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Impact of gaseous NO₂ on *P. fluorescens strain* in the membrane adaptation and virulence

S. DEPAYRAS¹, T. KONDAKOVA^{1,2}, N. MERLET-MACHOUR³, H.J. HEIPIEPER⁴, M. BARREAU¹, C. CATOVIC¹, M. FEUILLOLEY¹, N. ORANGE¹, C. DUCLAIROIR-POC¹

¹ Laboratory of Microbiology Signals and Microenvironment EA 4312, Normandie Univ., Univ. Rouen, IRIB, 27000 Evreux, France ² Cronan Lab, Department of Microbiology, University of Illinois, Urbana, USA

³ Team Modified to Surface and Interface Analysis (SIMA), UMR 6014 COBRA, Normandy Univ., Univ. Rouen, 55 rue St Germain, 27000 Evreux, France ⁴ Department of Environmental Biotechnology, UFZ Helmholtz Centre for Environmental Research, Permoserstr. 15, 04318 Leipzig, Germany

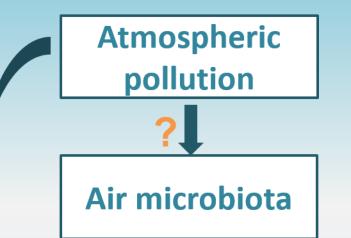


RÉGION NORMANDIE

Contacts : <u>segolene.depayras@etu.univ-rouen.fr</u>, <u>cecile.duclairoir@univ-rouen.fr</u>

Introduction:

Nowadays air pollution is clearly increasing due to anthropogenic activity despite more drastics regulations. Among all air pollutants, Nitrogen oxides (NOx), such as NO and NO₂, are predominant. It is well-known that those compounds exhibit direct high toxic effects on human health especially on skin and lung^{1,2}. However microorganisms are also exposed to them, but their with microorganisms on microbial virulence is still not synergy stated.



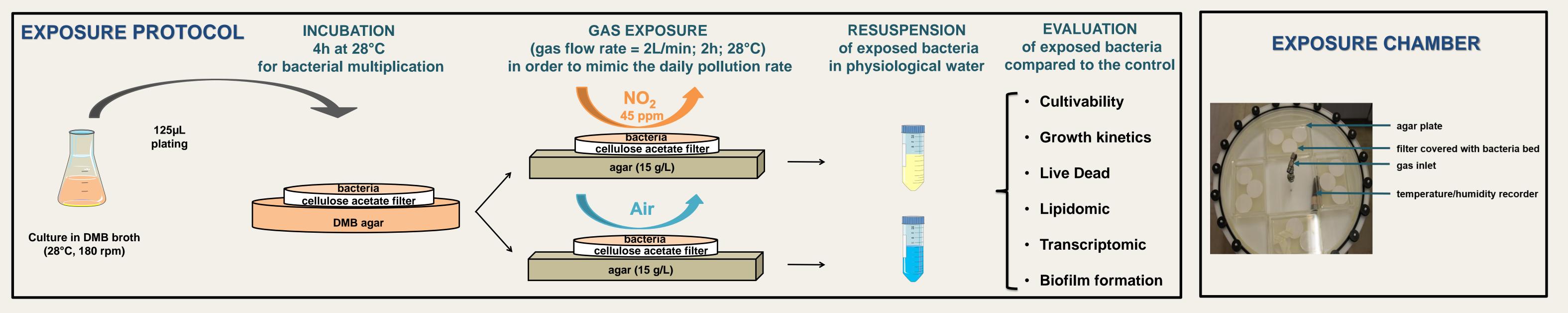
In this study, we tried to evaluate the response of an airborne strain of *P. fluorescens*, MFAF76a³ to a two hours exposure at 45ppm NO₂. The physiological behavior of the strain was measured using cultivability and growth kinetics. Moreover the membrane adaptation was assessed thanks to permeabilization tests and lipidomic studies. Then virulence factors such as biofilm formation and antibiotic resistance were studied.

HELMHOLTZ CENTRE FOR

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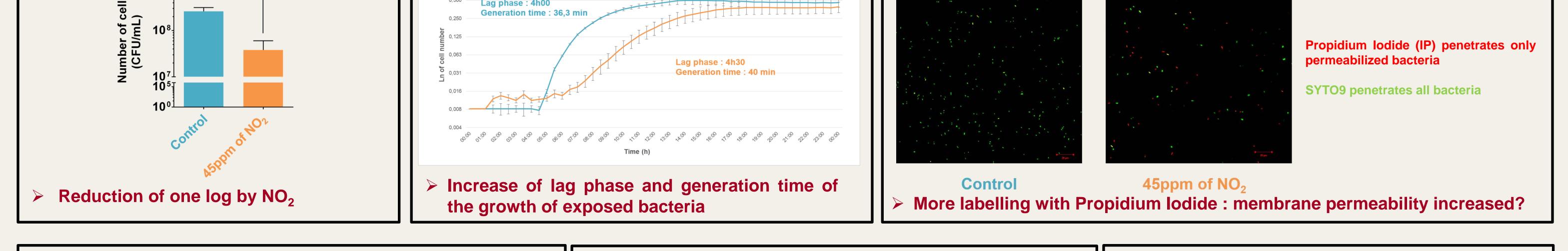


Material and methods:



Results:

CULTIVABILITY PHYSIOLOGY VIABILITY Quantification of colony forming units (CFU) from Observation of membrane permeability through Live Dead tests (PI and SYTO9) using confocal Growth kinetics of bacteria after exposure bacterial suspension microscopy Control 45ppm of NO2 10^{9.}



Control

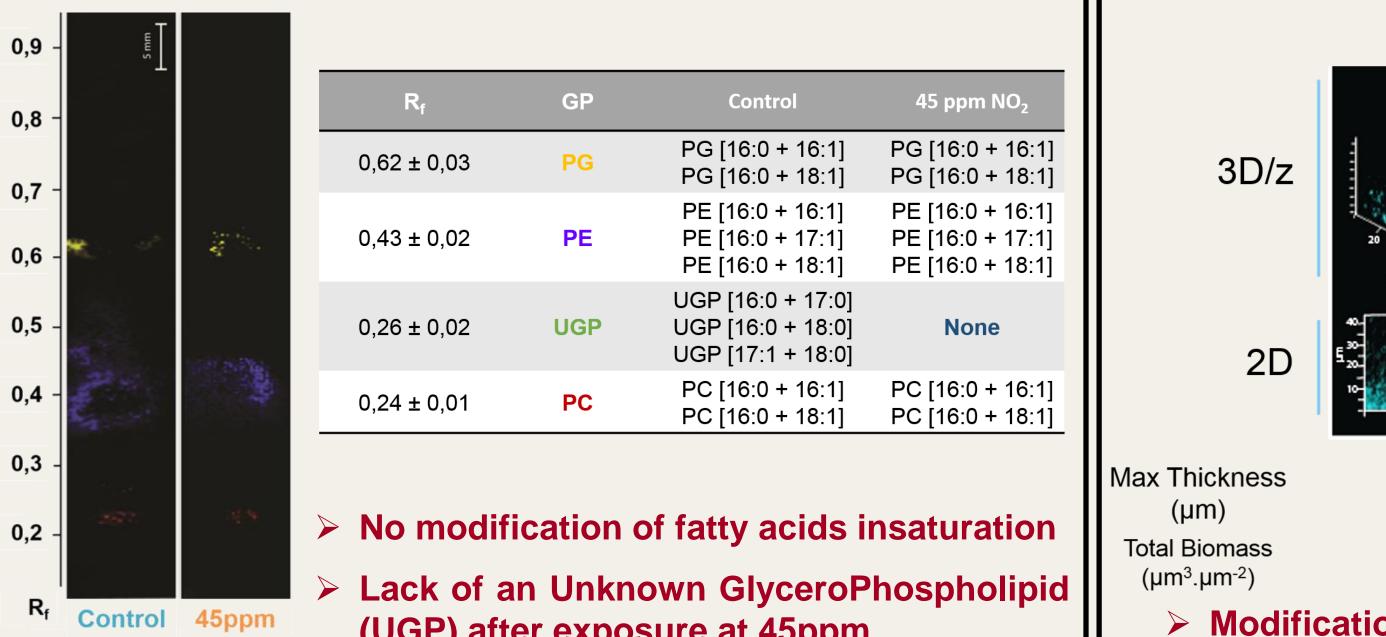
LIPIDOMIC STUDY⁴

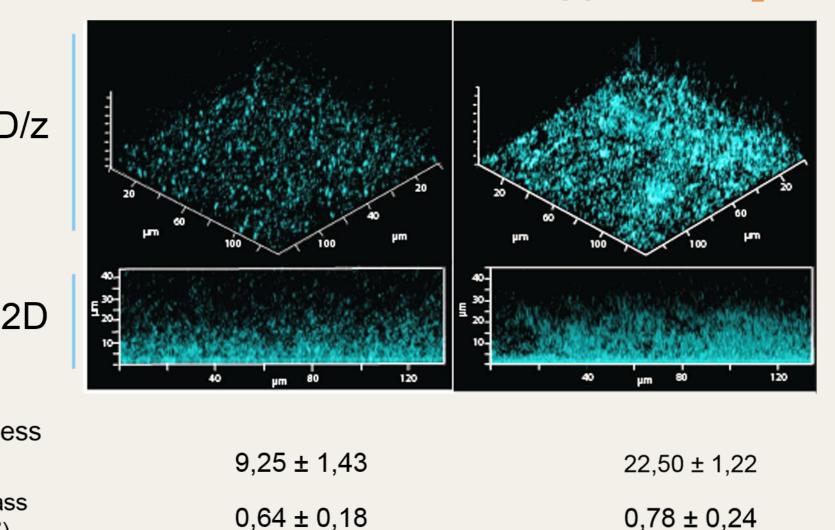
BIOFILM FORMATION⁴

Analysis of Glycerophospholipids (GP) content using HPTLC MALDI-TOF MS/MS 24h biofilm observed by confocal microscopy and image analysis using a Zeiss LSM710 confocal microscope and the Comstat2 software

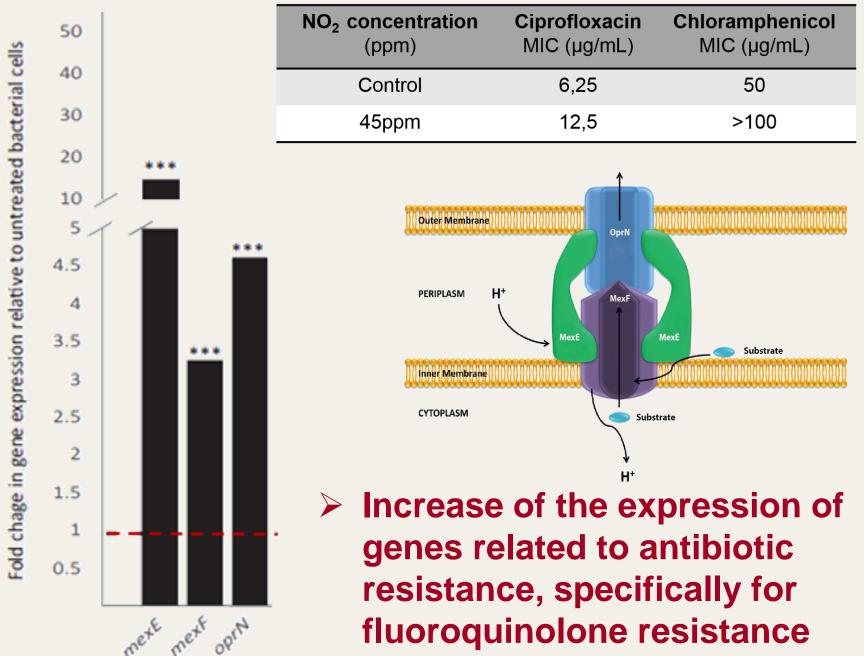
TRANSCRIPTOMIC⁴

RT-qPCR analysis on RND efllux pump correlated with MIC assays





45ppm of NO₂

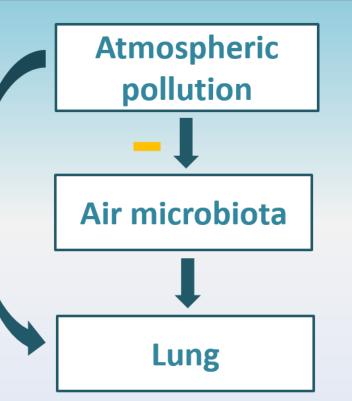


(UGP) after exposure at 45ppm

- \succ Modification of the sessile structure by NO₂
- Presence of prominences



Deleterious effects of NO_2 (45ppm, 2h) were noticed for an airborne strain *Pseudomonas fluorescens* MFAF76a, with an important loss of cultivablity and an increased lag-phase of the growth kinetics. A significant alteration of the membrane permeability was observed. However no significant modification of glycerophospholipids content was measured except for an Unknown GlyceroPhospholipid (UGP). The exposed strain seems to form more prominences highlighting the heterogeneity in the biofilm structure.



For virulence factors, NO_2 exposure increases the resistance of MFAF76a for fluoroquinolones confirmed by transcriptomic analysis and MIC assays.

Now our project is focused on the impact of lower concentration of NO₂ mimicking the daily pollution rate which could be less toxic for bacteria. In such conditions, more phenotypes related to virulence and adaptability could be enhanced.

Références : 1-WHO Ambient (outdoor) air quality health (2015) 3-Duclairoir Poc et al. Int. J. Curr. Microbiol. Appl. Sci. 2014, 708e22 2- INERIS Dioxyde d'azote: Données toxicologiques et environnementales (2015)

4-Kodakova *et al.*, Front. Microbiol. 2016, 7:379