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

Clamens T¹, Desriac F¹, Rosay T¹, Alexandre Crépin², Alain Dufour², Cornelis P¹, Bouffartigues E¹, Chevalier S¹, Feuilloley MGJ¹, Lesouhaitier O¹

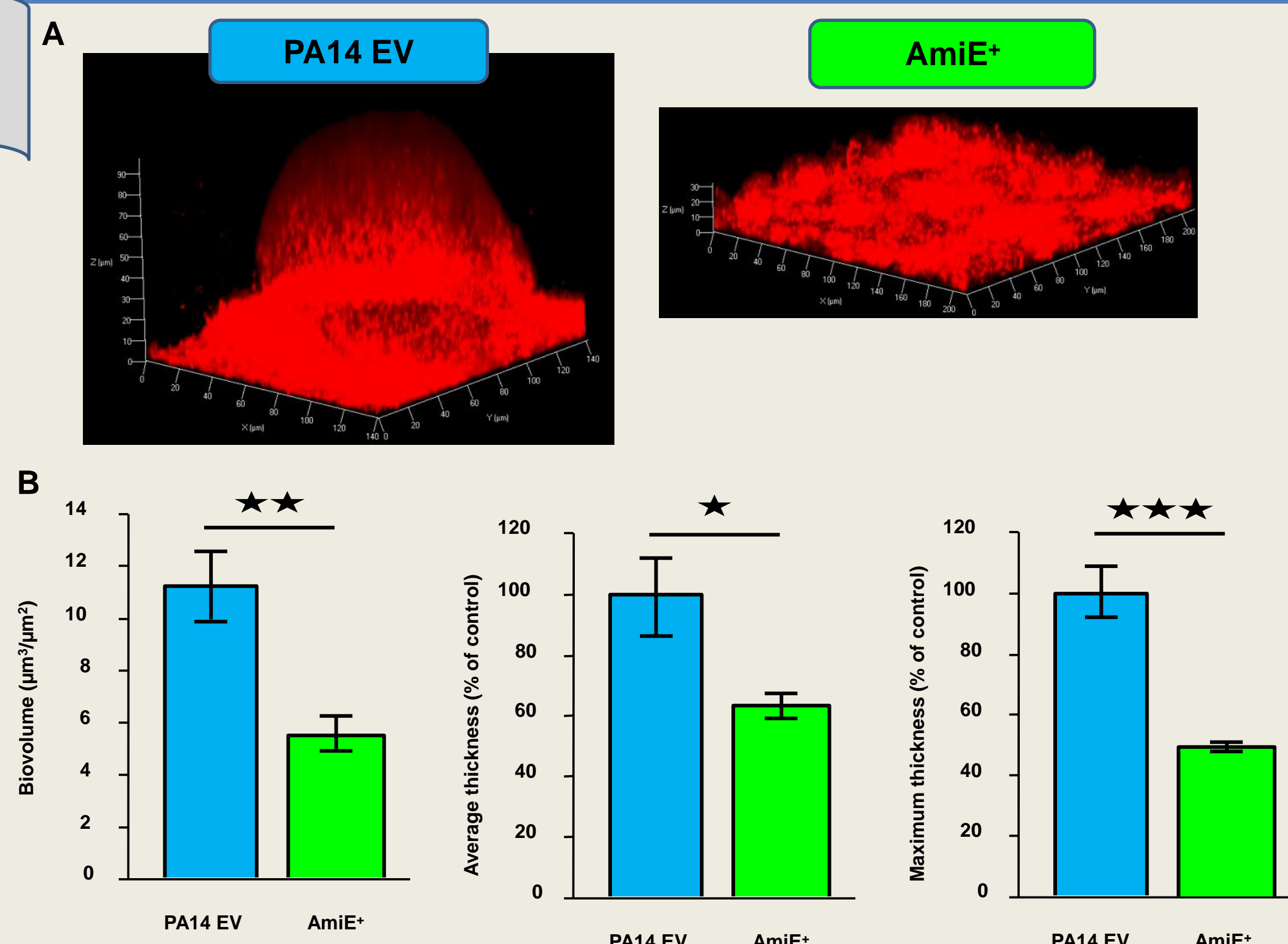
¹Laboratory of Microbiology Signals and Microenvironment EA 4312, Normandie Univ., Univ. Rouen; IRIB, Evreux, France.

²Univ. Bretagne-Sud, EA 3884, LBCM, IUEM, Lorient, France

Introduction

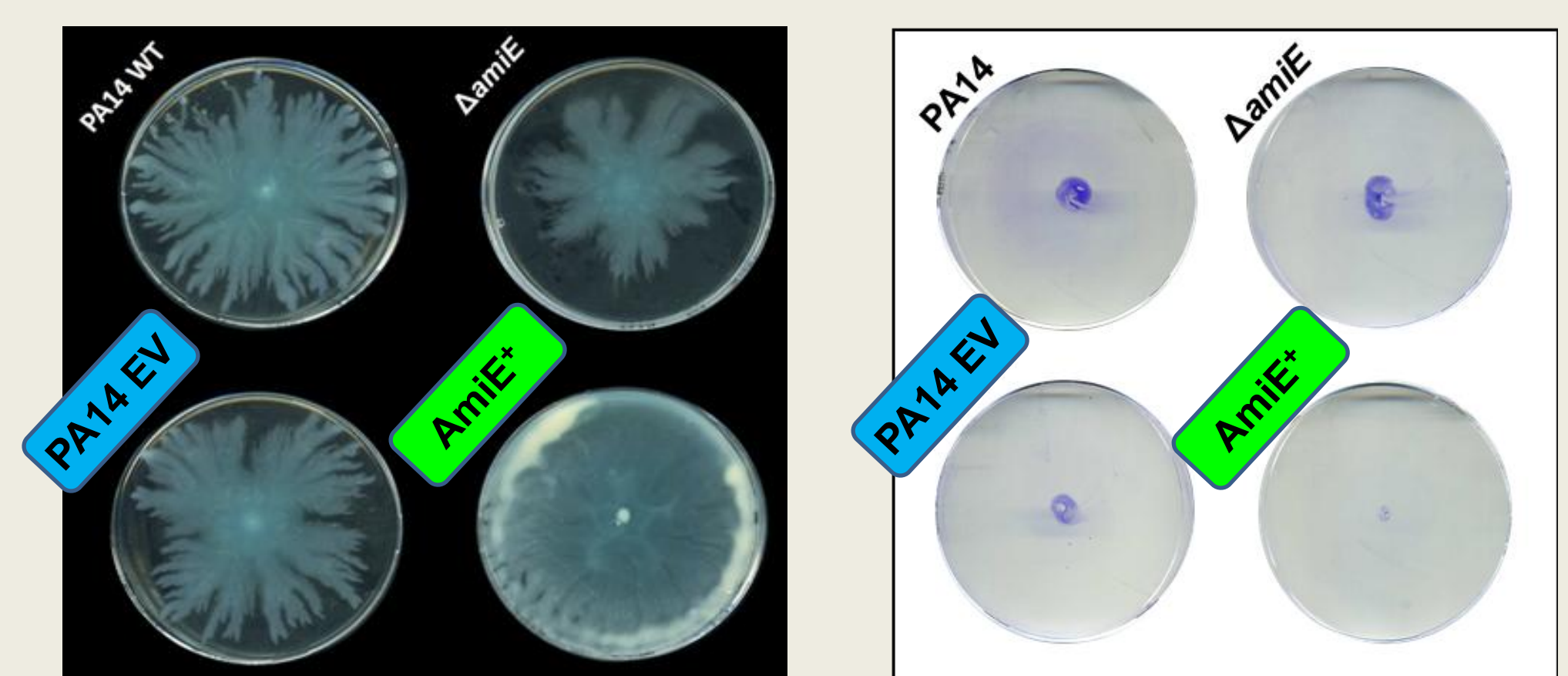
We observed that the «C-type Natriuretic Peptide», a human hormone produced in the lungs, is able to impact *P. aeruginosa* virulence and biofilm formation. We showed that these effects are caused by the binding of CNP to AmiC, a bacterial sensor coded by the *amiC* gene (Rosay *et al.*, 2015). *amiC* 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 Δ *amiE*.

Biofilm

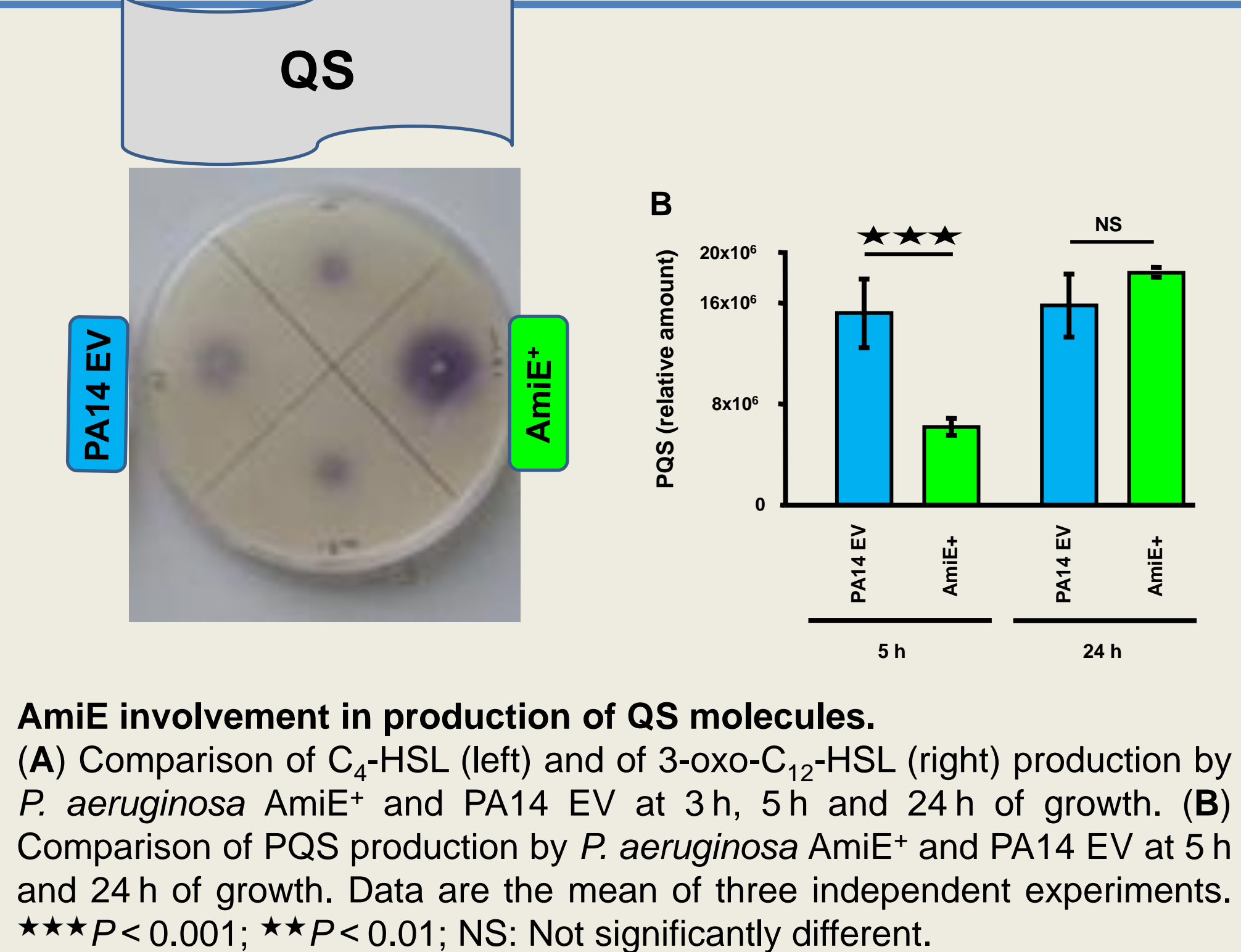
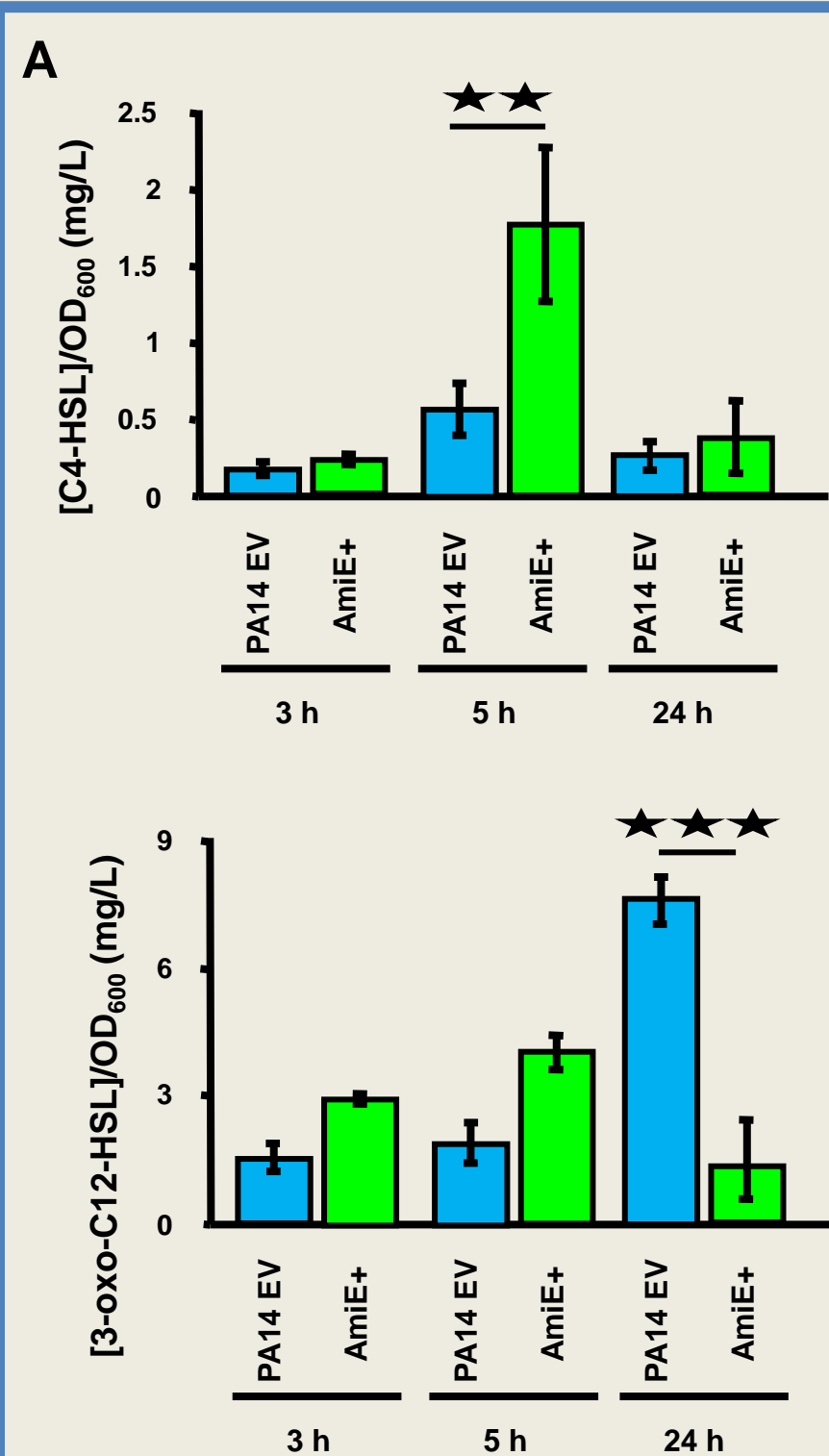


AmiE and *P. aeruginosa* biofilm formation. (A) 3D shadow representations of the biofilms developed by *P. aeruginosa* PA14 EV and AmiE⁺ under dynamic conditions at 37 °C for 24 h in LB broth. Biofilms were stained with Syto 61 red dye and observed by confocal laser scanning microscopy. (B) COMSTAT analyses 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

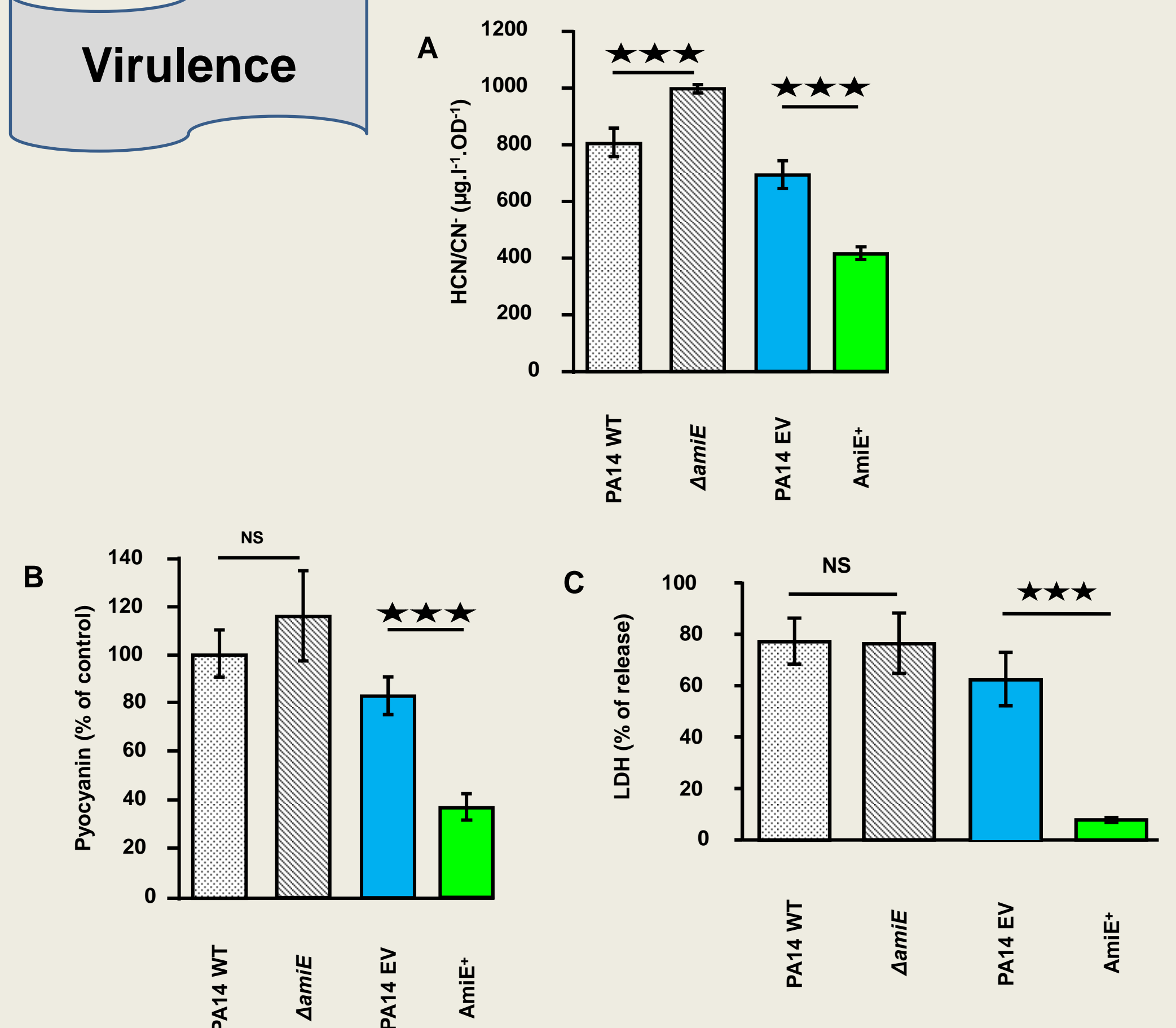


AmiE involvement in *P. aeruginosa* motility. (A) Pictures show swarming motilities of *P. aeruginosa* PA14 WT (control), PA14 Δ *amiE* mutant strain, PA14 strain harboring the empty vector (PA14 EV), and PA14 AmiE over-producing strain (AmiE⁺) after 18 h at 37 °C. (B) Pictures show twitching motilities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



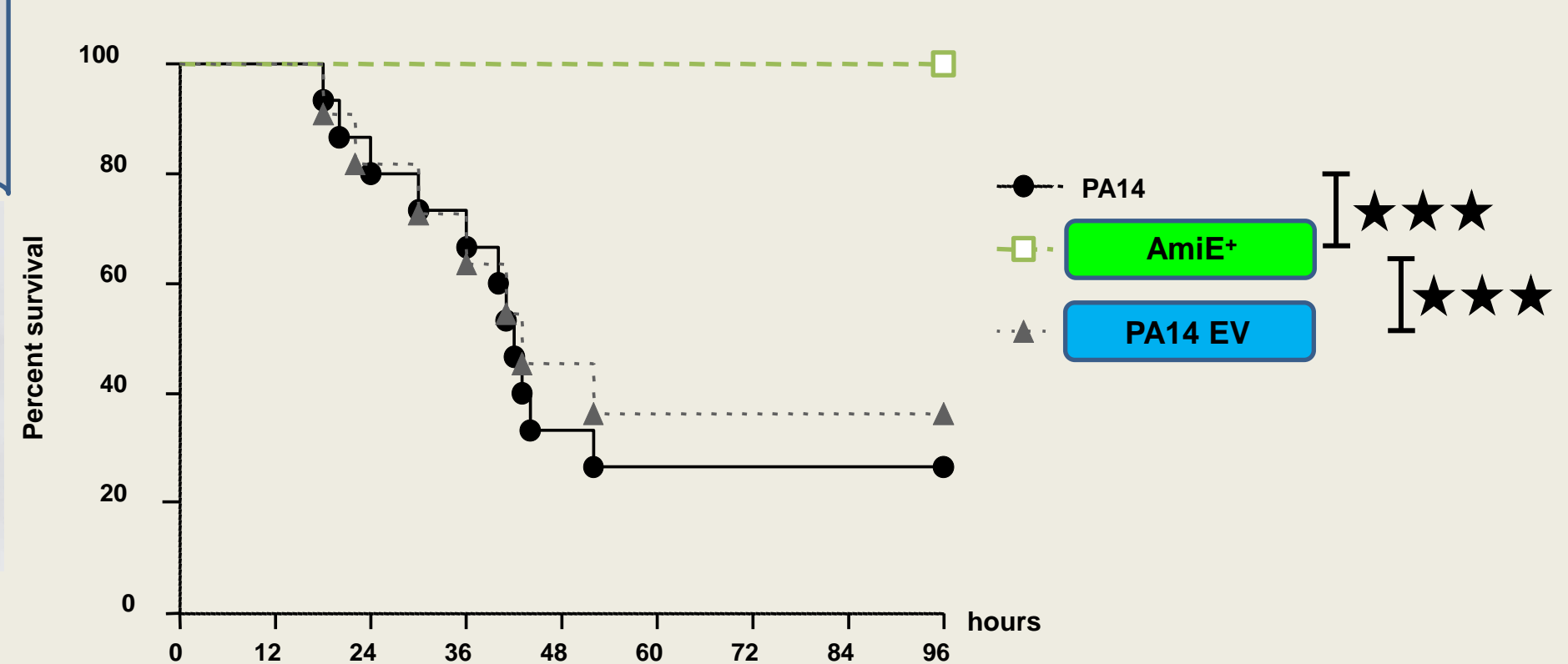
AmiE involvement in production of QS molecules. (A) Comparison of C₄-HSL (left) and of 3-oxo-C₁₂-HSL (right) production by *P. aeruginosa* AmiE⁺ and PA14 EV at 3 h, 5 h and 24 h of growth. (B) Comparison of PQS production by *P. aeruginosa* AmiE⁺ and PA14 EV at 5 h and 24 h of growth. Data are the mean 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 μg.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 1.10⁷ 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:



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