The involvement of the ami operon in Pseudomonas aeruginosa virulence regulation and biofilm formation reveals new functions for the amidase AmiE

T. Clamens, F. Desriac, T. Rosay, Alexandre Crépin, Alain Dufour, P Cornelis, E Bouffartigues, S Chevalier, M Feuilloley, Olivier Lesouhaitier

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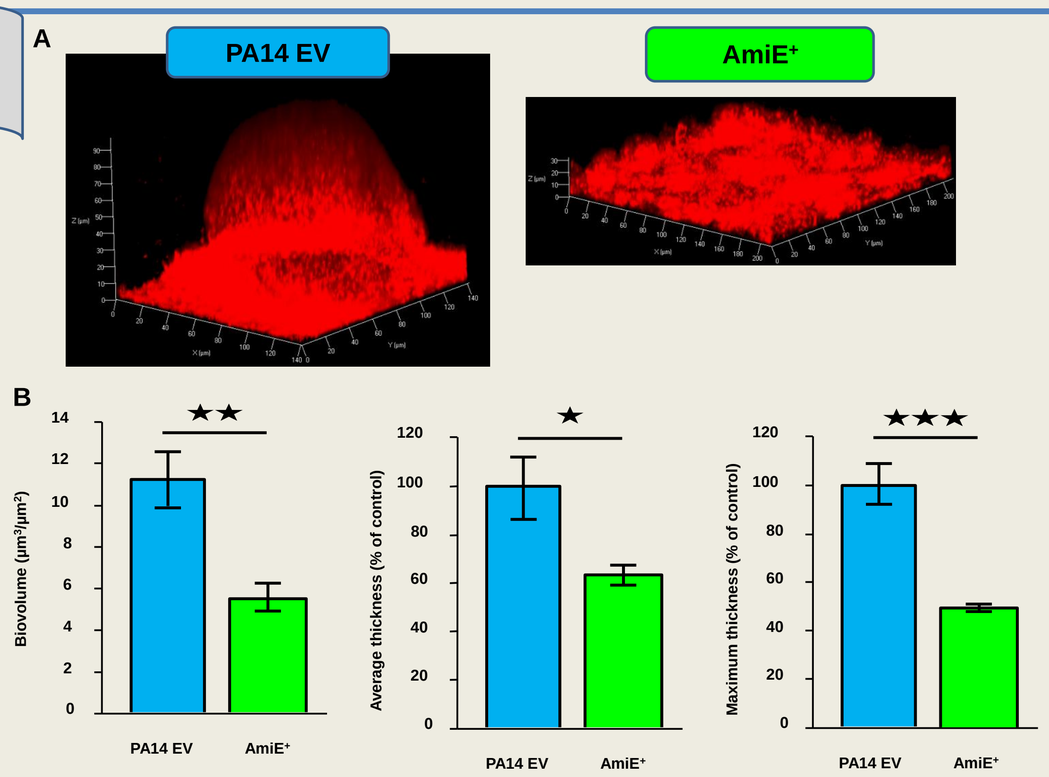
Clamens T[1], Desriac F[1], Rosay T[1], Alexandre Crépin[2], Alain Dufour[2], Cornelis P[1], Bouffartigues E[1], Chevalier S[1], Feuilloley MGJ[1], Lesouhaitier O[1]

[1]Laboratory of Microbiology Signals and Microenvironment EA 4312, Normandie Univ., Univ. Rouen; IRIB, Evreux, France.
[2]Univ. Bretagne-Sud, EA 3884, LBCM, IUEM, Lorient, France

Introduction

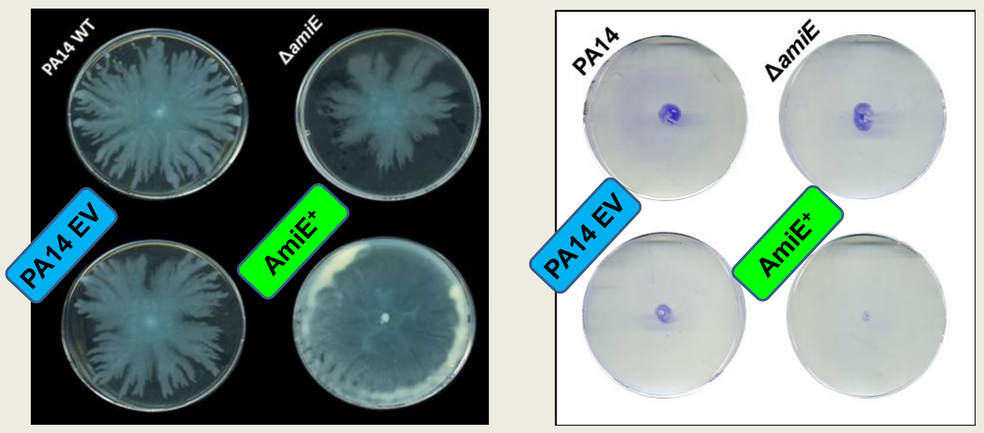
We observed that the «C-type Natriuretic Peptide», a human hormone produced in the lungs, is able to impact *P. aeruginosa* virulence and biofilm formation. We showed that these effects are caused by the binding of CNP to AmiC, a bacterial sensor coded by the *amiC* gene (Rosay *et al*., 2015). *amiC* belongs to the *ami* operon whose final product is the amidase enzyme AmiE coded by the *amiE* gene. The goal of the present work is to assess the impact of the AmiE protein on *P. aeruginosa* virulence regulation and the bacterial ability to form biofilm. In order to investigate AmiE impact on *P. aeruginosa* physiology we constructed a bacterial strain overproducing the enzyme AmiE (AmiE$^+$) and we compared the physiology of this strain with the control strains PA14 WT (wild-type) and PA14 EV (empty vector) and the mutant strain *ΔamiE*.

Biofilm



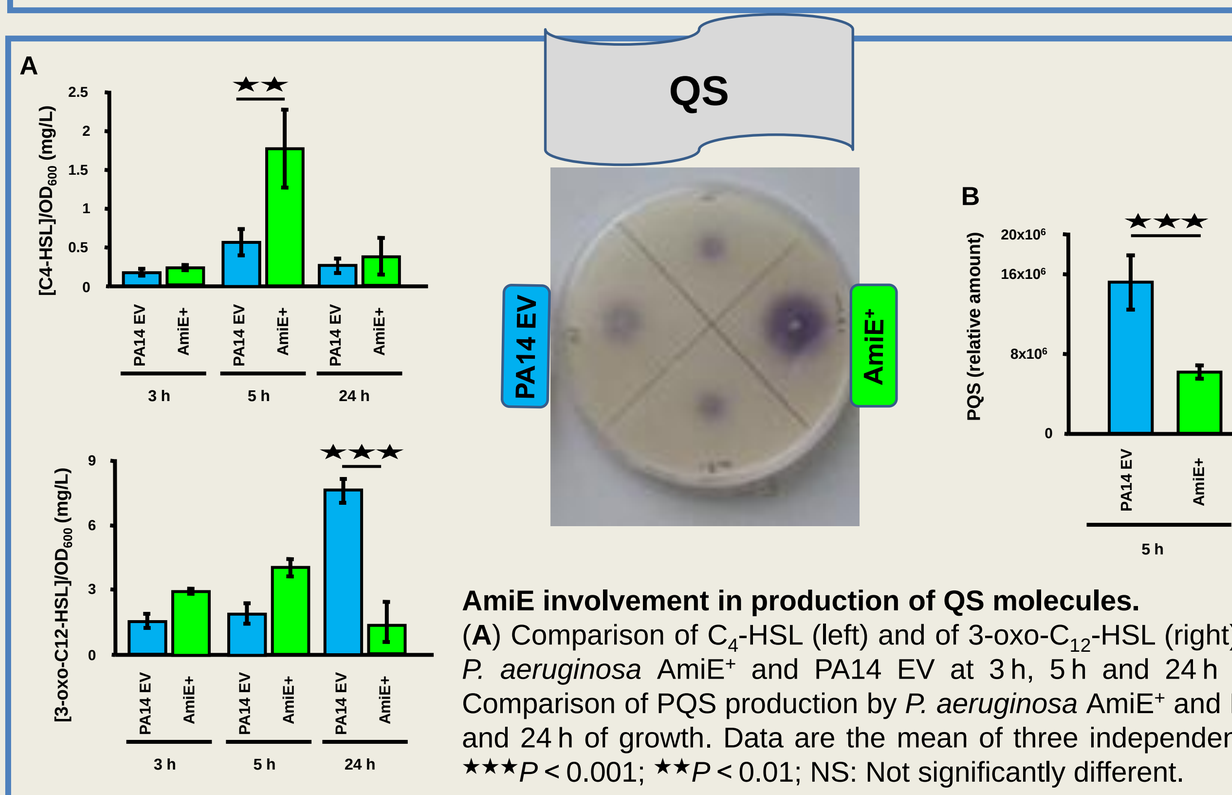
AmiE and *P. aeruginosa* biofilm formation. (**A**) 3D shadow representations of the biofilms developed by *P. aeruginosa* PA14 EV and AmiE+ under dynamic conditions at 37 °C for 24 h in LB broth. Biofilms were stained with Syto 61 red dye and observed by confocal laser scanning microscopy. (**B**) COMSTAT analyses of biofilms of *P. aeruginosa* PA14 EV and AmiE+ strains. Data are the mean of eighteen measures from six independent channels from two independent experiments. ★★★$P < 0.001$; ★★$P < 0.01$; NS: Not significantly different.

Motility



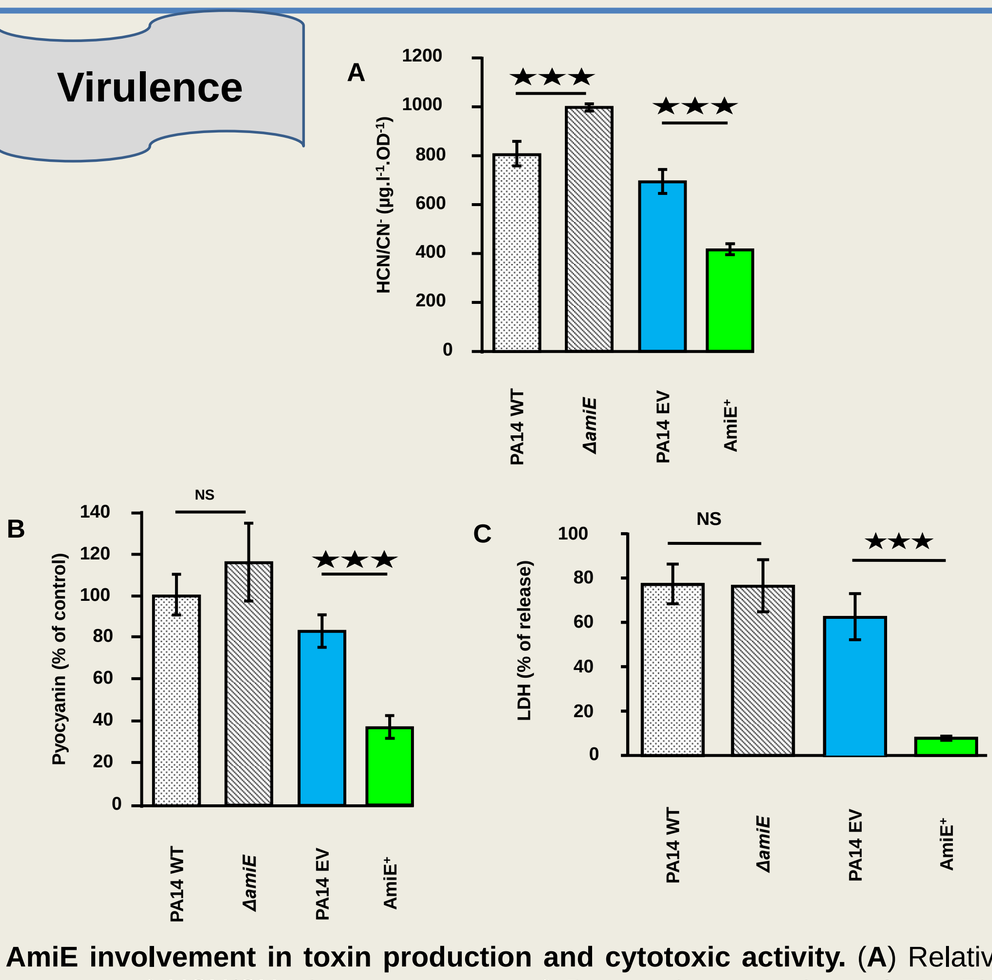
AmiE involvement in *P. aeruginosa* motility. (**A**) Pictures show swarming motilities of *P. aeruginosa* PA14 WT (control), PA14 Δ *amiE* mutant strain, PA14 strain harboring the empty vector (PA14 EV), and PA14 AmiE over-producing strain (AmiE+) after 18 h at 37 °C.
(**B**) Pictures show twitching motilities of *P. aeruginosa* strains after 16 h at 37 °C.

QS



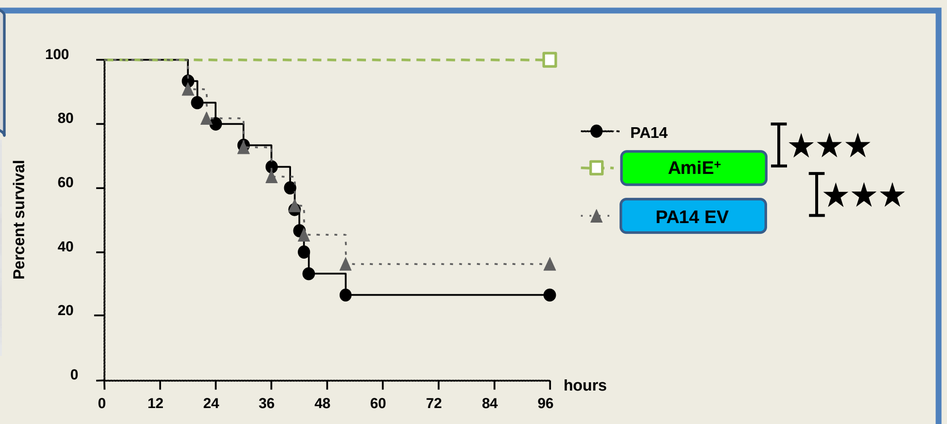
AmiE involvement in production of QS molecules.
(**A**) Comparison of C_4-HSL (left) and of 3-oxo-C_{12}-HSL (right) production by *P. aeruginosa* AmiE$^+$ and PA14 EV at 3 h, 5 h and 24 h of growth. (**B**) Comparison of PQS production by *P. aeruginosa* AmiE$^+$ and PA14 EV at 5 h and 24 h of growth. Data are the mean of three independent experiments. ★★★$P < 0.001$; ★★$P < 0.01$; NS: Not significantly different.

Virulence



AmiE involvement in toxin production and cytotoxic activity. (**A**) Relative amounts of HCN/CN− in supernatants of *P. aeruginosa* strains culture. The mean HCN/CN− level in the control was 1,834 +/- 134 µ g.l−1. (**B**) Relative amounts of pyocyanin in supernatants of *P. aeruginosa* strains culture. (**C**) Involvement of AmiE in *P. aeruginosa* cytotoxicity towards A549 lung cells. The measurement of LDH was done after 6 h of contact with PA14 WT, Δ *amiE*, PA14 EV and AmiE+ strains. All data are the mean of three independent experiments. ★★★$P < 0.001$; NS: Not significantly different.

In vivo



Effects of *P. aeruginosa* AmiE+ strain on acute lung injury model. Mice were infected with intranasal instillation of 1.10^7 CFU of *P. aeruginosa* PA14 WT, PA14 EV and AmiE+ strains (n = 11). Lethality was monitored for 96 h after *P. aeruginosa* infection. ★★★$P < 0.001$.

Conclusion

This work shows that the *P. aeruginosa ami* operon and particularly the enzyme AmiE could be involved in bacterial virulence regulation, suggesting that this operon could represent a new potential target for developing molecules able to disrupt *P. aeruginosa* pathogenicity. In order to decipher this new function putative partners for AmiE enzyme are currently being investigated through Bacterial Two-Hybrid screening.

Supported by :

References:
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Corresponding authors:
thomas.clamens1@univ-rouen.fr
olivier.lesouhait@univ-rouen.fr