

Supplementary Information

Titanium complexes affect *Bacillus subtilis* biofilm formation

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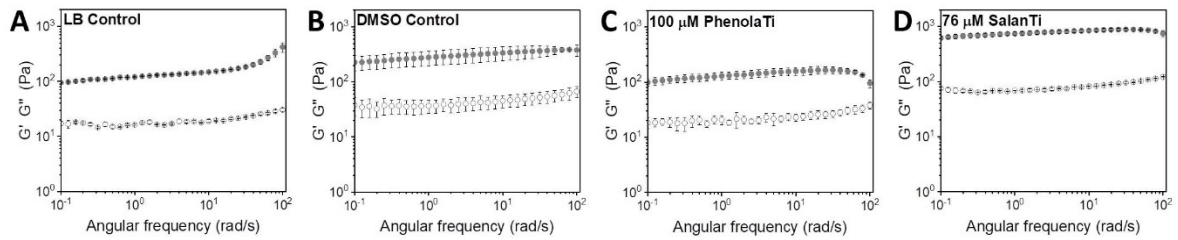
Figure 1. Mechanical properties of pellicles in the absence and presence of Ti(IV) complexes

Figure 2. Effect of Ti(IV) complexes on OD measurements of whole well content

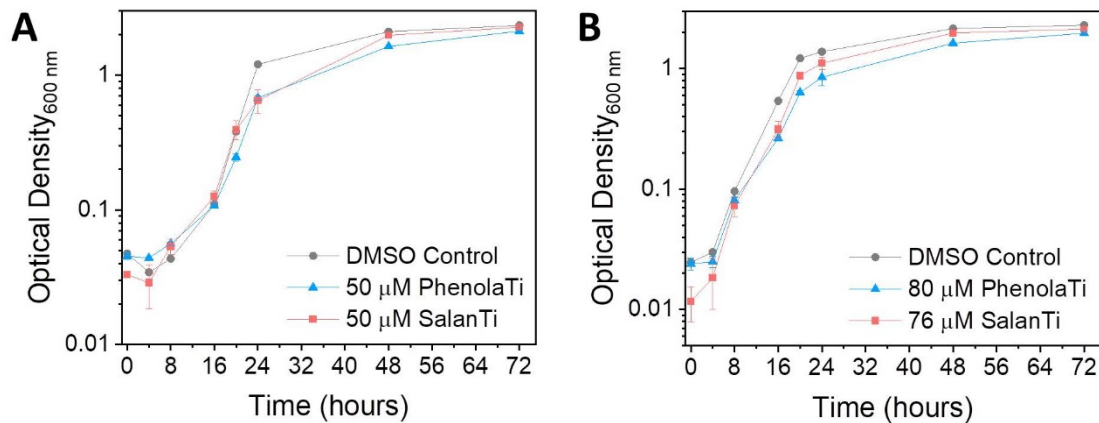
Figure 3. Effect of Ti(IV) complexes on pellicle growth only

Figure 4. Effect of Ti(IV) complexes on cells in biofilms

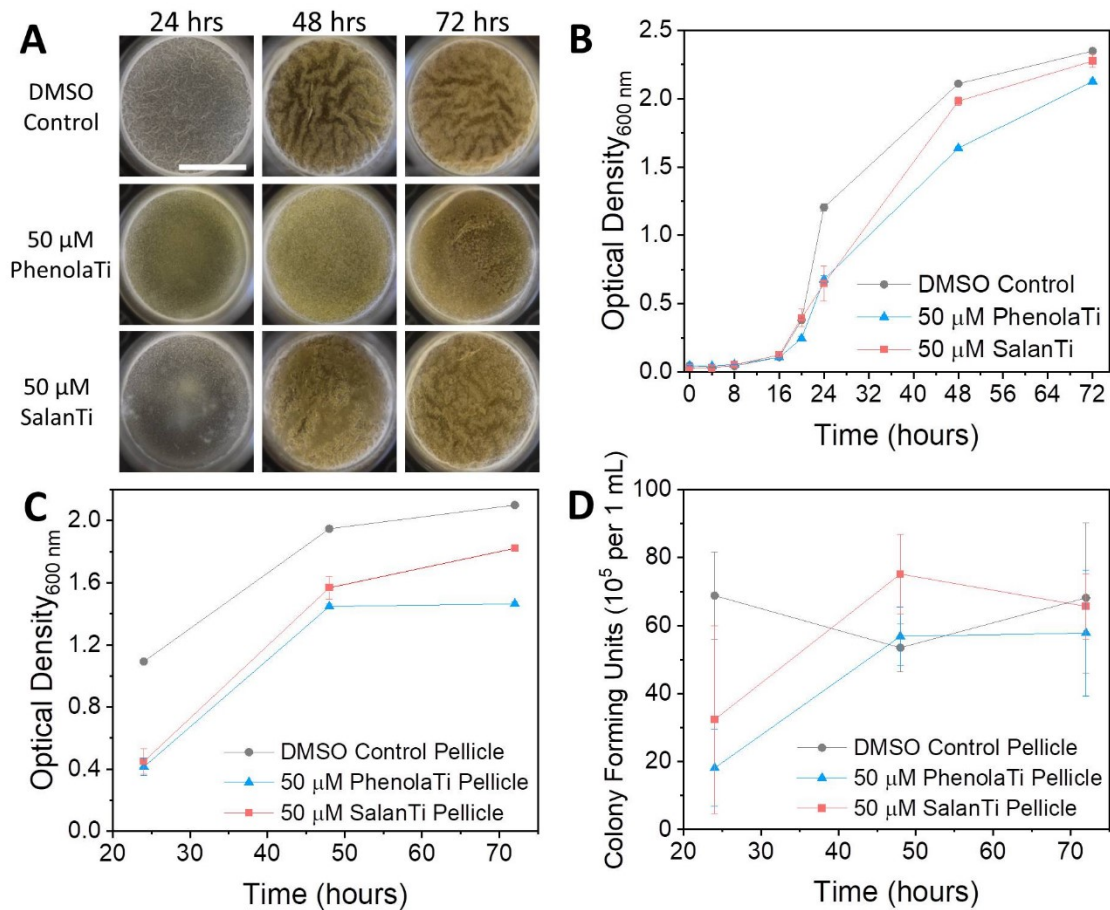
Figure 5. Effect of Ti(IV) complexes on *Escherichia coli* bacterial growth in shaken conditions and pellicle formation.



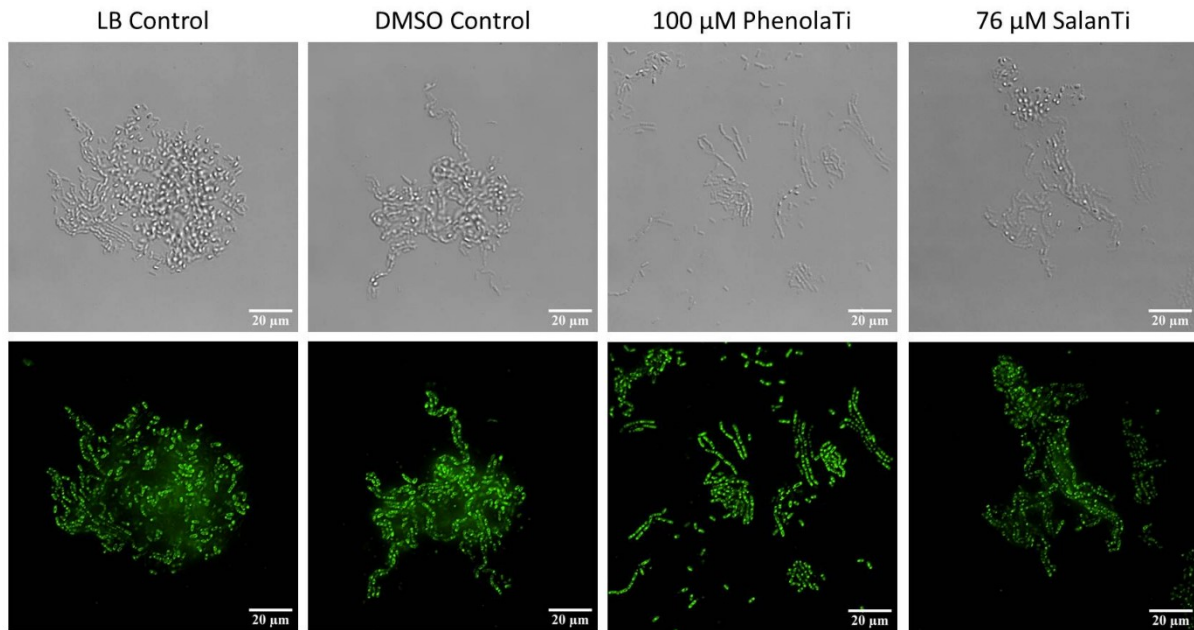
Supplementary Fig. 1 Mechanical properties of pellicles in the absence and presence of Ti(IV) complexes. Storage and loss modulus (G' , G'' , respectively) measurements of WT pellicles in the absence and presence of Ti(IV) complexes (A-D). Storage modulus G' – full symbols; loss modulus G'' – empty symbols.



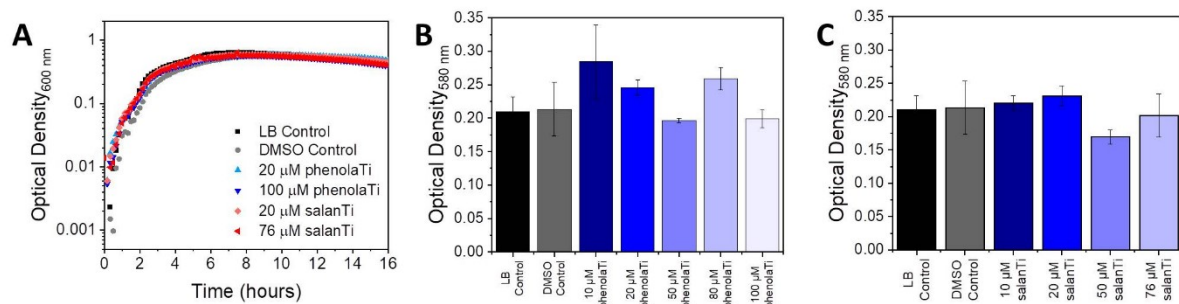
Supplementary Fig. 2 Effect of Ti(IV) complexes on OD measurements of whole well content. Semi-log graphs showing the OD of whole well content along time in the presence of 50 μM Ti(IV) complexes (A) and 80 μM Ti(IV) complexes (B). The whole well content was sonicated prior to the measurement of OD.



Supplementary Fig. 3 Effect of Ti(IV) complexes on pellicle growth only. Images of pellicles in the absence and presence of 50 μM Ti(IV) complexes (A), OD measurements of whole well content following disruption using sonication (B), OD measurements of pellicles only following disruption using sonication (C), and Colony forming units (CFU) counts of disrupted pellicles along time. Scale in (A) corresponds to 7.5 mm.



Supplementary Fig. 4 Effect of Ti(IV) complexes on cells in biofilms. Microscopy images of samples taken from 48 h old WT pellicles. Top row: DIC bright field images of cells from control and pellicles treated with Ti(IV) complexes. As control pellicles we used pellicles with no additives (LB control) and pellicles with DMSO added in a similar volume to that used with DMSO-dissolved Ti(IV) complexes. Bottom row: fluorescence imaging of samples from pellicles grown in the absence and presence of Ti(IV) complexes. Samples were exposed to BioTracker 510 Green C2(FM2-10) synaptic dye prior to imaging.



Supplementary Fig. 5 Effect of Ti(IV) complexes on *E. coli* K-12 bacterial growth in shaken conditions and pellicle formation. (A) Growth curves of *E. coli* along time in the presence of increasing concentrations of phenolaTi and salanTi. Growth curves in the absence of Ti(IV) complexes in pure LB culture media (LB control) and in media containing only DMSO (DMSO control) served as controls. *E. coli* pellicle formation was quantified with crystal violet staining and optical density measurement (OD_{580 nm}) of resuspended pellicles (see experimental section) in the absence and presence of increasing concentrations of phenolaTi (B) and salanTi (C).