

Table S1. Minimal inhibitory concentration (MIC) of ZnSO₄ in mM for *M. adhaerens* HP15 wild-type and mutant Δ czcCBA.1/2 in f/2 medium. The MIC was determined in a 2-fold dilution assay in liquid f/2 medium at 18°C without additional carbon or nitrogen source as described previously (Stahl et al. 2015). Due to a precipitation in the f/2 medium hindering OD₆₀₀ measurements, 10 μ l culture from each well were spotted on MB agar plates to check for surviving bacterial cells. The means and standard deviations were calculated from three replicates each.

<i>M. adhaerens</i> HP15 strain	Minimal inhibitory concentration (MIC) for ZnSO ₄ in mM
wild-type	0.63 \pm 0.00
Δ czcCBA.1/2	0.63 \pm 0.00

Table S2. *P* values of significance of the TEP quantification results presented in Figure 2 and section 3.2. *P* values lower than 0.05 were regarded as significant.

Compared cultures	<i>P</i> value	Significance
<i>Assays with zinc addition</i>		
HP15 WT + T.w. – HP15 Δ czcCBA.1/2 + T.w.	0.126	No
HP15 WT – HP15 Δ czcCBA.1/2	0.089	No
HP15 WT + T.w. – HP15 WT	0.026	Yes
HP15 Δ czcCBA.1/2 + T.w. – HP15 Δ czcCBA.1/2	0.017	Yes
HP15 WT + T.w. – T.w.	0.072	No
HP15 Δ czcCBA.1/2 + T.w. – T.w.	0.018	Yes
<i>Assays without zinc addition</i>		
HP15 WT + T.w. – HP15 Δ czcCBA.1/2 + T.w.	0.343	No
HP15 WT – HP15 Δ czcCBA.1/2	0.413	No
HP15 WT + T.w. – HP15 WT	1.000	No
HP15 Δ czcCBA.1/2 + T.w. – HP15 Δ czcCBA.1/2	0.237	No
HP15 WT + T.w. – T.w.	0.175	No
HP15 Δ czcCBA.1/2 + T.w. – T.w.	0.138	No

Table S3. Bacterial cell numbers at the start of incubation, in the separated bacterial fractions and bacterial cell density inside TEP in the attached fractions of co-cultures of *M. adhaerens* HP15 and *T. weissflogii*. CFU mL⁻¹ in the separated fractions were determined by serial dilution plating. The bacterial cell density inside TEP was calculated by the ratio of CFU mL⁻¹ to TEP in µg Xeq. mL⁻¹. Values for CFU mL⁻¹ and bacterial cell density represent the mean of three biological replicates with their respective standard deviation.

	f/2		f/2 + ZnSO ₄	
	<i>M. adhaerens</i> HP15 WT	<i>M. adhaerens</i> HP15 ΔczcCBA.1/2	<i>M. adhaerens</i> HP15 WT	<i>M. adhaerens</i> HP15 ΔczcCBA.1/2
<i>CFU mL⁻¹ at the start of the incubation</i>				
	1.57 ± 0.2 × 10 ⁷	1.33 ± 0.2 × 10 ⁷	3.39 ± 0.5 × 10 ⁷	3.31 ± 0.3 × 10 ⁷
<i>CFU mL⁻¹ in the separated bacterial fractions after 24 h incubation and filtration</i>				
Free-living	3.4 ± 1.3 × 10 ⁷	2.7 ± 1.3 × 10 ⁷	3.1 ± 2.3 × 10 ⁶	6.0 ± 4.4 × 10 ⁶
Attached	1.0 ± 0.7 × 10 ⁶	8.1 ± 5.0 × 10 ⁵	2.7 ± 1.5 × 10 ⁶	1.5 ± 1.4 × 10 ⁶
Total cells mL ⁻¹	3.5 ± 1.3 × 10 ⁷	2.8 ± 1.3 × 10 ⁷	5.8 ± 2.8 × 10 ⁶	8.1 ± 3.4 × 10 ⁶
<i>Bacterial cell density inside TEP (CFU/ TEP (µg Xeq))</i>				
	4.6 ± 0.007 × 10 ⁶	3.0 ± 0.003 × 10 ⁶	5.5 ± 0.1 × 10 ⁵ *	1.5 ± 1.0 × 10 ⁵ *

* significant difference (p < 0.05)

Table S4. *P* values of significance of the zinc quantification results presented in Figure 4 and section 3.4. *P* values lower than 0.05 were regarded as significant.

Compared cultures	P value	Significance
<i>Attached fractions of assays with zinc addition</i>		
HP15 WT + T.w. – HP15 ΔczcCBA.1/2 + T.w.	0.097	No
HP15 WT – HP15 ΔczcCBA.1/2	0.016	Yes
HP15 WT + T.w. – HP15 WT	0.132	No
HP15 ΔczcCBA.1/2 + T.w. – HP15 ΔczcCBA.1/2	0.046	Yes
HP15 WT + T.w. – T.w.	0.029	Yes
HP15 WT + T.w. – f/2	0.027	Yes
HP15 WT – T.w.	0.115	No
HP15 WT – f/2	0.170	No
HP15 ΔczcCBA.1/2 + T.w. – T.w.	0.128	No
HP15 ΔczcCBA.1/2 + T.w. – f/2	0.249	No
HP15 ΔczcCBA.1/2 – T.w.	0.076	No
HP15 ΔczcCBA.1/2 – f/2	0.050	No

Table S5. Zinc concentrations in the attached fractions of assays with zinc addition before normalization to TEP in mM. The concentration of zinc in the samples was quantified using a photometric ligand-binding assay. Concentrations given are the mean of three biological replicates with their standard deviation. HP15 + T. w.: co-cultures of *M. adhaerens* HP15 and *T. weissflogii*; HP15: *M. adhaerens* HP15 control; T. w.: *T. weissflogii* control; f/2: medium control.

Culture	Zinc concentration in mM
HP15 WT + T.w.	0.15 ± 0.02*
HP15 WT	0.09 ± 0.03*
HP15 Δ czcCBA.1/2 + T. w.	0.23 ± 0.04**
HP15 Δ czcCBA.1/2	0.06 ± 0.05**
T. w.	0.24 ± 0.05
f/2	0.11 ± 0.04

* significant difference (p = 0.029)

** significant difference (p = 0.009)

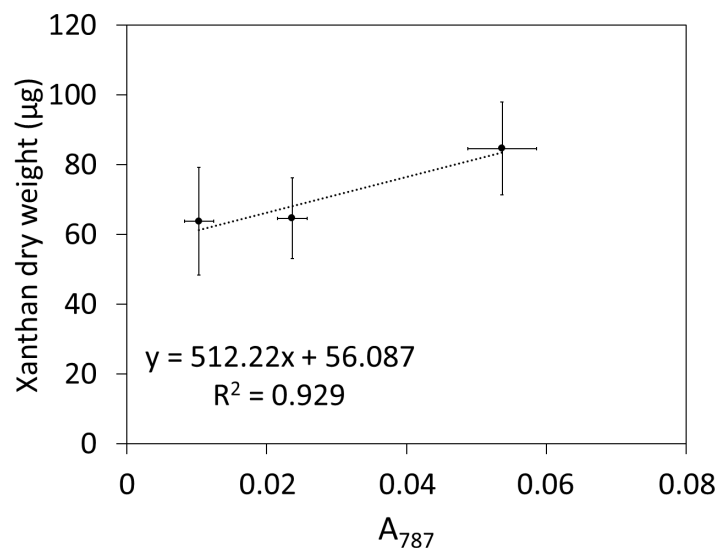


Figure S1. Gum xanthan calibration curve used for TEP quantification. Different amounts of Xanthan gum were filtered onto 0.4 µm pore-sized polycarbonate filters and the dry weights were determined. The same amounts of gum Xanthan were used for spectrophotometric measurements after staining with Alcian Blue. The slope of the calibration curve (512.22) equals the calibration factor used for calculations during the TEP quantification.

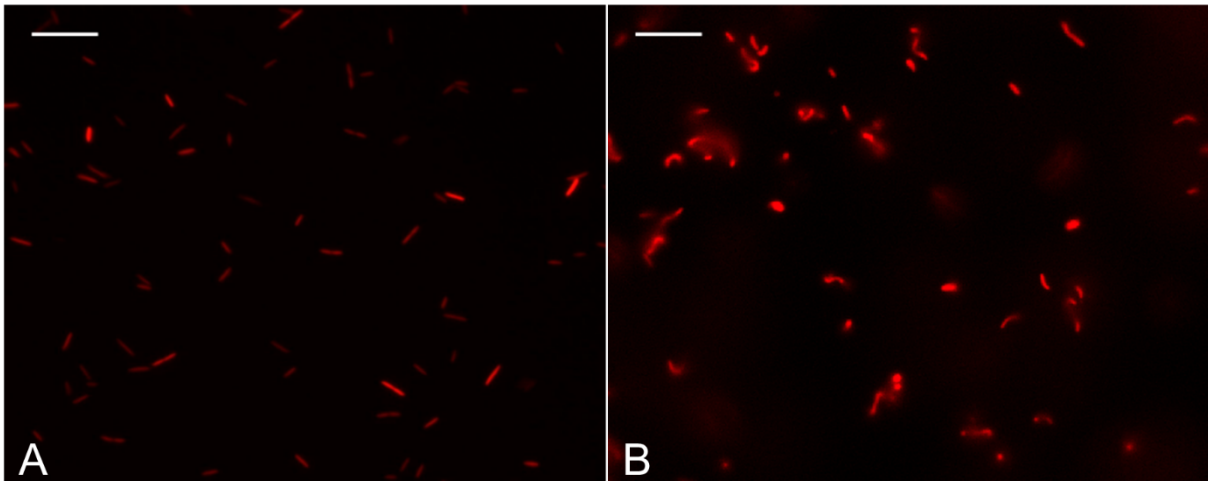


Figure S2. Fluorescently labelled *M. adhaerens* HP15 strains. The plasmid pBBR-4-DsRed was brought into *M. adhaerens* HP15 by biparental conjugation. Fluorescence microscopy was done at a 1,000x magnification. Panel A. *M. adhaerens* HP15 wild-type + pBBR-4-DsRed; Panel B. *M. adhaerens* HP15 $\Delta czcCBA.1/2$ + pBBR-4-DsRed. Scale bars: 10 μ m.

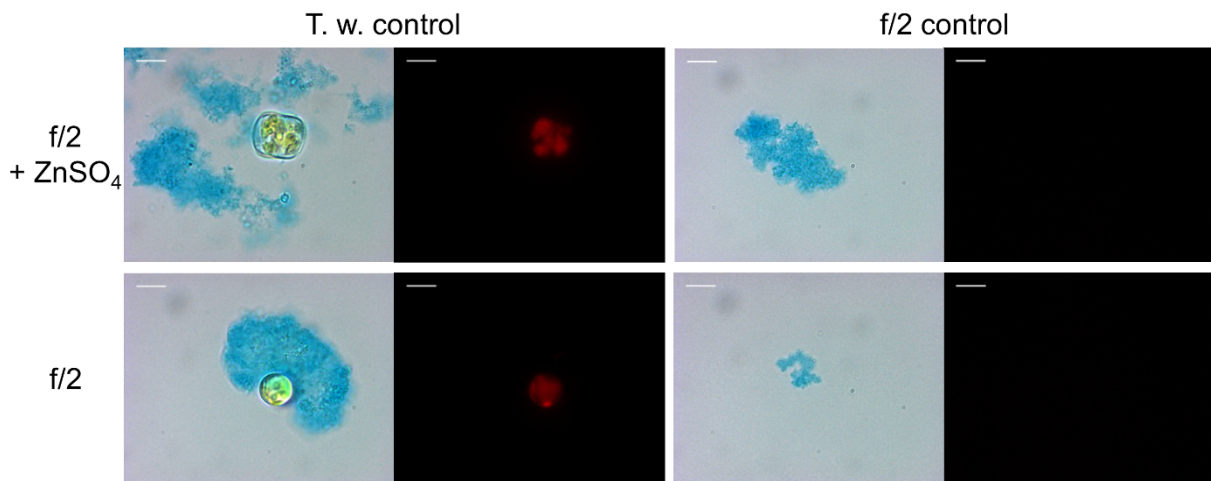


Figure S3. Light and fluorescence microscopy of the attached fractions of the *T. weissflogii* and the f/2 medium controls. The TEP in the samples were stained with Alcian Blue. Pictures were taken at a 1,000x magnification under phase contrast (light microscopy) and an Alexa546 filter (fluorescence microscopy). ZnSO₄ was added at a concentration of 0.3 mM. Scale bars: 10 μ m.

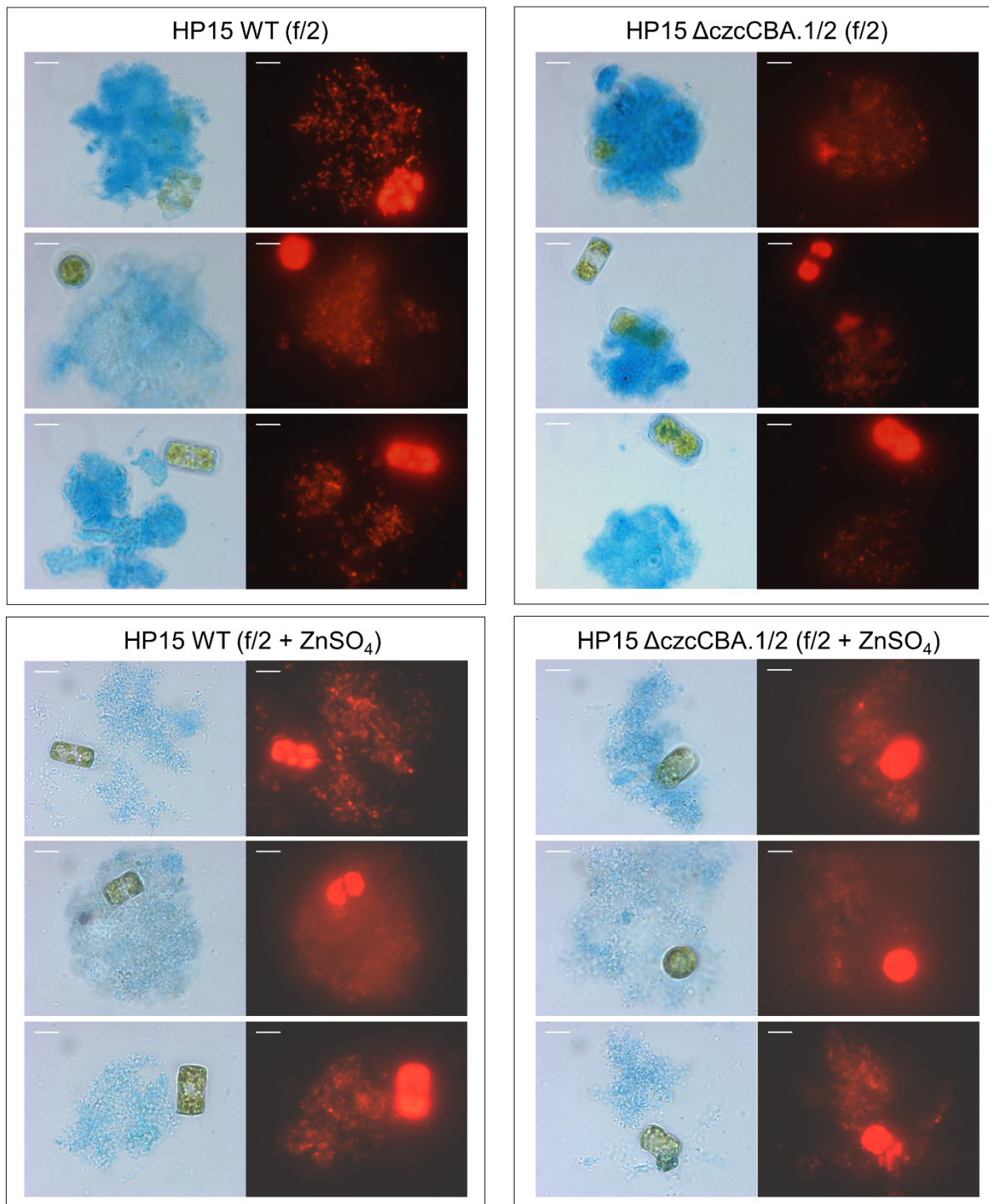


Figure S4. Additional light and fluorescence microscopy images of the attached fractions of co-cultures of *M. adhaerens* HP15 and *T. weissflogii*. The TEP in the samples were stained with Alcian Blue. Pictures were taken at a 1,000x magnification under phase contrast (light microscopy) and an Alexa546 filter (fluorescence microscopy). ZnSO₄ was added at a concentration of 0.3 mM. Scale bars: 10 μ m.

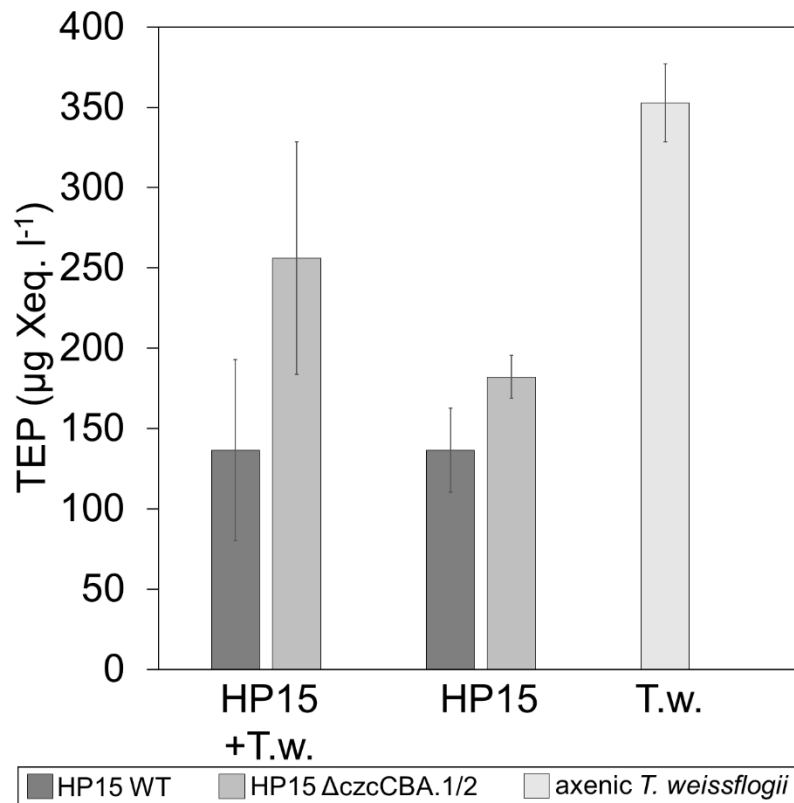


Figure S5. Quantification of transparent exopolymer particles (TEP) in the attached fractions of the attachment assays without ZnSO₄ supplementation. TEP were quantified using the dye-binding photometric Alcian Blue assay and are given in gum Xanthan equivalents (Xeq). HP15 + T. w.: co-cultures of *M. adhaerens* HP15 and *T. weissflogii*; HP15: *M. adhaerens* HP15 control; T. w.: *T. weissflogii* control. In assays with presence of *M. adhaerens* HP15 the wild-type is indicated by dark grey and the mutant ΔczcCBA.1/2 by light grey. Results are shown as mean ± SD, n=3. (Complement of assays shown in Fig. 2 but without zinc addition).

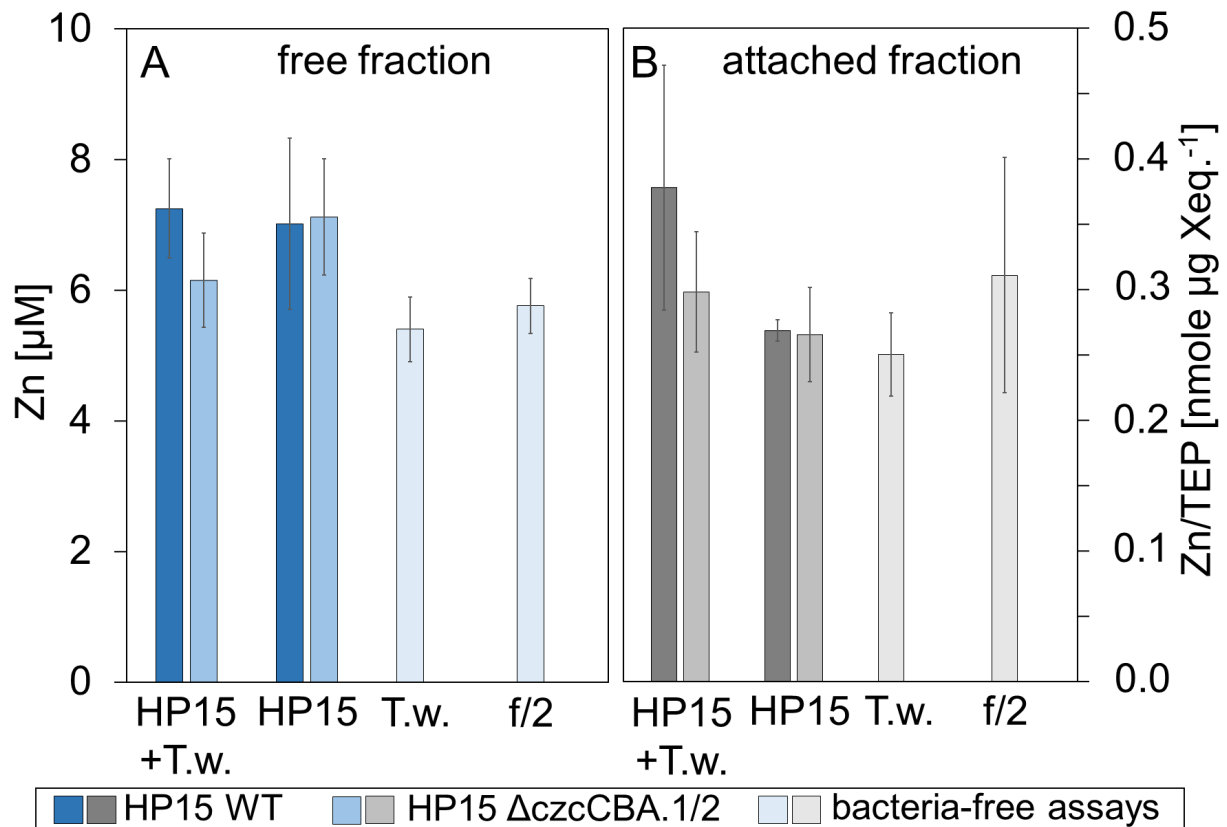


Figure S6. Zinc concentrations in the free-living and attached fractions of the attachment assays without supplementation of $ZnSO_4$. The zinc in the samples was quantified using a photometric ligand-binding assay. Panel A: Zinc concentration in the fraction of free-living cells; panel B: Zinc concentrations in the attached fractions shown relative to the TEP concentration. HP15 + T. w.: co-cultures of *M. adhaerens* HP15 and *T. weissflogii*; HP15: *M. adhaerens* HP15 control; T. w.: *T. weissflogii* control; f/2: f/2 medium control. In assays with presence of *M. adhaerens* HP15 the wild-type is indicated with dark blue or grey and the mutant $\Delta czcCBA.1/2$ with light blue or grey. Results are shown as mean \pm SD, $n=3$.

LITERATURE CITED

Stahl A, Pletzer D, Mehmood A, Ullrich MS (2015) *Marinobacter adhaerens* HP15 harbors two CzcCBA efflux pumps involved in zinc detoxification. *Antonie Van Leeuwenhoek* 108:649-658 <https://doi.org/10.1007/s10482-015-0520-5>