Supplemental Material

The inhibition of biofilm formation was evaluated as follows. 50 µl of a bacterial suspension at 5×10^5 CFU/ml were added to 50 µl of MHB containing Bac7(1-35) at 1/2, 1/4 and 1/8 MIC or no peptide as control on a flat-bottom 96-well polystyrene microtiter plate. After incubation at 37°C for 20 h, non-adherent bacteria were removed by washing three times with 150 µl sterile MHB. Subsequently 100 µl of a 1-(4, 5-Dimethylthiazol-2-yl)-3, 5-diphenylformazan (MTT) solution in MHB at a final concentration of 1 mg/ml were added to each well and the plate was incubated for additional 4 h at 37° C. Each well was then washed with 150 µl sterile PBS and biofilms were destained by treatment with 100 µl of Lysis Buffer (20% SDS in 50% water and 50% dimethylformamide) for 16 h at 37°C. The absorbance was read at 570 nm with a Tecan Infinite Pro200 instrument.

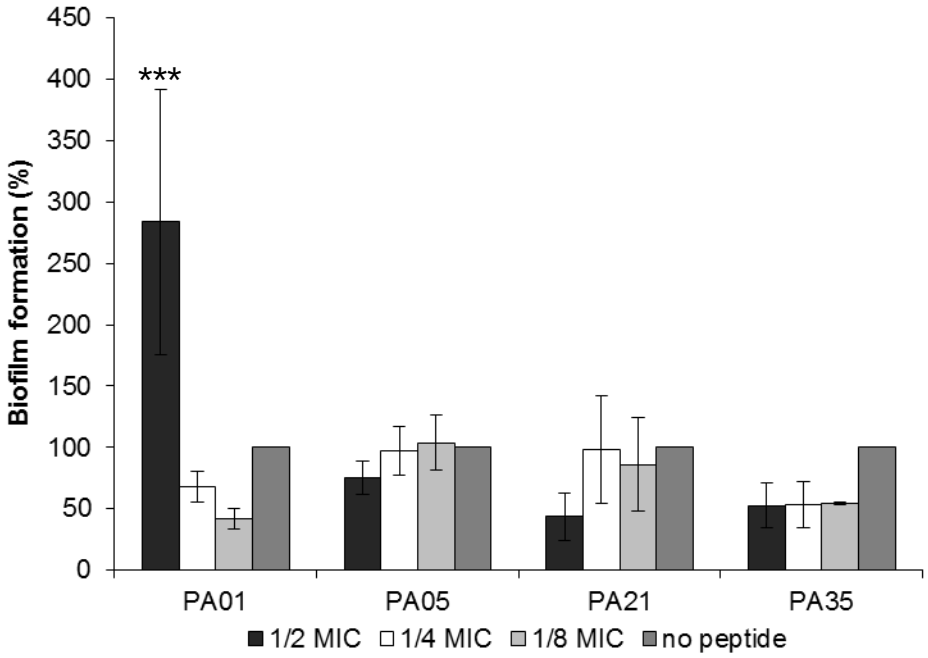
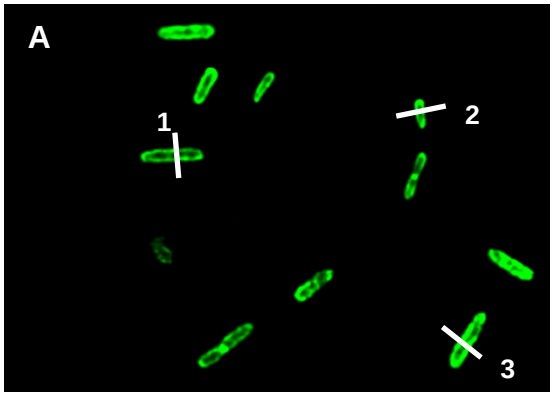
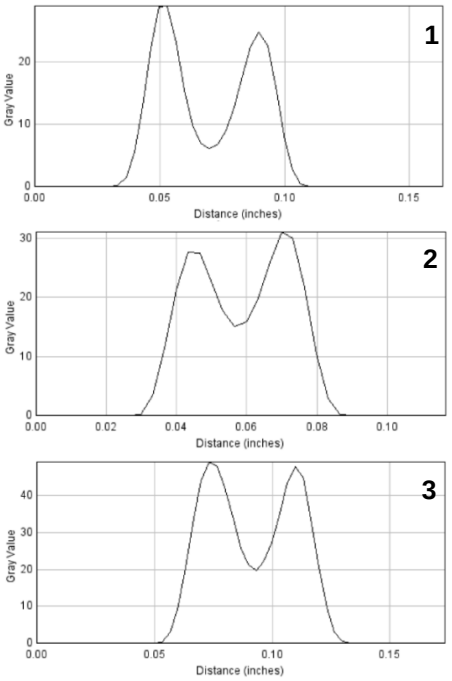
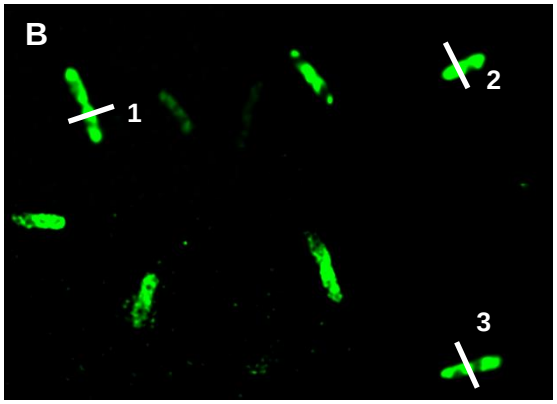


Figure S1. Inhibition of biofilm formation in presence of Bac7(1-35) on *P. aeruginosa* strains. Biofilm production was evaluated by the MTT assay on bacterial cells incubated with sub-inhibitory concentrations of peptide for 24 h. The results are expressed as percentage of the biofilm formed with respect to untreated control taken as 100%. Data are the mean ± standard deviation of four independent experiments performed in duplicate. *** $p < 0.001$ *vs* no peptide, ANOVA with post-test Tukey-Kramer.

CSLM analysis were performed as described in the Materials and Methods section of the main text. After the deconvolution process of the images, the distribution of the fluorescence signal was evaluated by putting a transversally marker line and then analyzing the fluorescence plot profile with the Image J 1.40g software. The plot profile shows a two-dimensional graph of the intensities of pixels along the line within the image. The x-axis represents distance along the line and the y-axis is the pixel intensity.







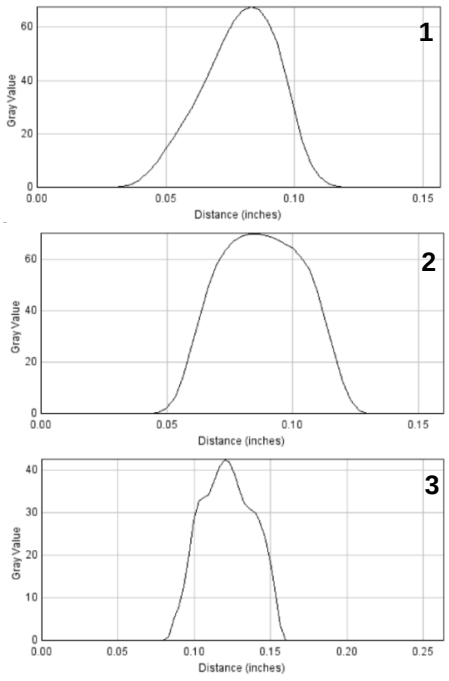


Figure S2. Evaluation of fluorescence distribution by confocal microscopy and analysis of the plot profile.

Confocal microscopy images of PAO1 (**A**) and PAO1 [psbmA1] (**B**) treated with 1 µM Bac7(1-35)-BY for 30 min and relative plots of fluorescence profile. All images are representative sections from the middle of the bacterial cell. Many fields were examined and, for each experiment, over 95% of the cells displayed the pattern of the respective representative cell shown here. For the numbered cells, it is reported the plot profile of fluorescence relatively to the marker.