



Identifying the range of sub-inhibitory concentrations of antibiotics triggering bacterial functions of relevant interest

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Marie-Noëlle Pons, Petitcolas Kévin, Cecile Lemaitre, Cédric Pradalier, Hélène Guilloteau, et al.. Identifying the range of sub-inhibitory concentrations of antibiotics triggering bacterial functions of relevant interest. Xenowac-II, Oct 2018, Limassol, Cyprus. hal-02334772

HAL Id: hal-02334772

<https://hal.univ-lorraine.fr/hal-02334772>

Submitted on 29 Oct 2019

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Background

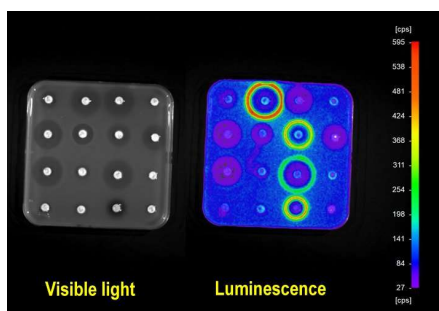
Beyond their selective role, some antibiotics continue to exert an effect below their minimum inhibitory concentration level, where they can act as signaling molecules to modulate various bacterial activities: biofilm formation, exopolymers production, virulence, quorum sensing, cell mobility, and even the dissemination of specific antibiotic resistance genes. This clearly asks the question of the possible effects antibiotics can have once diluted after the disposal of reclaimed wastewater into the aquatic environment or during its reuse. Lately we developed a biosensor approach to identify antibiotics interfering with gene regulation. This approach lies on the use of bacteria genetically modified to express a bioluminescence once the promoter of a relevant function is induced by a modulating antibiotic. Rather than testing arbitrary chosen concentrations, the biosensor is exposed to diffusion gradients of antibiotics in agar plate (as for antibiograms) while luminescence profiles are recorded by CCD camera. With such approach we could detect several antibiotics able to trigger the activation of promoters controlling the mobility functions of "integrative and conjugative elements" (ICEs) such as *Tn916* and *SXT*. An automated image analysis algorithm combined with a modelling of the diffusion process in the agar has been developed to convert the luminescence profile into a concentration profile information, enabling to estimate the level of induction and the range of sub-MIC concentration at which an antibiotic remain effective.

Experimental setup

The biosensor strains are first spread onto the agar of a square Petri dish before receiving one to sixteen antibiotics loaded disks deposited in an equispaced manner. The agar plate is incubated at 30°C allowing the antibiotics to diffuse and luminescence and transmitted light images are regularly acquired over time. An automated image processing system has been developed to detect the induction and inhibition zones around each antibiotic loaded disk.

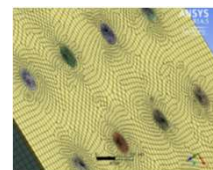
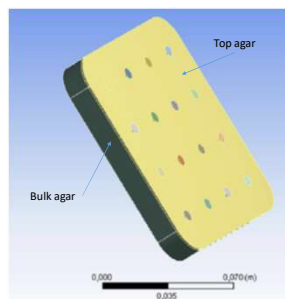
For the present experiment two antibiotics were used: ciprofloxacin (CIP) (broad spectrum) and nalidixic acid (NAL) (limited spectrum). Both inhibit the DNA gyrase while CIP inhibits also topoisomerase IV.

Commercially available discs (BioRad) were used (30 µg for NAL and 5 µg for CIP). Additional discs were prepared by loading cellulose discs with antibiotic solutions. The MIC values were determined in separate experiments in liquid phase for each antibiotic.



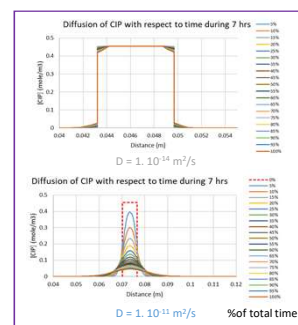
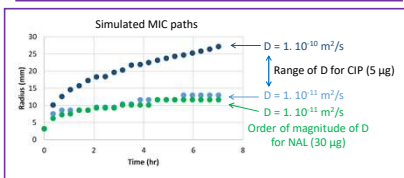
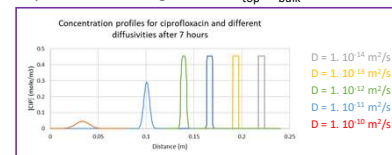
Modelling

The diffusion of antibiotics in agar is modelled through a finite element computational model based on Fick's second law of diffusion. The simulations are performed using a Computational Fluid Dynamics software (Fluent by ANSYS). The mesh contains ≈ 80,000 cells. It is assumed that the antibiotics deposited in the thin top agar layer exhibit higher diffusivities in this layer than in the bulk solid agar.



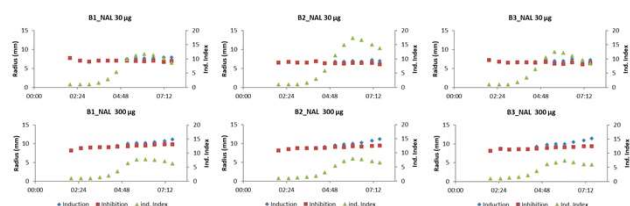
Modelling results

The key parameters are the antibiotic diffusivity in the top agar and the diffusivity ratio between the top and the bulk agar. Here $D_{\text{top}}/D_{\text{bulk}} = 1000$.

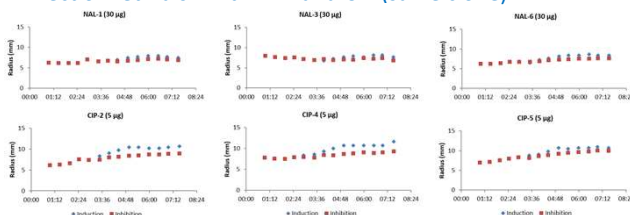


Experimental results

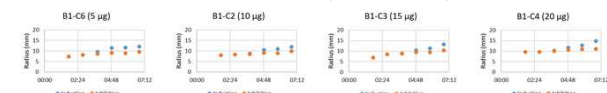
✓ Effect of clone and concentration with NAL



✓ Effect of Petri dish with NAL and CIP (same clone)



✓ Effect of concentration on CIP (same clone)

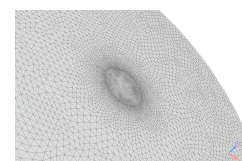


MIC (CIP) = 0.015 µg/mL = $4.53 \cdot 10^{-5}$ mole/m³

MIC (NAL) = 3 µg/mL = $1.29 \cdot 10^{-2}$ mole/m³

Conclusions & perspectives

- ✓ The effect of experimental parameters such as clone and antibiotic type and concentration has been tested. An improvement of the spatial resolution would be useful for antibiotics such as NAL with a high MIC.
- ✓ First simulation results show that it is possible to estimate the diffusivity of an antibiotic in gelose media. A refinement of the simulation mesh is however necessary, especially for high-MIC antibiotics, to estimate properly sub-inhibitory concentrations. Such refinement is currently tested.



245,000 cells for a gelose surface of 25 cm², against 80,000 cells for a gelose surface of 144 cm² in the presented results