Impact of gaseous NO_2 on *P. fluorescens strain* in the membrane adaptation and virulence

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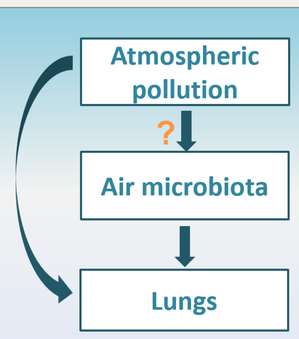
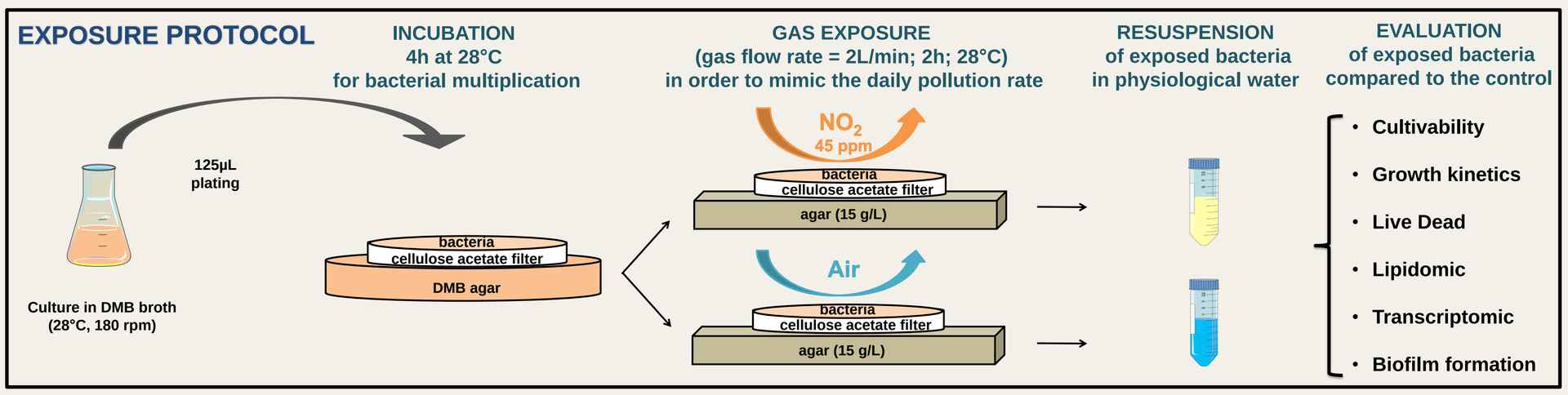
Introduction:

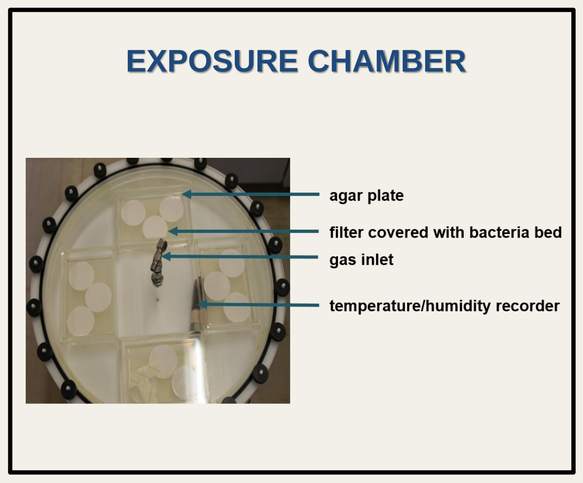
Nowadays air pollution is clearly increasing due to anthropogenic activity despite more drastics regulations. Among all air pollutants, Nitrogen oxides (NOx), such as NO and NO_2, are predominant.
It is well-known that those compounds exhibit direct high toxic effects on human health especially on skin and lung[1,2].
However microorganisms are also exposed to them, but their synergy with microorganisms on microbial virulence is still not stated.



In this study, we tried to evaluate the response of an airborne strain of *P. fluorescens,* MFAF76a[3] to a two hours exposure at 45ppm NO_2. 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.

Material and methods:

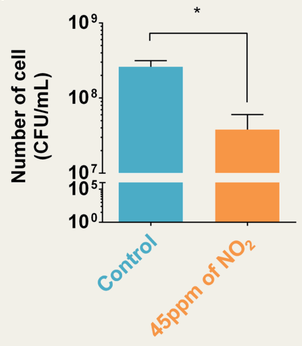




Results:

CULTIVABILITY

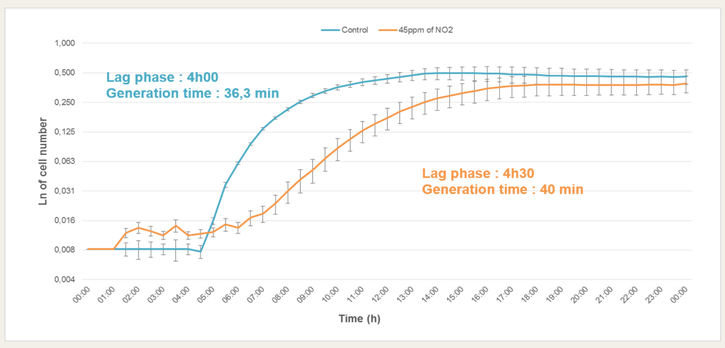
Quantification of colony forming units (CFU) from bacterial suspension



➢ **Reduction of one log by NO_2**

PHYSIOLOGY

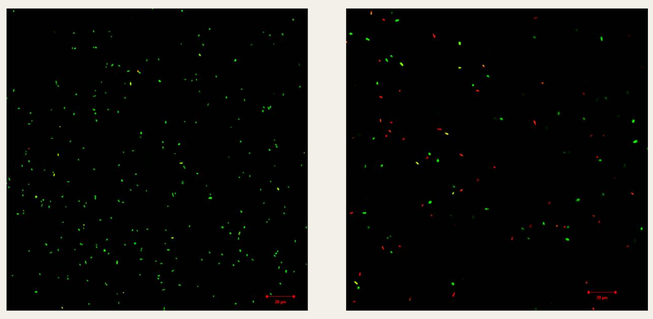
Growth kinetics of bacteria after exposure



➢ **Increase of lag phase and generation time of the growth of exposed bacteria**

VIABILITY

Observation of membrane permeability through Live Dead tests (PI and SYTO9) using confocal microscopy



Propidium Iodide (IP) penetrates only permeabilized bacteria

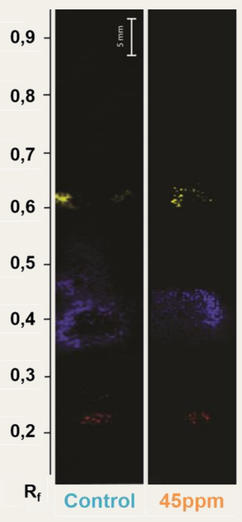
SYTO9 penetrates all bacteria

Control 45ppm of NO_2

➢ **More labelling with Propidium Iodide : membrane permeability increased?**

LIPIDOMIC STUDY[4]

Analysis of Glycerophospholipids (GP) content using HPTLC MALDI-TOF MS/MS



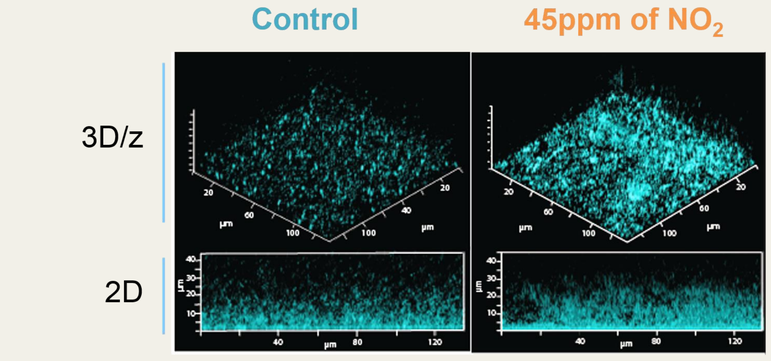
R_f	GP	Control	45 ppm NO_2
0,62 ± 0,03	PG	PG [16:0 + 16:1] PG [16:0 + 18:1]	PG [16:0 + 16:1] PG [16:0 + 18:1]
0,43 ± 0,02	PE	PE [16:0 + 16:1] PE [16:0 + 17:1] PE [16:0 + 18:1]	PE [16:0 + 16:1] PE [16:0 + 17:1] PE [16:0 + 18:1]
0,26 ± 0,02	UGP	UGP [16:0 + 17:0] UGP [16:0 + 18:0] UGP [17:1 + 18:0]	None
0,24 ± 0,01	PC	PC [16:0 + 16:1] PC [16:0 + 18:1]	PC [16:0 + 16:1] PC [16:0 + 18:1]

➢ **No modification of fatty acids insaturation**

➢ **Lack of an Unknown GlyceroPhospholipid (UGP) after exposure at 45ppm**

BIOFILM FORMATION[4]

24h biofilm observed by confocal microscopy and image analysis using a Zeiss LSM710 confocal microscope and the Comstat2 software



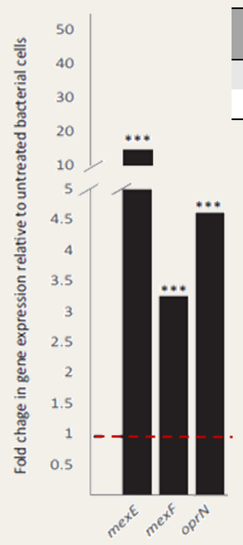
	Control	45ppm of NO_2
Max Thickness (µm)	9,25 ± 1,43	22,50 ± 1,22
Total Biomass ($\mu m^3.\mu m^{-2}$)	0,64 ± 0,18	0,78 ± 0,24

➢ **Modification of the sessile structure by NO_2**

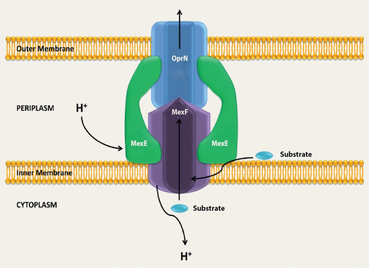
➢ **Presence of prominences**

TRANSCRIPTOMIC[4]

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



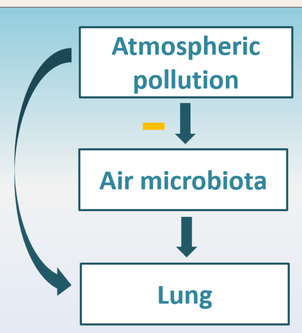
NO_2 concentration (ppm)	Ciprofloxacin MIC (µg/mL)	Chloramphenicol MIC (µg/mL)
Control	6,25	50
45ppm	12,5	>100



➢ **Increase of the expression of genes related to antibiotic resistance, specifically for fluoroquinolone resistance**

Conclusion:

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 NO_2 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) 2- INERIS Dioxyde d'azote: Données toxicologiques et environnementales (2015) 3-Duclairoir Poc *et al.* Int. J. Curr. Microbiol. Appl. Sci. 2014, 708e22 4-Kodakova *et al.*, Front. Microbiol. 2016, 7:379