Does nicotine influence the kinetics of biofilm development in reference and clinical strains *of Streptococcus pneumoniae* ?

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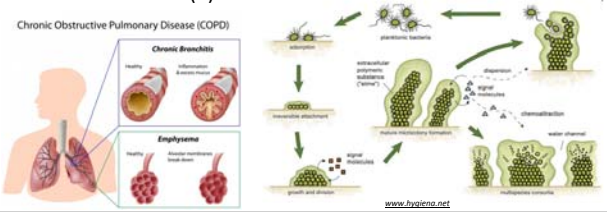
Introduction

- *S. pneumoniae* (SP) is a human pathogen frequently causing acute exacerbations of chronic bronchitis (AECB).
- Biofilms are involved in persistent infections such as AECB.
- Tobacco smoking is one of the main risk factors to develop chronic bronchitis (**1**).

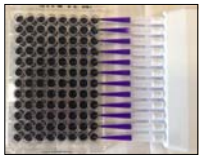


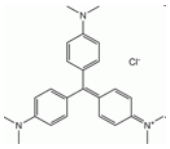
Aim of the study

Our objective was to study the impact of nicotine on the development of SP biofilm, using *in vitro* models of young to very mature biofilms.

Methods

- Strains : reference strain ATCC49619 and 2 clinical isolates, N1, coming from a smoking COPD patient and SP1337, isolated from a child suffering from CAP (non smoking patient).
- Biofilm : SP strains were cultivated for 2 to 16 days in 96-well plates using cation-ajusted Mueller Hinton broth with lysed horse blood (5%), glucose (2%) and supplemented or not with nicotine 15,3mg/L [human urinary concentration in smokers; **2**] as growth medium.
- Biofilm mass : measured by crystal violet staining and OD measurement at 570 nm (**3**).





References

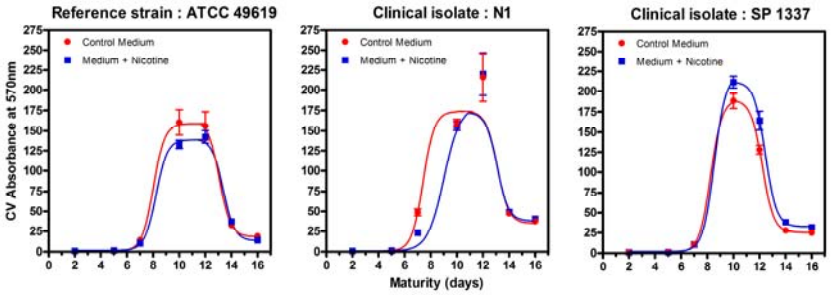
(1) Fabbri *et al.* (2008). *European Respiratory Journal*. 31, 204-212.

(2) Feyeraband *et al.* (1982). *British Medical Journal.* 284, 1002-1004.

(3) Roveta *et al.* (2007). *International Journal of Antimicrobial Agents*. 30, 415-421.

(4) Blaise *et al.* (2011). *Trends in Microbiology*. 19, 449-455.

Results



Kinetics of biofilm development in *S. pneumoniae* strains, as quantified by measurement of crystal violet OD_{570nm} (mean with SD, n=12)

- Biofilms developed overtime following a three-steps process:
  - Firstly, there was a lag phase of approx. 6-7 days during which there was no or minimal increase in biomass.
  - Secondly, biomass increased following a sigmoid function (with an Hillslope of 1) to reach a plateau at day 10, with the knee point of the curve being observed at day 7-8.
  - Thirdly, biomass decreased following again a sigmoid function (with a Hillslope of -1), to reach a lower plateau value at day 14.
- There was no significant difference in biomass between strains (one way-Anova with Tuckey post-test)
- The addition of nicotine did not significantly modify the extent or the rate of the whole process (unpaired t-test Welch corrected at each time point).

Conclusion

- Biofilm development in *S. pneumoniae* occurs after a latency phase, suggesting a slow adhesion of the bacteria to the support and/or a progressive adaptation of bacterial metabolism.
- A disassembling process seems to take place in very mature biofilms (> 12 days), which occurs at a rate similar to the assembling process.
  To our knowledge, this phenomenon, already described for other bacterial species (**4**), has never been previously observed in other *in vitro* models of pneumococcal biofilms. It may play a role in the spreading of the infection.
- Biofilm formation is not affected by the origin of the strain (smoker or non smoker), neither by the presence of nicotine in the culture medium. This may suggest that the preferential adhesion of bacteria in the respiratory tract of COPD patients may rely rather on the physiopathological environment to which bacteria are exposed *in vivo* than on a direct stress induced by nicotine on bacteria.

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