Growth curve & MIC determination

Aim of the growth curve:

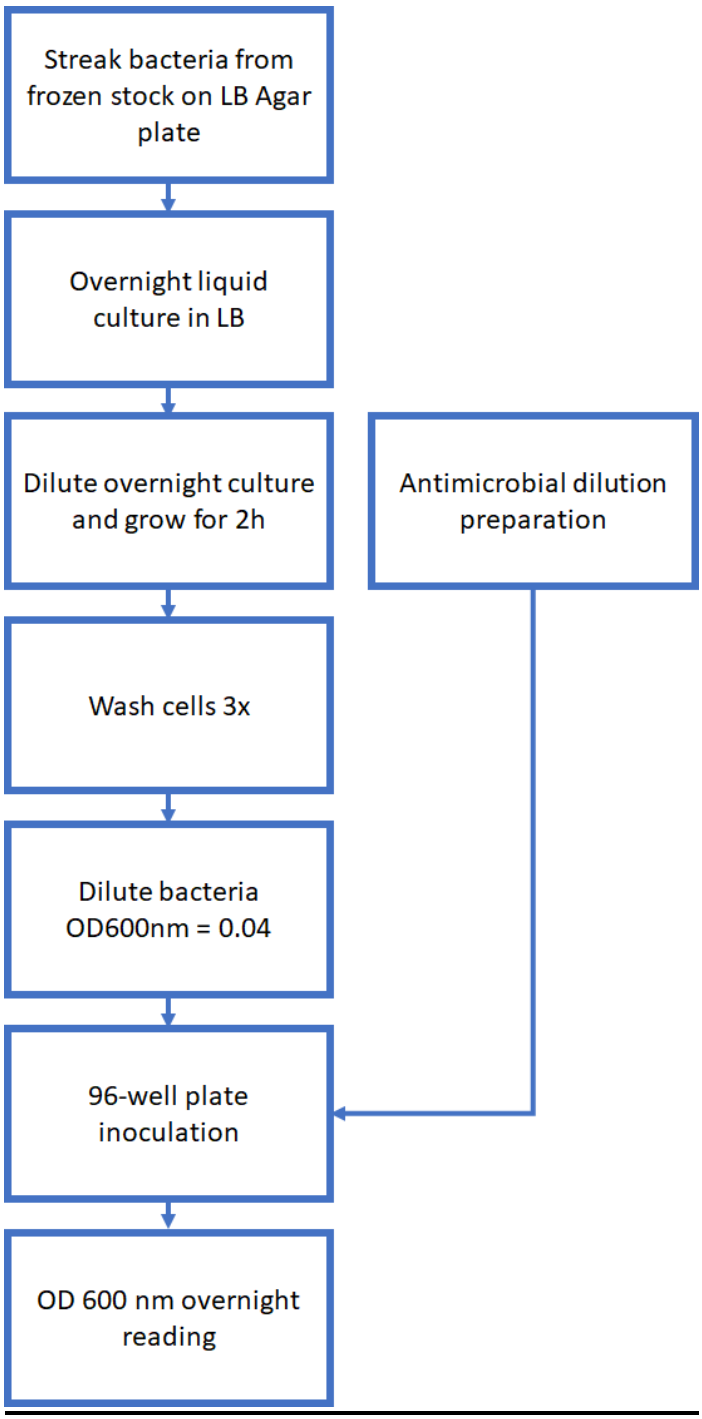
Evaluate the impact of antimicrobial compounds on the growth of bacteria.

Aim of the MIC determination:

Evaluate the antimicrobial efficiency of the constructs on bacteria.

Workflow:

Duration: 4 Day (3 Day for preparation + 1 Day for the measurement) : Day N°3 : 4h



Materials :

- Bacteria
- Antibiotics at stock solutions (see notes)
- 50 mL Falcon tubes
- 50 mL Erlenmeyer
- Sterile Loops
- 96-well plates
- LB agar plate
- LB Broth
- Müeller-Hinton Broth (MHB)
- Multichannel pipette (50 µL and 100 µL)
- PBS
- Incubator
- 96-well plate reader TECAN M200 Infinite PRO

Protocols:

Day 1

- Streak out the bacterial from the glycerol stock in a LB agar plate and incubate overnight at 37°C

Day 2

- Pick a single colony from the overnight plate with a sterile loop and inoculate it into 5 mL of LB media in a 50 mL Falcon tube. Incubate overnight at 37°C with agitation at 220 rpm.

Day 3

- In an Erlenmeyer flask, put 5 mL of LB media and 125 µL of the overnight culture (40 X dilution of the overnight culture).
- Incubate 2 h at 37°C with agitation at 220 rpm.

During this time

- Prepare the antibiotic stock solution and dilution range. The list of antimicrobials used in this study is shown in Table 1.
- From the appropriate stock solutions, prepare a range of suitable dilutions for each antibiotic to be tested. In general, a maximal concentration of 512 µg/ml and serial diluted concentrations in MHB solution are used. The lowest dilution concentration is depended on the possible minimal inhibitory concentration. 0.125 µg/ml is the normally the lowest possible dilution concentration.

After 2 h

- Collect 1 mL of bacterial culture in an eppendorf.
- Centrifuge for 5 min at 6 000 rpm
- Remove the supernatant and wash three times with PBS buffer
    - 1 wash
    - 2 wash
    - 3 wash
- Resuspend the pellet in 1 mL of PBS.
- Make a 10 X dilution of the cell suspension and measure the OD at 600 nm. Adjust the OD to 0.03 - 0.04.

- Inoculate the 96 well plate with different antibiotic concentrations prepared previously with 1 µL of the bacterial cell suspension with a sterilized pin tool.
- Monitor the OD at 600 nm overnight in a 96 well plate reader. The parameters used are described below. A representative growth curve of *E. coli* in the absence and presence of different concentrations of the antibiotic nalidixic acid is shown in Fig. 1.
- The MIC was defined as the lowest concentration of antibiotic at which there was no visible growth of bacteria (no solution turbidity on naked eyes) after 20-24 h of growth, and the difference of measured and background OD600 was less than 0.01. A representative MIC of nalidixic acid on *E. coli* is shown in Fig. 2.

96 Well Plate Reader Parameters :

Temperature: 37°C

Number of Cycles : 500
Kinetic interval: 15 min

Wavelength: 600 nm
Number of flashes: 5

Shaking duration: 140 s
Shaking mode : Linear
Shaking amplitude: 1 mm

Notes :

Table 1. Antibiotics, antimicrobial peptides, and StarCores used in the present study, with their respective stock solution concentration, preparation, storage conditions and working concentration. StarCores were used at the stock solution.

	Stock solution (mg/ml)	Solvant	Storage temp (C)	Working concentration (µg/ml)
Antibiotics				
Ampicillin	50	H2O	-20	50
Chloramphenicol	35	EtOH or MetOH	-20	35
Erythromycin	20	EtOH	-20	20
Gentamycin	10	H2O	-20	10
Kanamycin	50	H2O	-20 to + 4	50
Nalidixic acid	30	H2O: pH to 11 w/ NaOH	-20	30
Neomycin	10	H2O	-20	800
Rifampicin	50	MetOH	-20	100
Streptomycin	100	H2O	-20	100
Trimethoprim	10	10% EtOH or DMSO	-20	10
Tetracycline	15	70% EtOH	-20	15
Reference AMP				
Ovispirin	2.26	Ultra-pure	-20	2.26

		water		
StarCores				
Ferritin-Alyteserin 2A	0.337	Ultra-pure water	-20	/
Ferritin-Ovispirin	0.607	Ultra-pure water	-20	/
Pyruvate dehydrogenase-Ovispirin	0.511	Ultra-pure water	-20	/
Pyruvate dehydrogenase-Bacteriocin	0.833	Ultra-pure water	-20	/
Pyruvate dehydrogenase-Pediocin PA1	0.346	Ultra-pure water	-20	/
Pyruvate dehydrogenase-Enterocin A	0.630	Ultra-pure water	-20	/
Lyase cage-Bactofencin	0.759	Ultra-pure water	-20	/
Lyase cage-Bacterocin	2.151	Ultra-pure water	-20	/
Lyase cage-Enterocin 1	0.951	Ultra-pure water	-20	/
Cg-defensin-Ovispirin	0.473	Ultra-pure water	-20	/

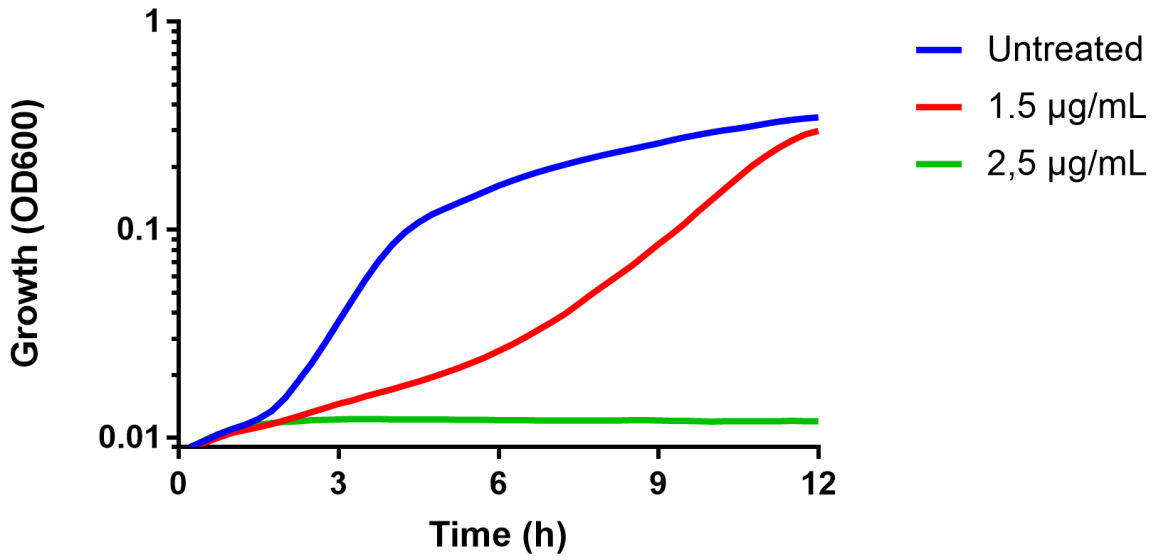


Fig. 1. Representative growth curve of *E. coli* in the absence and presence of different concentrations of the antibiotic nalidixic acid.

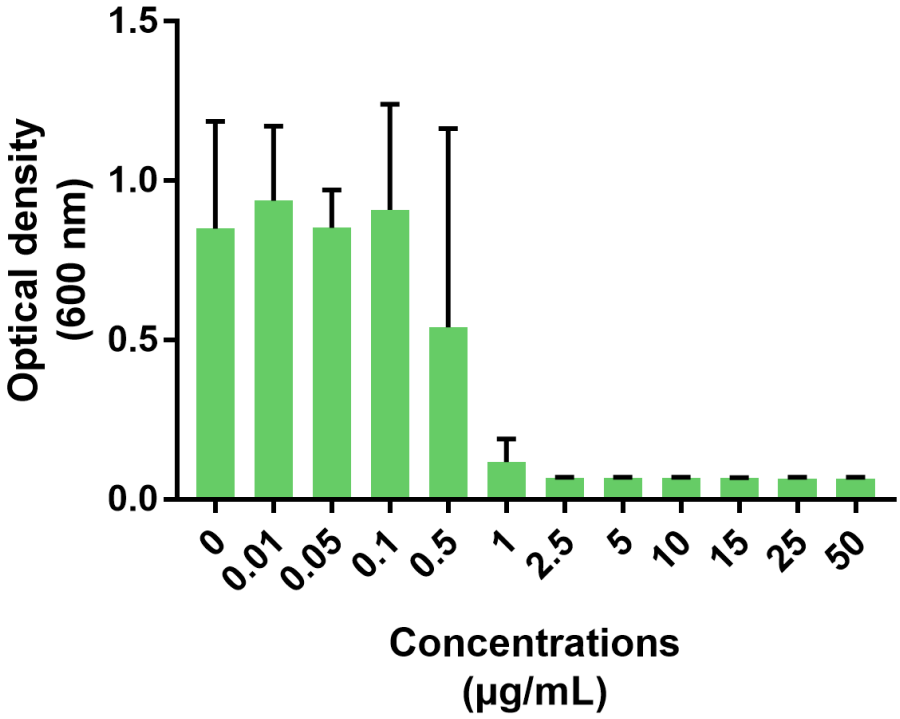


Fig. 2. Representative result from the MIC determination for nalidixic acid in *E. coli*. The MIC is defined as the lowest antimicrobial concentration resulting in no obvious growth compared to the background. In this case, the MIC was determined to be 1 µg/mL. Error bars represent standard error of at least 4 replicate measurements.