-lapse captured with oCelloScope (imaging of typical serpentine cords)

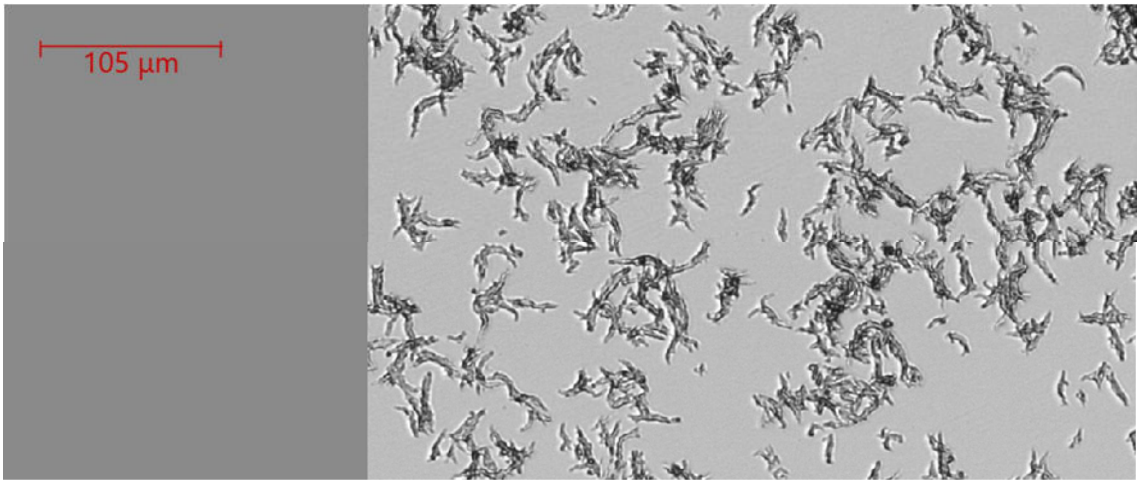
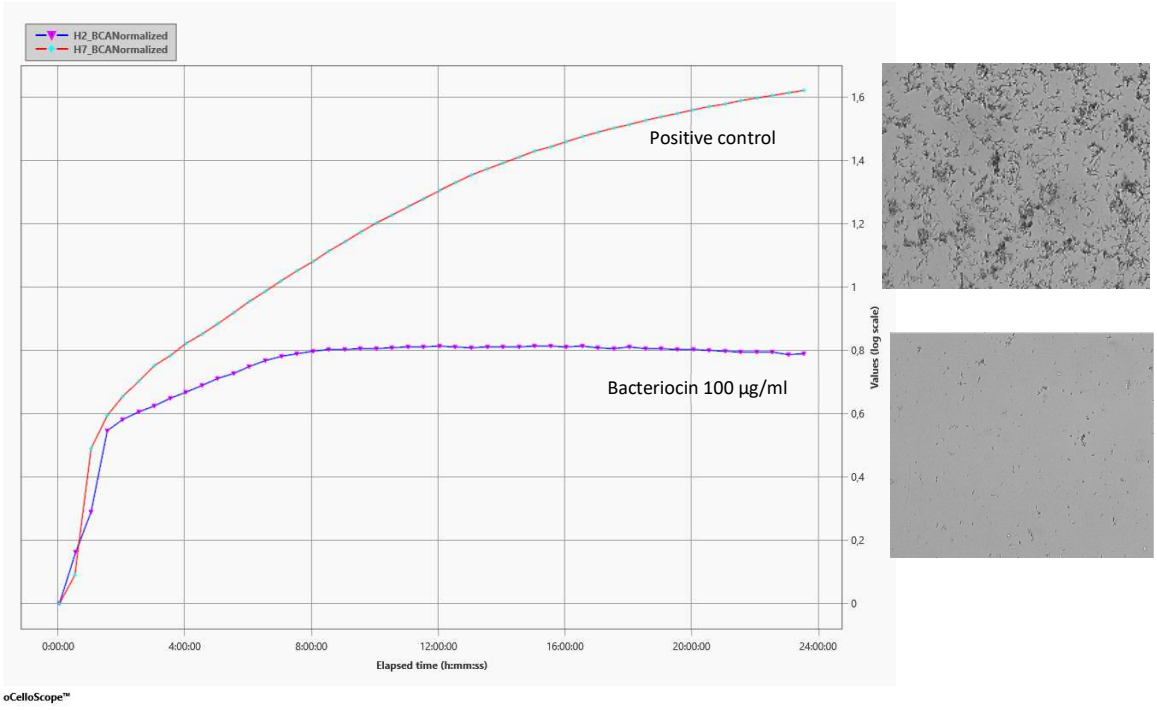


Figure 3 - *Mycobacterium abscessus* growth curve with oCelloScope



Time (h)

Figure 4 - Images of *Mycobacterium abscessus* (control and with bacteriocin) at different time point (6 to 24 hours)

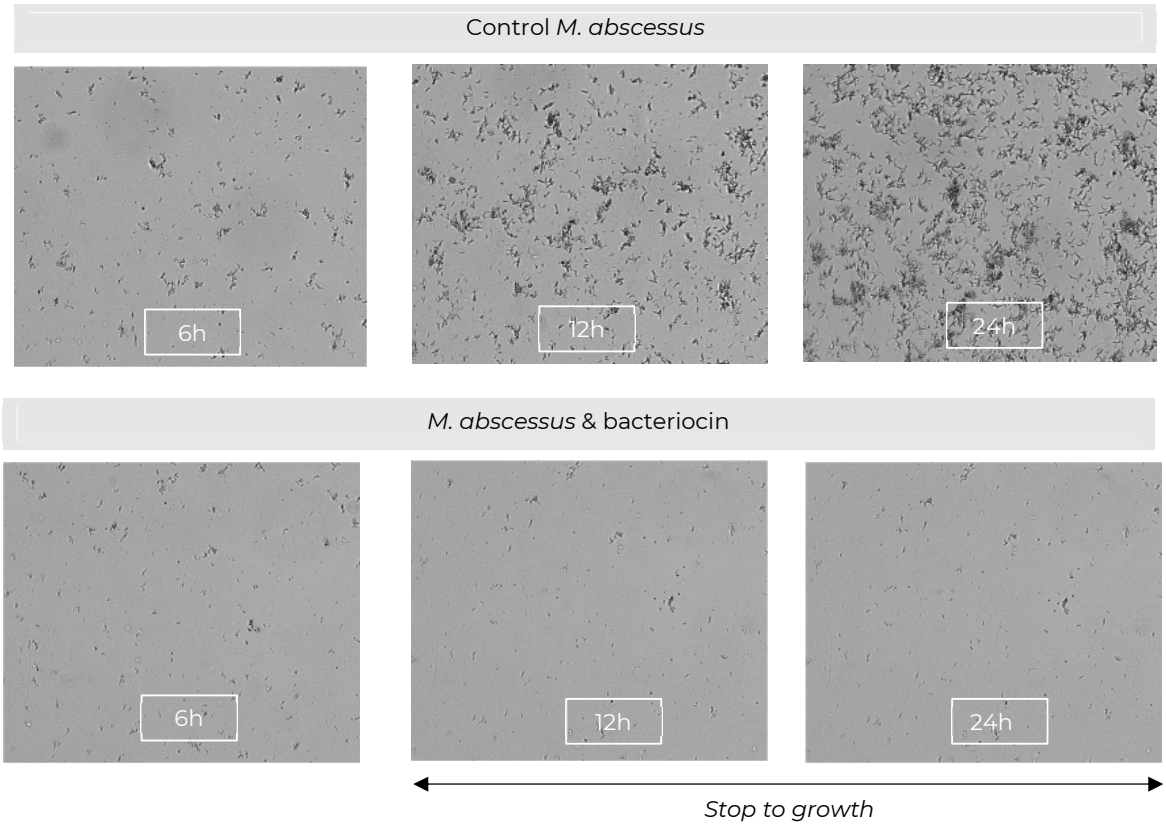
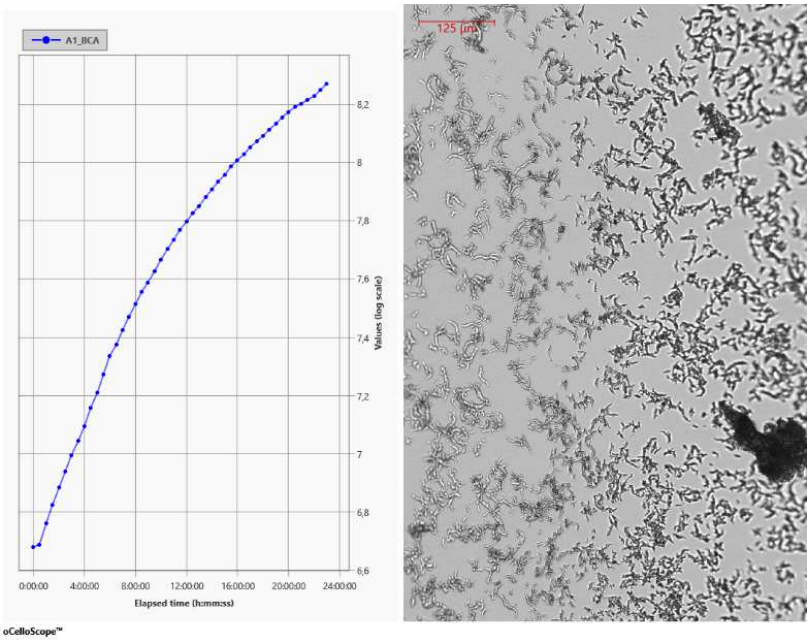


Figure 5 - *Mycobacterium abscessus* growth curve and live cell image



Publication in preparation

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