Bacterial marginolactones trigger formation of algal gloeocapsoids, protective aggregates on the verge of multicellularity

1. Supplementary Materials and Methods

1.1. Strain maintenance and cultivation

All microbial strains used in this study are listed in Supplementary Table 1. *Streptomyces iranensis* DSM41954 (HM35^T) was grown in TSB medium at 28 °C and 180 rpm in an Erlenmeyer flask with cotton wool plugs to allow for sufficient gas exchange. Typically, $5 \cdot 10^8$ spores were inoculated into 50 mL medium to obtain a culture grown to a high density. Spores were generated by plating 200 µL of a liquid culture on oatmeal agar and incubation for 14 days at 28 °C.

Streptomyces macronensis UC 8271 (NRRL12566) was grown in TSBY medium (1) at 28 °C and 180 rpm in Erlenmeyer flasks with cotton wool plugs. For liquid cultures, $5 \cdot 10^8$ spores were inoculated into 50 mL TSBY. For harvesting of spores, 200 µL of a liquid culture was spread on MS agar (2) and the agar plates were incubated for 14 days at 28 °C.

Streptomyces mashuensis DSM40896 was grown by inoculation of $2.5 \cdot 10^7$ spores into 50 mL GYM medium (4 g L^{-1} glucose, 4 g L^{-1} yeast extract, 10 g L^{-1} malt extract, pH 7.2) and incubation at 28 °C at 180 rpm overnight. Spores were generated by plating 200 µL of a liquid culture on oatmeal agar and incubation for 14 days at 28 °C.

Chlamydomonas reinhardtii SAG73.72 was inoculated into TAP medium (3) by scraping colonies from a TAP 1.5 % (w/v) agar plate. Cultures were incubated at 26 °C with 120 rpm and constant illumination of 30 µE m^{-2} s^{-1}. TAP agar plates with *C. reinhardtii* strains were maintained at room temperature. *C. reinhardtii* cells treated with various concentrations of azalomycin F, monazomycin (Santa Cruz Biotechnology, Dallas, USA), desertomycin A (Santa Cruz Biotechnology, Dallas, USA), amphotericin B (Thermo Fisher Scientific, Darmstadt, Germany) and daptomycin (Santa Cruz Biotechnology, Dallas, USA) were incubated in 96-well plates with 200 µL per well.

Induction of palmelloids by NaCl stress was carried out essentially as described by Khona, *et al.* (4). 50 mL TAP containing 100 mM NaCl were inoculated with a *C. reinhardtii* preculture to obtain a final OD_{750} of 1. The culture was incubated at 26 °C, 120 rpm and an illumination of 30 µE m^{-2} s^{-1} for 5 days. Then, the cells were harvested by centrifugation with 2,000 × *g* for 2 min and inoculated into fresh TAP + 100 mM NaCl and adjusted to a final OD_{750} of 1. 200 µL of this culture was inoculated into a 96-well plate and incubated at 26 °C, 120 rpm and 30 µE m^{-2} s^{-1}. Palmelloid induction by sodium citrate was carried out as for

NaCl but instead 15 mM trisodium citrate dihydrate (Roth, Karlsruhe, Germany) was used (5). Lipids were stained with Nile red (Carl Roth, Karlsruhe, Germany). Formation of gloeocapsoids in synchronous culture was induced as explained in Materials & Methods in the main text. 1.6 µg/mL azalomycin F were used in order to minimize stress-dependent delay of cell division. The cells were cultured in clear 96-well plates under 30 µE m^{-2} s^{-1} illumination for 12 h followed by 12 h of no illumination.

To follow the kinetics of disassembly of gloeocapsoids, 7 days-old gloeocapsoids were pooled and centrifuged with 2,000 × *g* for 2 min. Then, the cells were washed in TAP medium and centrifuged again with 2,000 × *g* for 2 min. The pellet was resuspended in TAP and 200 µL were inoculated in 96-well plates per well. The cells were checked daily for release of single cells from gloeocapsoids.

Gloeothece membranacea SAG26.84 was grown either in BG11 (6) or Bold's Basal Medium (3N-BBM+V modified from Bold (7)) at 26 °C, 100 rpm and an illumination of 40 µE m^{-2} s^{-1} in a 12h dark 12h light cycle.

1.2. Extraction and detection of monazomycin and desertomycin A

Co-cultivations of *C. reinhardtii* with *S. mashuensis* and *S. macronensis* in order to detect overproduction of monazomycin and desertomycin A were carried out as previously described for the co-cultivation of the alga with *S. iranensis* (8). The culture broth containing bacterial mycelium with *C. reinhardtii* was homogenized using an ULTRA-TURRAX (IKA-Werke, Staufen, Germany). Homogenized cultures were extracted twice with a total of 100 mL ethyl acetate, dried with sodium sulfate and concentrated under reduced pressure. For LC-MS analysis, the dried extracts were dissolved in 1 mL of methanol and loaded onto an ultrahigh-performance liquid chromatography (LC)–MS system consisting of an UltiMate 3000 binary rapid-separation LC with photodiode array detector (Thermo Fisher Scientific, Dreieich, Germany) and an LTQ XL linear ion trap mass spectrometer (Thermo Fisher Scientific, Dreieich, Germany) equipped with an electrospray ion source. The extracts (injection volume, 10 µl) were analyzed on a 150 mm by 4.6 mm Accucore reversed-phase (RP)-MS column with a particle size of 2.6 µm (Thermo Fisher Scientific, Dreieich, Germany) at a flow rate of 1 ml min^{-1}, with the following gradient over 21 min: initial 0.1% (v/v) HCOOH-MeCN/0.1% (v/v) HCOOH-H_2O 0/100, which was increased to 80/20 in 15 min and then to 100/0 in 2 min, held at 100/0 for 2 min, and reversed to 0/100 in 2 min. Identification of desertomycin A and monazomycin was achieved by comparison with authentic references purchased from Santa Cruz Biotechnology (Dallas, USA).

1.3. Staining methods

1.3.1. Cell wall staining of *C. reinhardtii* by concanavalin A

Concanavalin A tetramethylrhodamine conjugate was purchased from Thermo Fisher Scientific (Darmstadt, Germany). For staining, 50 µL of *C. reinhardtii* vegetative cells or gloeocapsoids were withdrawn and centrifuged with 2,000 × *g* for 2 min. The supernatant was discarded, and the cells were resuspended in 50 µL PBS + 4 % (v/v) formaldehyde. Then, 1 µL of concanavalin A stock solution (2 mg mL^{-1} in water) was added to the sample and the sample was cultivated in the dark for 10 min at room temperature. After incubation, the cells were centrifuged with 2,000 × *g* for 2 min and the supernatant was discarded. The cells were resuspended in 50 µL PBS + 4 % (v/v) formaldehyde and analyzed microscopically. Concanavalin A tetramethylrhodamine conjugate was excited at 553 nm and emission was recorded for a wavelength of 577 nm.

1.3.2. Staining of the plasma membrane using CellMask stain

CellMask™ Orange plasma membrane stain was purchased from Thermo Fisher Scientific (Darmstadt, Germany). 45 µL per well of *C. reinhardtii* were withdrawn and 5 µL 37 % (v/v) formaldehyde solution were added. CellMask plasma membrane stain was diluted 1:100 in DMSO and 5 µL of the working solution was added to 50 µL of the prepared cells and incubated for 5 min at room temperature in the dark. For fluorescence microscopy CellMask™ Orange plasma membrane stain was excited at a wavelength of 556 nm and emission was recorded for 572 nm.

1.3.3. Alcian blue staining of the matrix of gloeocapsoids

Alcian blue was purchased from Carl Roth (Karlsruhe, Germany). A 0.1 % (w/v) solution was prepared by dissolving Alcian blue in either 0.5 M HCl (pH 0.5) or 0.5 M acetic acid (pH 2.5), as described by Van Boekel (9). For staining, 50 µL of a gloeocapsoid solution was centrifuged with 2,000 × *g* for 2 min and the pellet was resuspended in 100 µL of the staining solution. After 20 min incubation in the dark, the cells were centrifuged with 2,000 × *g* for 2 min and resuspended in 500 µL PBS + 4 % (v/v) formaldehyde. For microscopical inspection, the cells were centrifuged with 2,000 × *g* for 2 min and resuspended in 50 µL PBS + 4 % (v/v) formaldehyde.

1.3.4. Staining of dead *C. reinhardtii* cells using SYTOX Blue

SYTOX Blue Dead Cell Stain was purchased from Thermo Fisher Scientific (Darmstadt, Germany). 50 µL of the culture to be tested was withdrawn and 6.25 µL of 37% (v/v) formaldehyde was added. Staining was carried out by addition of 1 µL of 500 µM SYTOX Blue dye and further incubation of the cells for 15 min at room temperature in the dark. SYTOX Blue was excited at 353 nm and emission was measured at 465 nm.

1.3.5. Nile Red staining for visualization of lipids

Lipophillic Nile Red dye was purchased from Carl Roth (Karlsruhe, Germany). A 0.1 mg mL^{-1} stock solution in methanol was generated and applied in a final concentration of 0.1 µg mL^{-1}. For staining of lipids, 50 µL of the culture to be tested were withdrawn and 6.25 µL of 37% (v/v) formaldehyde was added. The culture was then centrifuged with 2,000 × *g* for 2 min and the supernatant was discarded. The cells were resuspended in 50 µl PBS + 4 % (v/v) formaldehyde. Nile red was added to a final concentration of 0.1 µg mL^{-1} and it was incubated for 10 min in the dark prior to microscopy. Nile red fluorescence was excited at 559 nm and emission was measured at 636 nm.

1.4. Electron microscopy

Sample preparation for electron microscopy

For electron microscopy sample preparation, 500 µL of *C. reinhardtii* vegetative cells or gloeocapsoids were centrifuged for 10 min with 500 × *g* and the supernatant was discarded. The pellet was resuspended in 10 mL TAP freshly supplied with 4 % (v/v) formaldehyde and 2.5 % (v/v) glutaraldehyde and incubated for one hour at room temperature. Then, the cells were centrifuged again for 10 min with 500 × *g* and the supernatant was discarded. The pellet was resuspended in fresh 0.1 M sodium cacodylate buffer pH 7.4 and incubated for 10 min at room temperature. The washing in 0.1 M sodium cacodylate buffer was repeated and subsequently the pellet was resuspended in 50 µL sodium cacodylate buffer.

Transmission electron microscopy

The cells in 50 µL cacodylate buffer were pelleted (500 × *g*, 10 min) and post-fixed with 2 % (w/v) osmiumtetroxide for 1 h at room temperature. During the following dehydration in ascending ethanol series post-staining with 1 % (w/v) uranylacetate was performed. Afterwards the samples were embedded in epoxy resin (Araldite) and sectioned using a Leica

Ultracut S (Leica, Wetzlar, Germany). Finally, ultrathin sections were mounted on filmed Cu grids, post-stained with lead citrate, and studied in a transmission electron microscope (EM 900, Zeiss, Oberkochen, Germany) at 80 kV and magnifications of 3,000 × *g* to 50,000 × *g*. For image recording a 2K slow scan CCD camera (TRS, Moorenweis, Germany) was used.

Scanning electron microscopy

Small droplets of cell suspensions were placed on poly-L-lysine-coated glass cover slips. After sedimentation for 1 h at 4 °C the droplets were removed, and the cover slips were immersed in fixative (2.5 % (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer) for 1 h at room temperature. Then, the slips were rinsed three times with fresh sodium cacodylate buffer for 15 min each. After post-fixation with 2 % (w/v) osmiumtetroxide for 1 h the samples were dehydrated in ascending ethanol concentrations (30, 50, 70, 90, 96 % (v/v) and 100 %) for 15 min each. Subsequently, the samples were critical-point dried using liquid CO_2 and sputter coated with gold (thickness approx. 2 nm) using a CCU-010 SCD005 sputter coater (Safematic GmbH, Zizers, Switzerland). The specimens were investigated with a field emission SEM LEO-1530 Gemini (Carl Zeiss NTS GmbH, Oberkochen, Germany).

1.5. Sample preparation for matrix-assisted laser desorption ionization imaging mass spectrometry (MALDI-IMS)

Samples for MALDI-IMS were prepared according to Krespach *et al*. (8). In short, glass slides covered with indium tin oxide (ITO, Bruker Daltonics, Bremen, Germany) were sterilized over a Bunsen burner. Then, they were covered with 1 % (w/v) TAP agar containing 10 g L^{-1} glucose, 0.5 g L^{-1} $MgSO_4 \cdot 7H_2O$, 1 mL L^{-1} trace elements (22 g L^{-1} $FeSO_4 \cdot 7H_2O$, 5 g L^{-1} $ZnSO_4 \cdot 7H_2O$, 1.6 g L^{-1} $CuSO_4 \cdot 5H_2O$, 5 g L^{-1} $MnSO_4 \cdot 7H_2O$, 11 g L^{-1} $Na_2BO_4 \cdot 7H_2O$, 1.1 g L^{-1} $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$), 3 mL L^{-1} 10 mM $FeSO_4$ and 3 mL L^{-1} 0.1 % (w/v) *p*-aminobenzoic acid. For embedding of *C. reinhardtii* cells in the agar, the cells were first centrifuged with 2,000 × *g* for 2 min and the pellet was dissolved in approximately 35 °C liquid agar to a final OD_{750} of 1. *S. macronensis* and *S. mashuensis* were precultured as described above and washed in TAP medium (14,000 × *g*; 1 min). Then, 15 µL were spot inoculated for co-cultivation. MALDI-IMS analysis was carried out according to Krespach *et al*. (8).

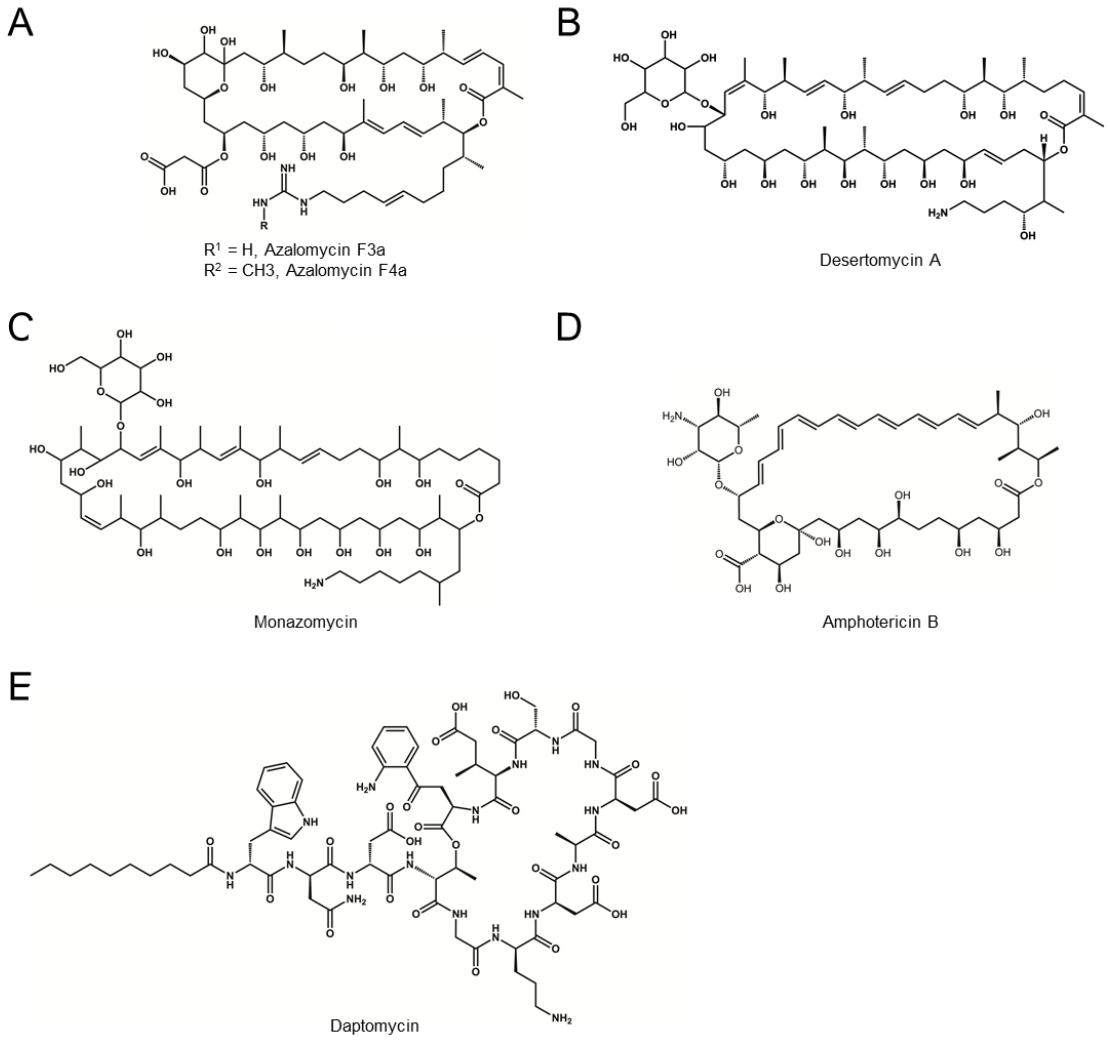
2. Supplementary Tables and Figures

2.1. Microorganisms used in this study

Supplementary Table 1: Microorganisms, their genotype and reference.

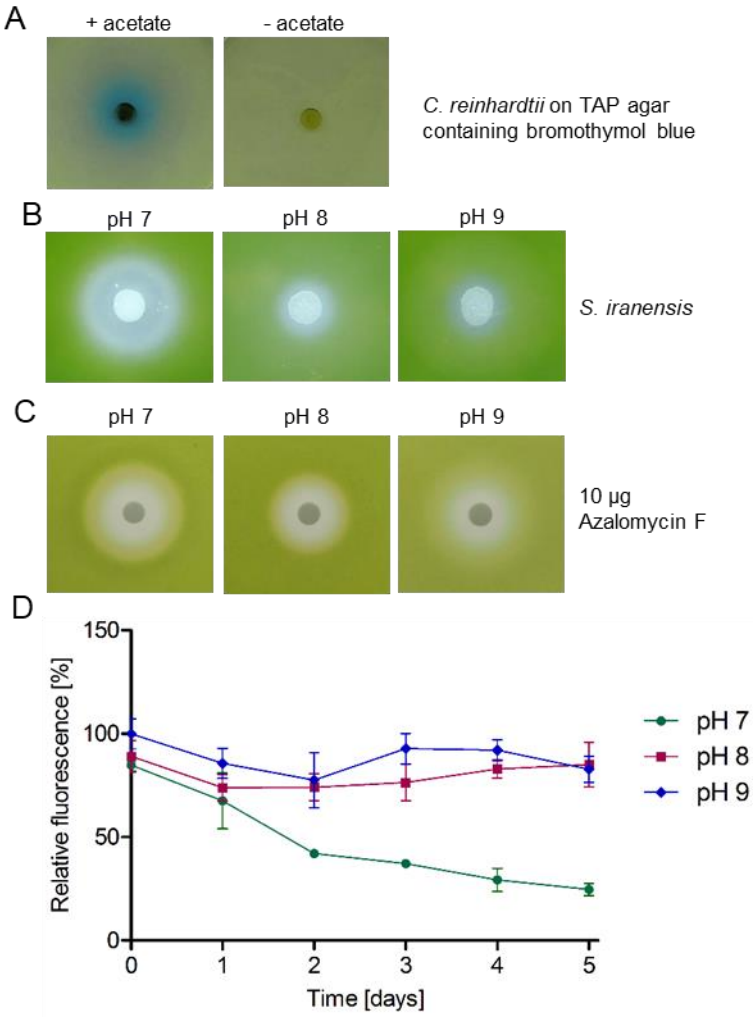
Organism	Relevant genotype	Reference
Streptomyces iranensis DSM41954 (HM35^{T})	Wild-type strain	Hamedi, *et al.* (10)
Streptomyces macronensis UC 8271 (NRRL12566)	Wild-type strain	Dolak, *et al.* (11)
Streptomyces mashuensis DSM40896	Wild-type strain	German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany
Chlamydomonas reinhardtii SAG73.72	Wild-type strain	Algal culture collection Göttingen
Gloeothece mebranacea SAG26.84 (*Gloeocapsa* sp. PCC 6501)	Wild-type strain	Algal culture collection Göttingen

2.2. Chemical structures of natural products used in this study

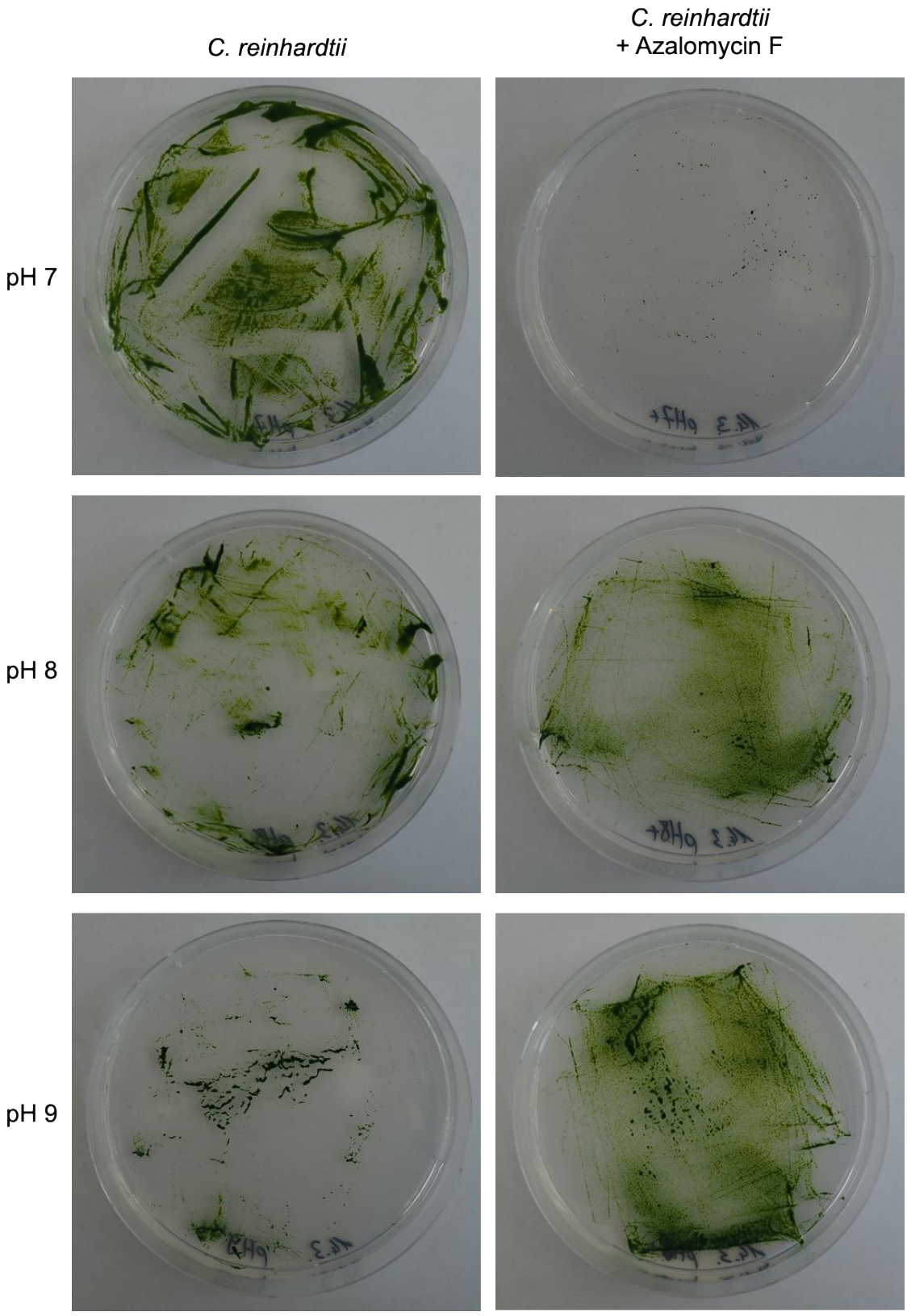


Supplementary Figure 1: Chemical structures of azalomycin F (**A**), desertomycin A (**B**), monazomycin (**C**), amphotericin B (**D**) and daptomycin (**E**).

2.3. Azalomycin F activity is reduced at alkaline pH

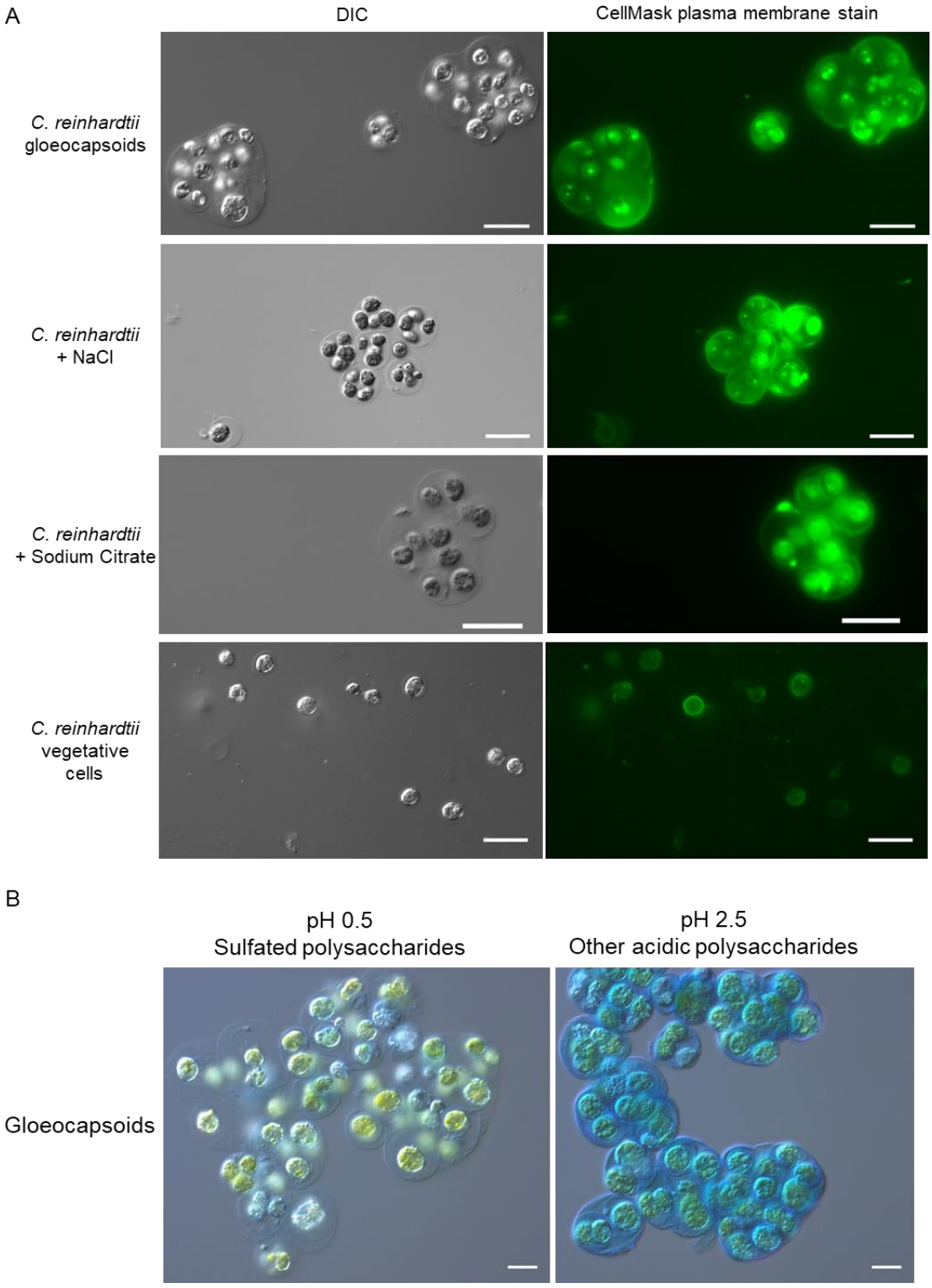


Supplementary Figure 2: *C. reinhardtii* alkalizes the environmental pH, which decreases the algicidal activity of azalomycin F. (**A**) *C. reinhardtii* increases local pH by consumption of acetate. *C. reinhardtii* was plated on TAP agar with or without acetate at pH 6. Bromothymol blue was used as pH indicator. Blue coloration around algal colonies indicates rise of pH. (**B**) Killing of *C. reinhardtii* by *S. iranensis* is reduced at elevated pH. *S. iranensis* spot inoculated onto TAP agar of pH 7 to 9, containing *C. reinhardtii*. A halo around *S. iranensis* indicates killed algae. At pH 7, the diameter of the killing zone is larger (1.97 ± 0.25 cm) than at pH 8 (1.43 ± 0.3 cm) and pH 9 (1.2 ± 0 cm). n = 3 plates. (**C**) Filter discs loaded with 10 µg azalomycin F were placed on TAP agar containing *C. reinhardtii*. The killing zone at pH 7 was bigger (2.4 ± 0.12 cm) than that of pH 8 (1.67 ± 0.32 cm) and 9 (1.2 ± 0.1 cm). n = 3 plates. (**D**) Autofluorescence of *C. reinhardtii* at pH 7 to 9 in presence of 5 µg mL^{-1} azalomycin F in liquid culture. At pH 7 the autofluorescence collapses, while at pH 8 and 9, the autofluorescence recovered after 3 to 4 days to levels of the untreated controls.



Supplementary Figure 3: Alkaline pH increased survival of *C. reinhardtii* treated with azalomycin F. *C. reinhardtii* plated on TAP medium after five days of incubation with or without 5 µg mL^{-1} azalomycin F at pH 7 to 9. *C. reinhardtii* incubated at pH 7 and azalomycin F only showed little growth on agar, while *C. reinhardtii* treated with azalomycin F at pH 8 and 9 exhibited easily detectable growth.

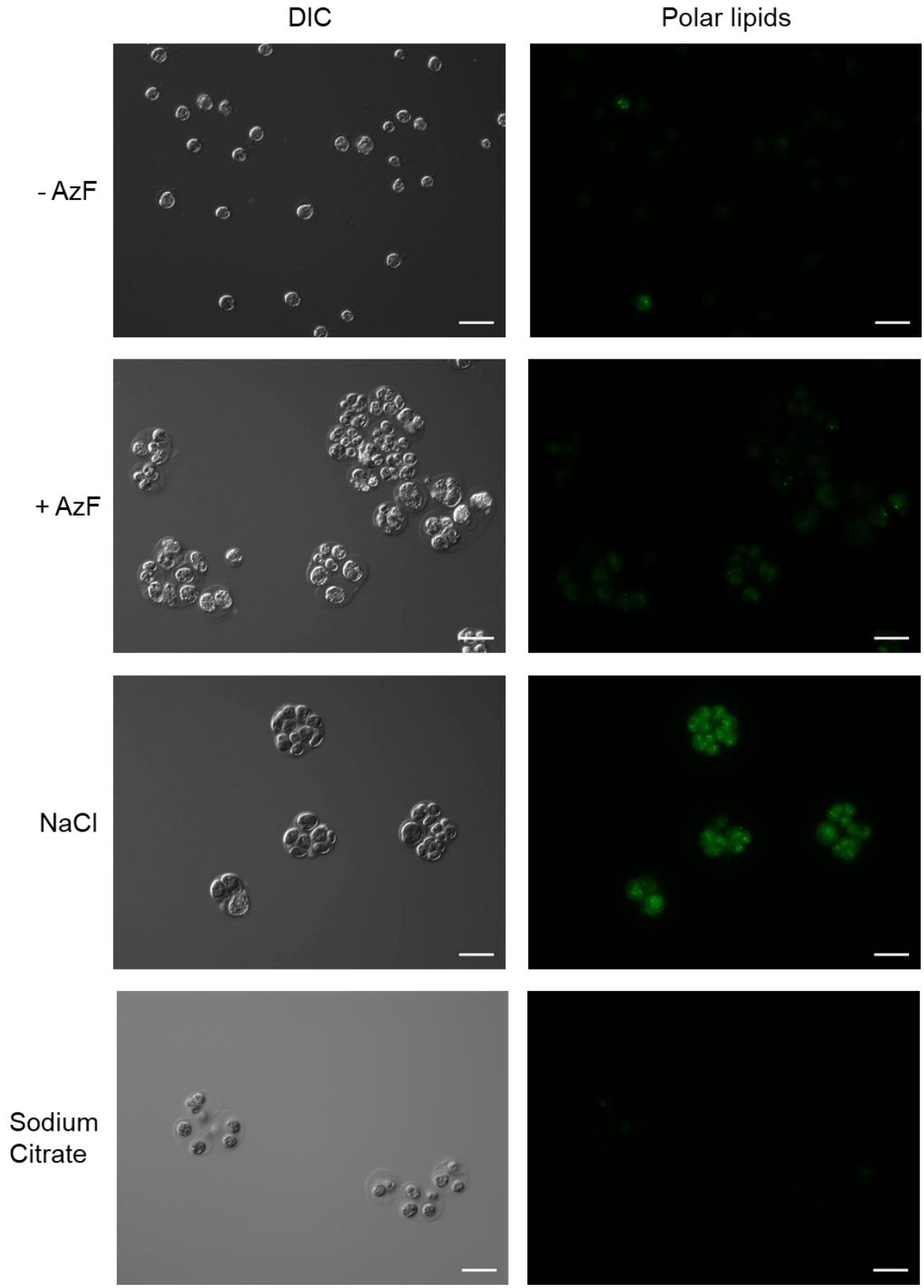
2.4. Gloeocapsoids are enveloped by multiple membranes and a matrix consisting of acidic polysaccharides



Supplementary Figure 4: CellMask membrane stain indicates multiple membranes enveloping individual cells of gloeocapsoids. NaCl- and sodium citrate-induced palmelloids can also be enveloped by a single membrane. However, multi-layered cell membranes were not observed. Scale bar: 20 µm (**A**). The matrix of gloeocapsoids consists of acidic but not sulfonated polysaccharides. Alcian blue at pH 0.5 (in 0.5 M HCl) stains sulfate groups of polysaccharides and at pH 2.5 (in 0.5 M acetic acid) it stains other acidic functional groups. Blue staining of

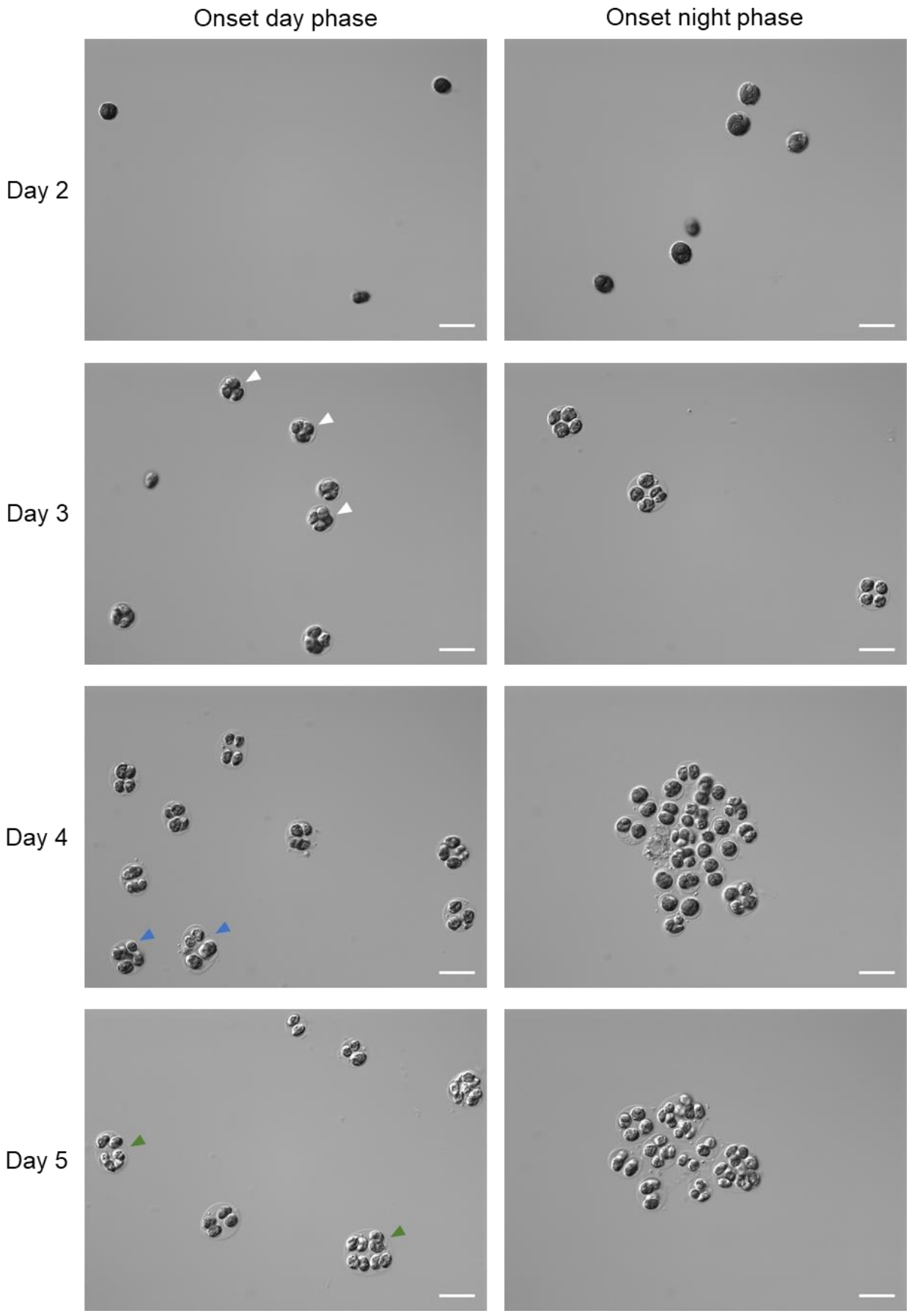
the matrix was only observed at pH 2.5 which indicates non-sulfate acidic polysaccharides. Scale bar: 10 µm. (**B**)

2.5. Gloeocapsoids do not accumulate lipids



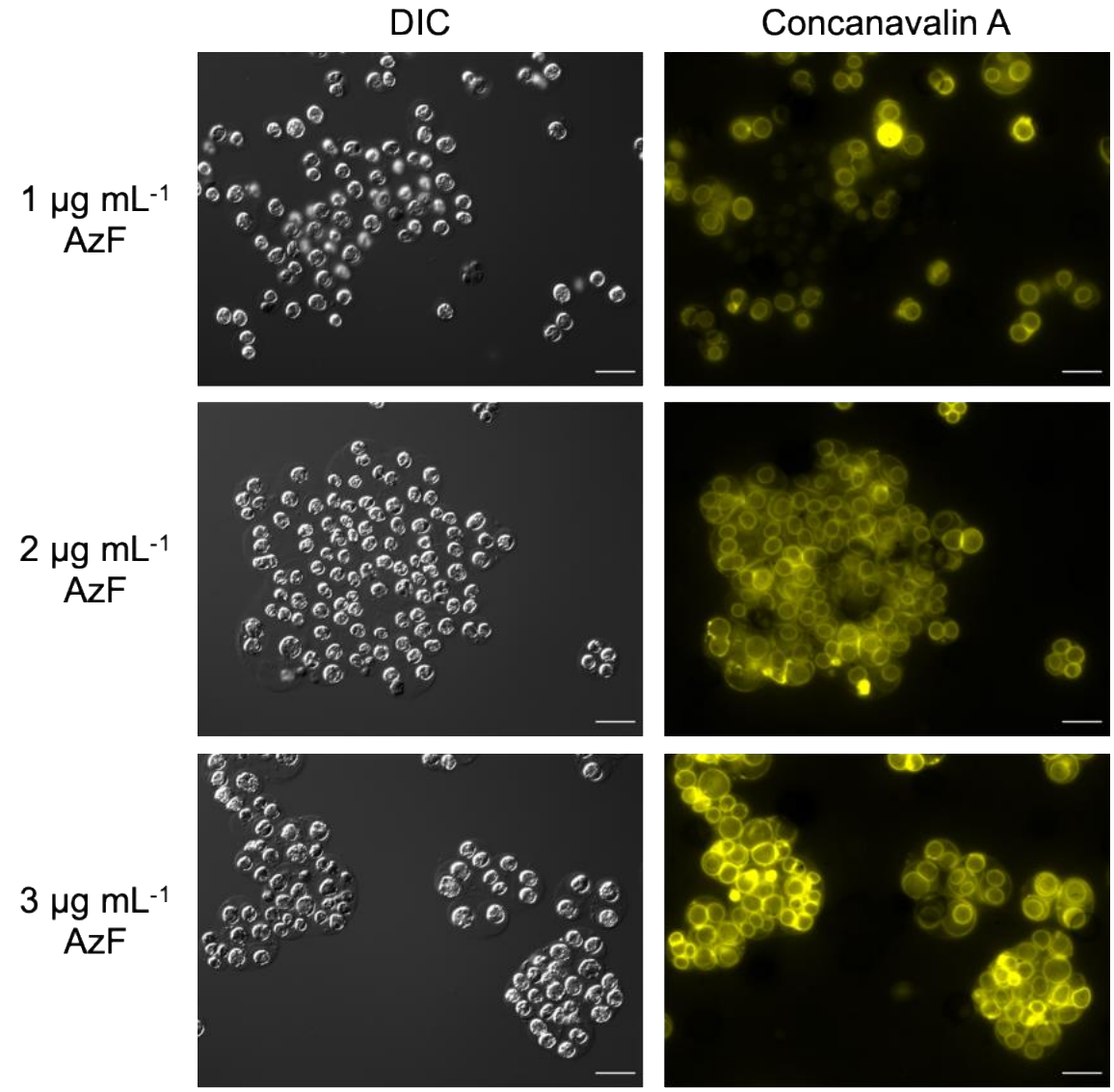
Supplementary Figure 5: Gloeocapsoids do not accumulate lipids. *C. reinhardtii* treated with 0 µg mL^{-1}, 2 µg mL^{-1} azalomycin F, 150 mM NaCl or 15 mM sodium citrate. Azalomycin F-treated cells formed gloeocapsoids. Nile red staining showed low accumulation of lipids in gloeocapsoids in contrast to cells treated with 150 mM NaCl. 15 mM sodium citrate palmelloids did not accumulate lipid bodies. Scale bars: 20 µm.

2.6. Gloeocapsoids in synchronized cultures of *C. reinhardtii* are formed during the night phase



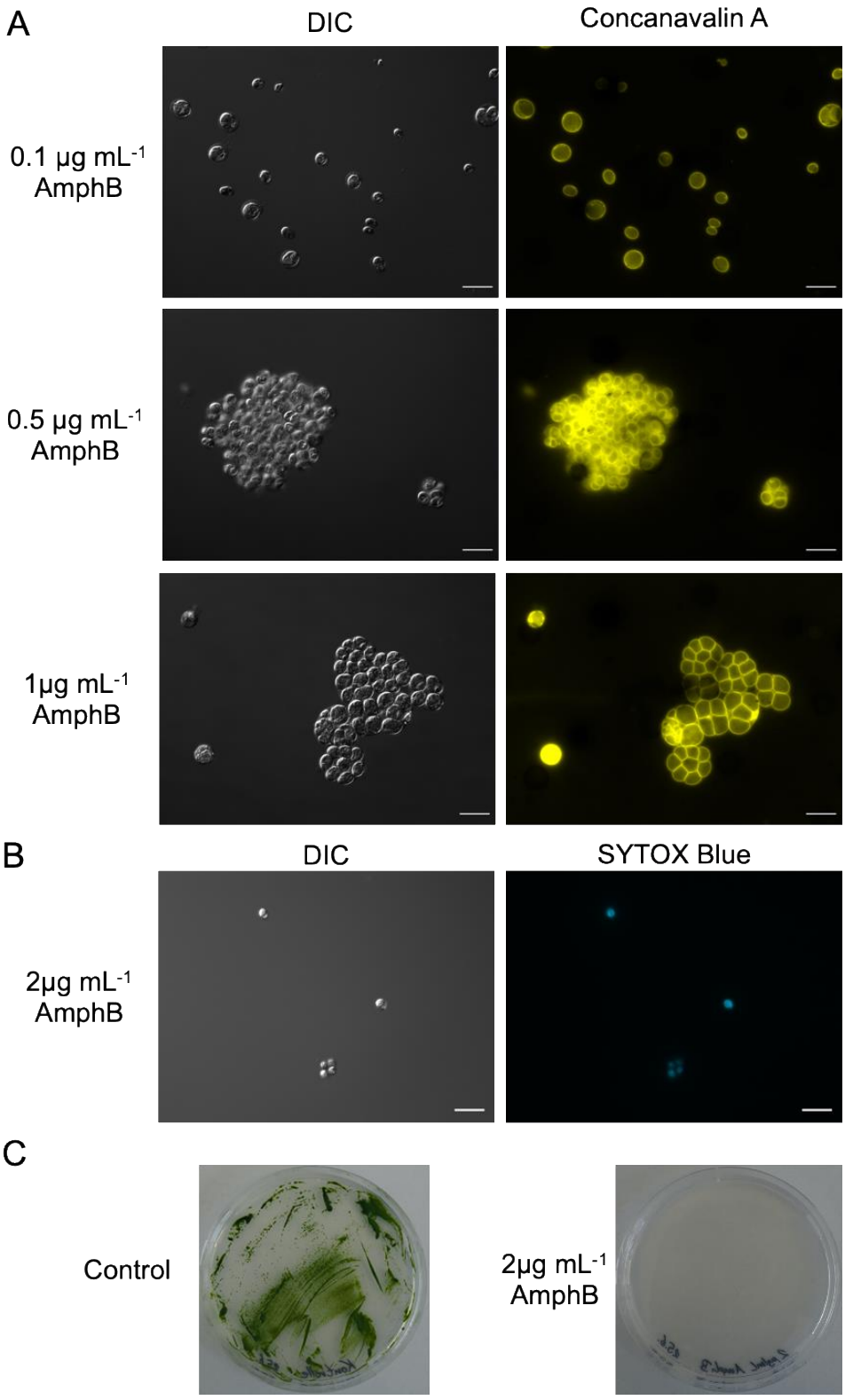
Supplementary Figure 6: *C. reinhardtii* treated with 1.6 µg/mL azalomycin F to induce gloeocapsoids in synchronized culture. Samples were taken and inspected using microscopy at the onset of the day phase (left) and the night phase (right). As for palmelloids, *C. reinhardtii* mother cells grow during the day phase and divide during the night phase. Division is indicated after the dark night phase using white arrows. Here, mostly 3 – 4 cells are found within one gloeocapsoid. After the following dark phase, indicated by blue arrows, gloeocapsoids with more than 4 cells per aggregate can be found as well as gloeocapsoids with the typical polysaccharide matrix. At the onset of day 5, 6 – 8 cells per gloeocapsoid are indicated by the green arrows. This finding indicates that gloeocapsoids are formed by a failure of the daughter cells to detach from each other.

2.7. Gloeocapsoid formation of *C. reinhardtii* depends on azalomycin F concentration



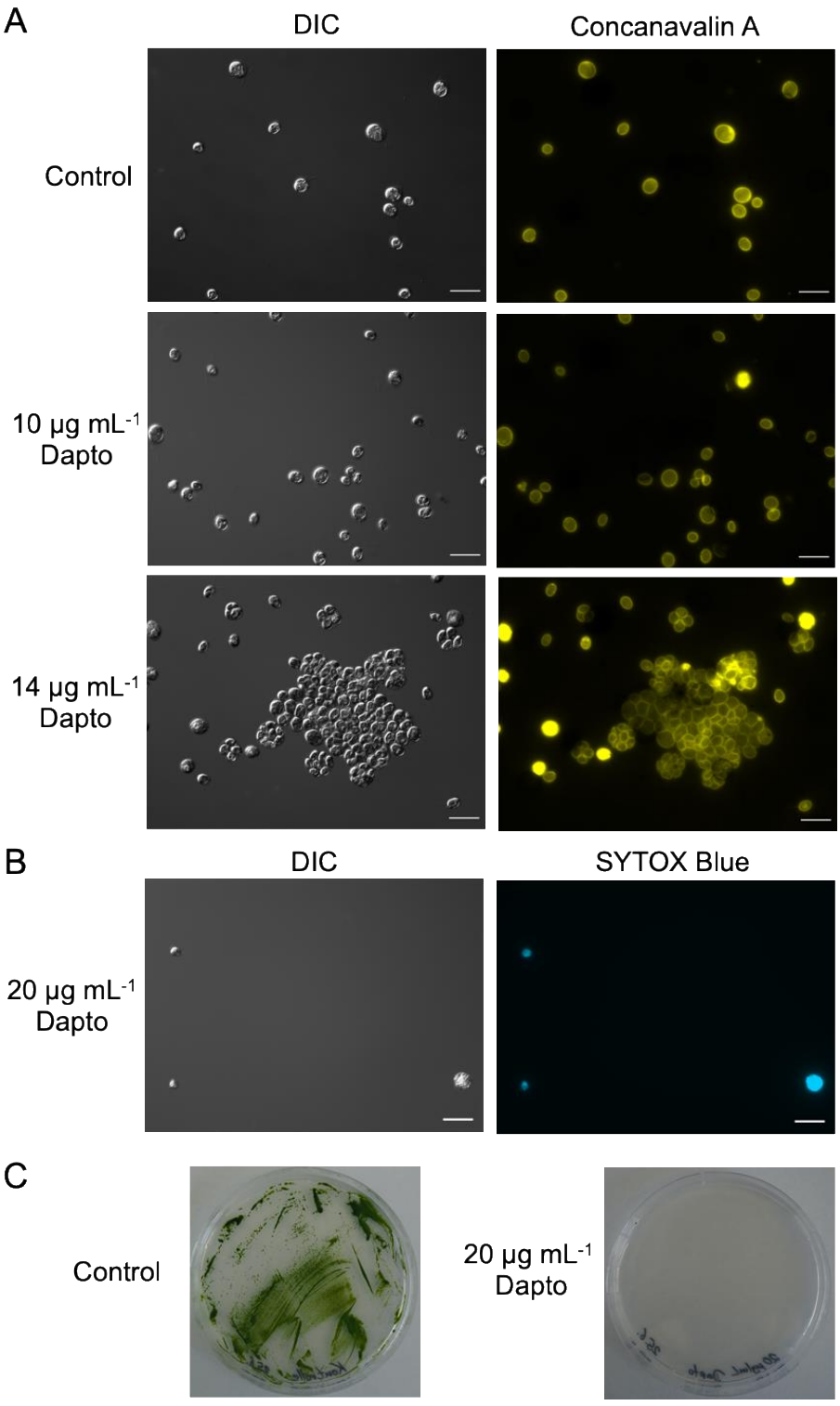
Supplementary Figure 7: Dependence of gloecapsoid formation on azalomycin F (AzF) concentration. Microscopy pictures of *C. reinhardtii* treated with 1 to 3 µg mL^{-1} azalomycin F. 2 and 3 µg mL^{-1} azalomycin F triggered formation of aggregates with a spacious extracellular matrix and multiple cell envelopes. Scale bars: 20 µm.

2.8. Influence of amphotericin B on *C. reinhardtii*



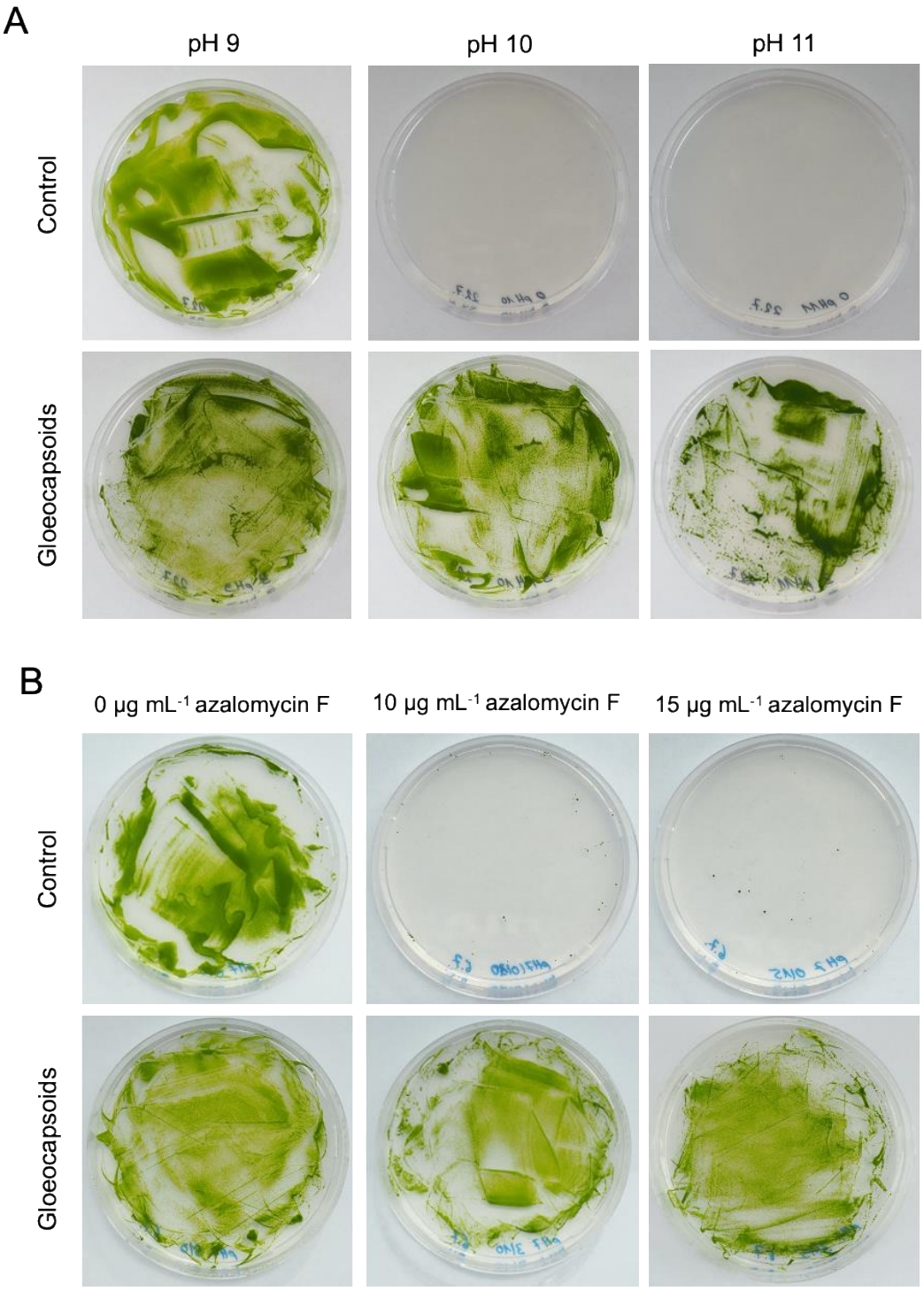
Supplementary Figure 8: *C. reinhardtii* treated with various amounts of amphotericin B (AmphB) produced densely packed aggregates. (**A**) Microscopic pictures of *C. reinhardtii* treated with 0.1 to 2 µg mL^{-1} amphotericin B. 0.1 µg mL^{-1} did not induce any aggregation. 0.5 and 1 µg mL^{-1} AmphB triggered production of densely packed aggregates. 2 µg mL^{-1} amphotericin B was lethal as shown by SYTOX blue staining (**B**) and plating of treated cells on agar plates (**C**). Scale bars: 20 µm.

2.9. Influence of daptomycin on *C. reinhardtii*



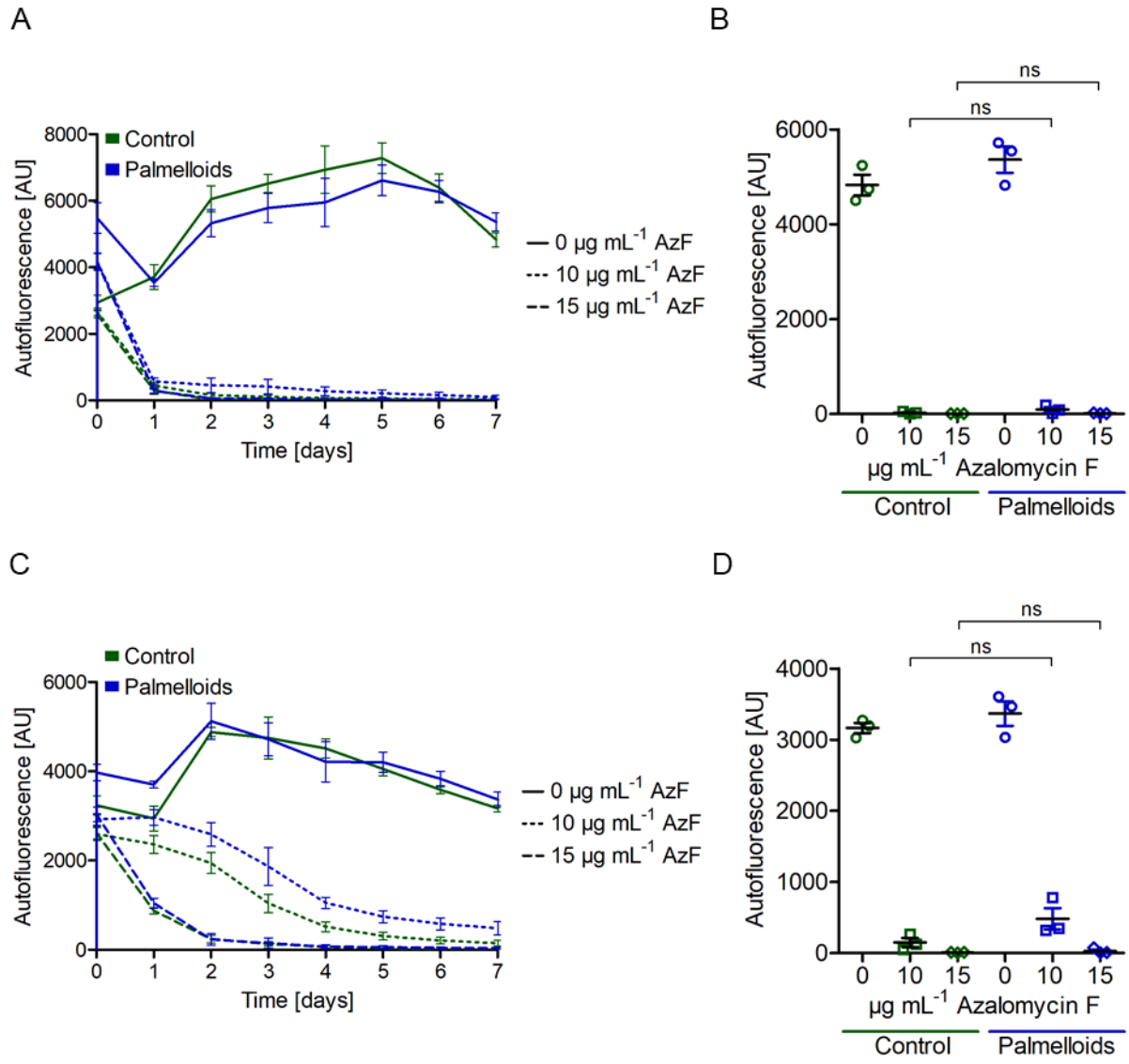
Supplementary Figure 9: Daptomycin (Dapto) triggered intra-specific aggregation in *C. reinhardtii* in a dose-dependent manner. (**A**) Microscopic images of *C. reinhardtii* treated with 0, 10 and 14 µg mL^{-1} daptomycin. At concentrations higher than 10 µg mL^{-1}, *C. reinhardtii* produced aggregates. 20 µg mL^{-1} daptomycin were lethal as shown by SYTOX Blue staining (**B**) and plating of the treated cells on agar (**C**). Scale bars: 20 µm.

2.10. Gloeocapsoids increased survival of *C. reinhardtii* at alkaline pH and during azalomycin F treatment

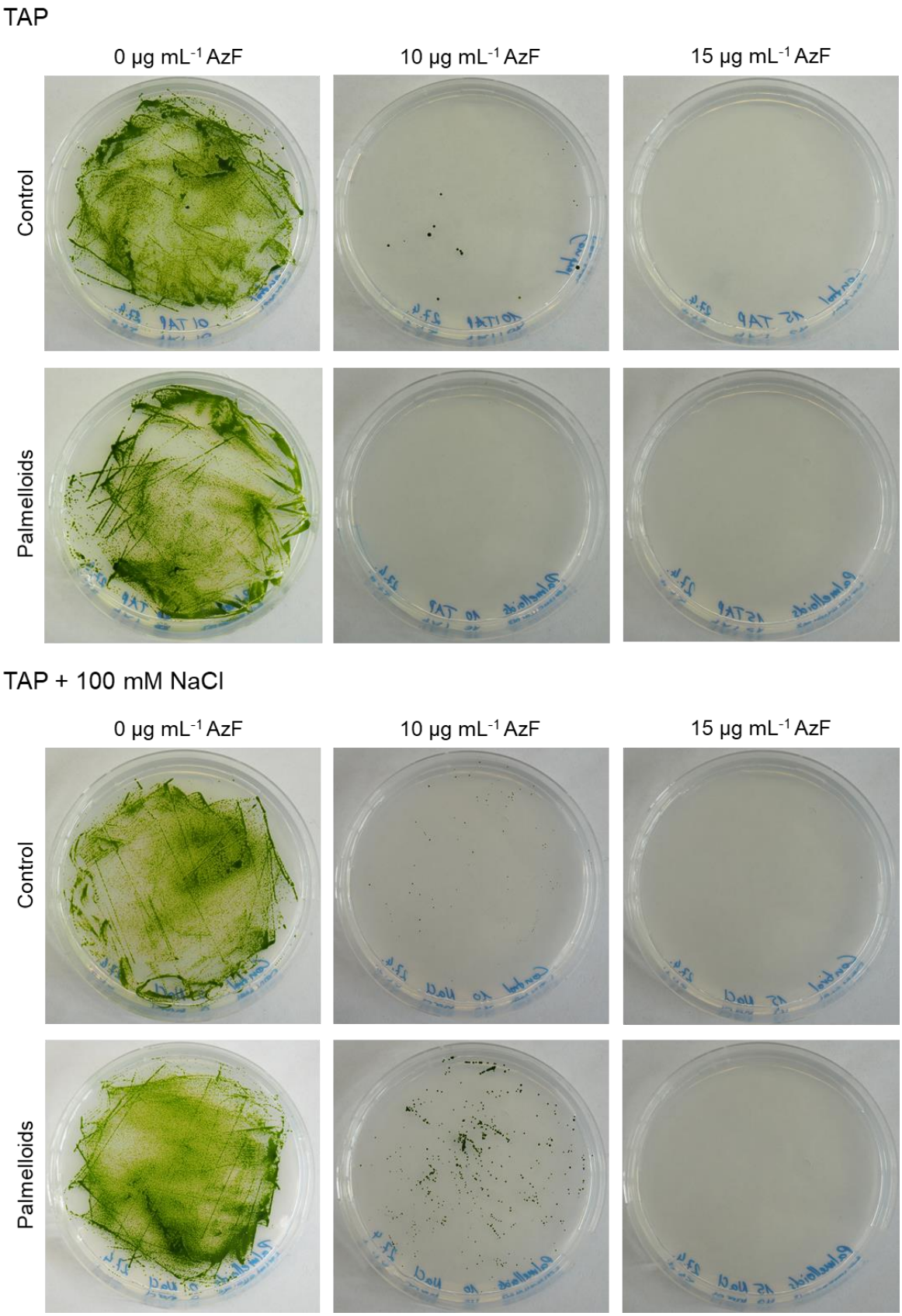


Supplementary Figure 10: Gloeocapsoids protected *C. reinhardtii* against alkaline pH (**A**) and lethal azalomycin F concentrations (**B**). After 7 days of incubation at pH 9 to 11 untreated cells or gloeocapsoids were plated on TAP agar (**A**). After 7 days of incubation with lethal azalomycin F concentrations untreated cells or gloeocapsoids were plated on TAP agar (**B**). Cells pre-treated with sublethal concentrations of azalomycin F formed gloeocapsoids and survived pH 10 and 11 as well as otherwise lethal azalomycin F concentrations and were able to re-grow on solid medium.

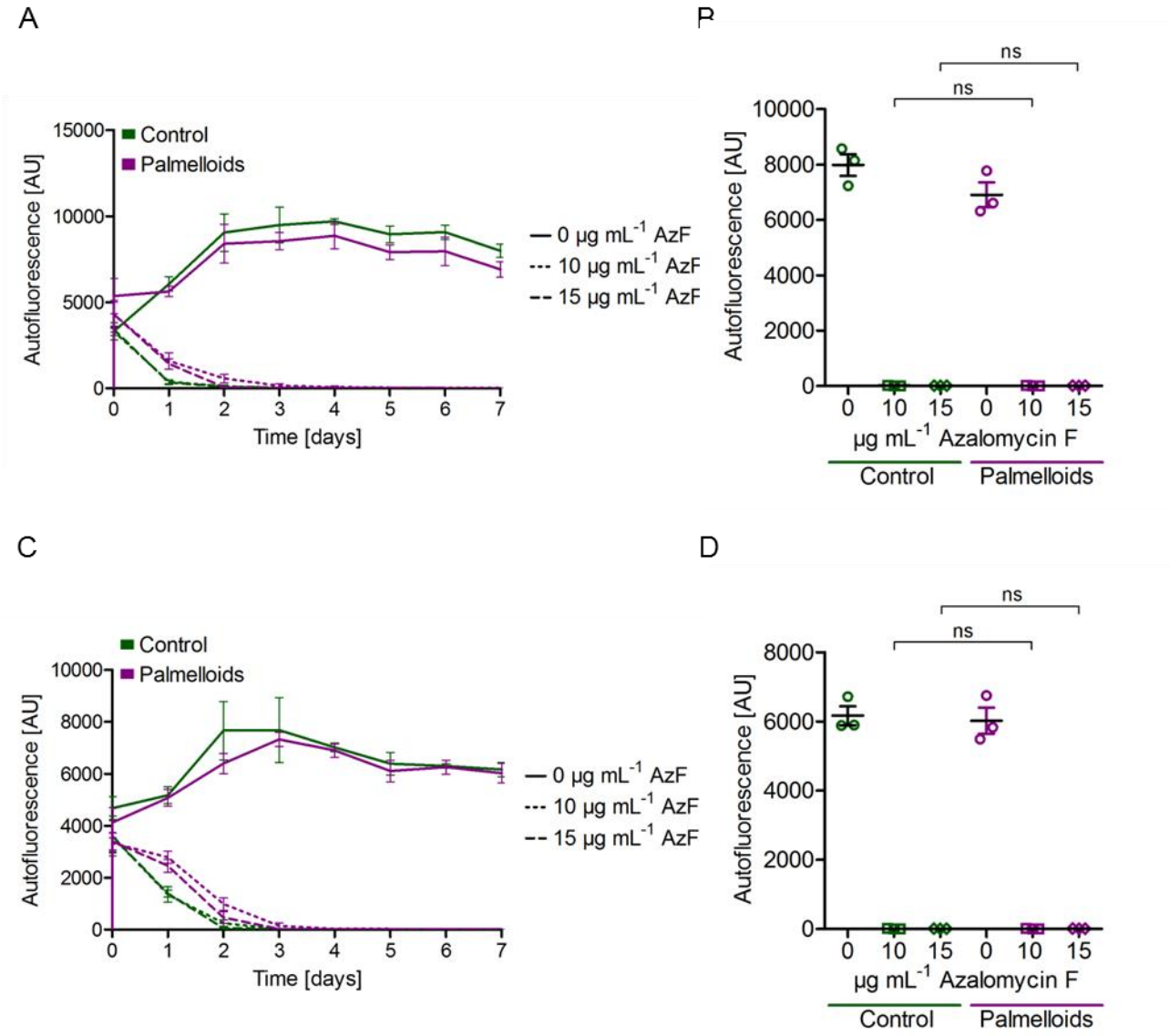
2.11. NaCl- and sodium citrate-induced palmelloids do not protect *C. reinhardtii* from azalomycin F



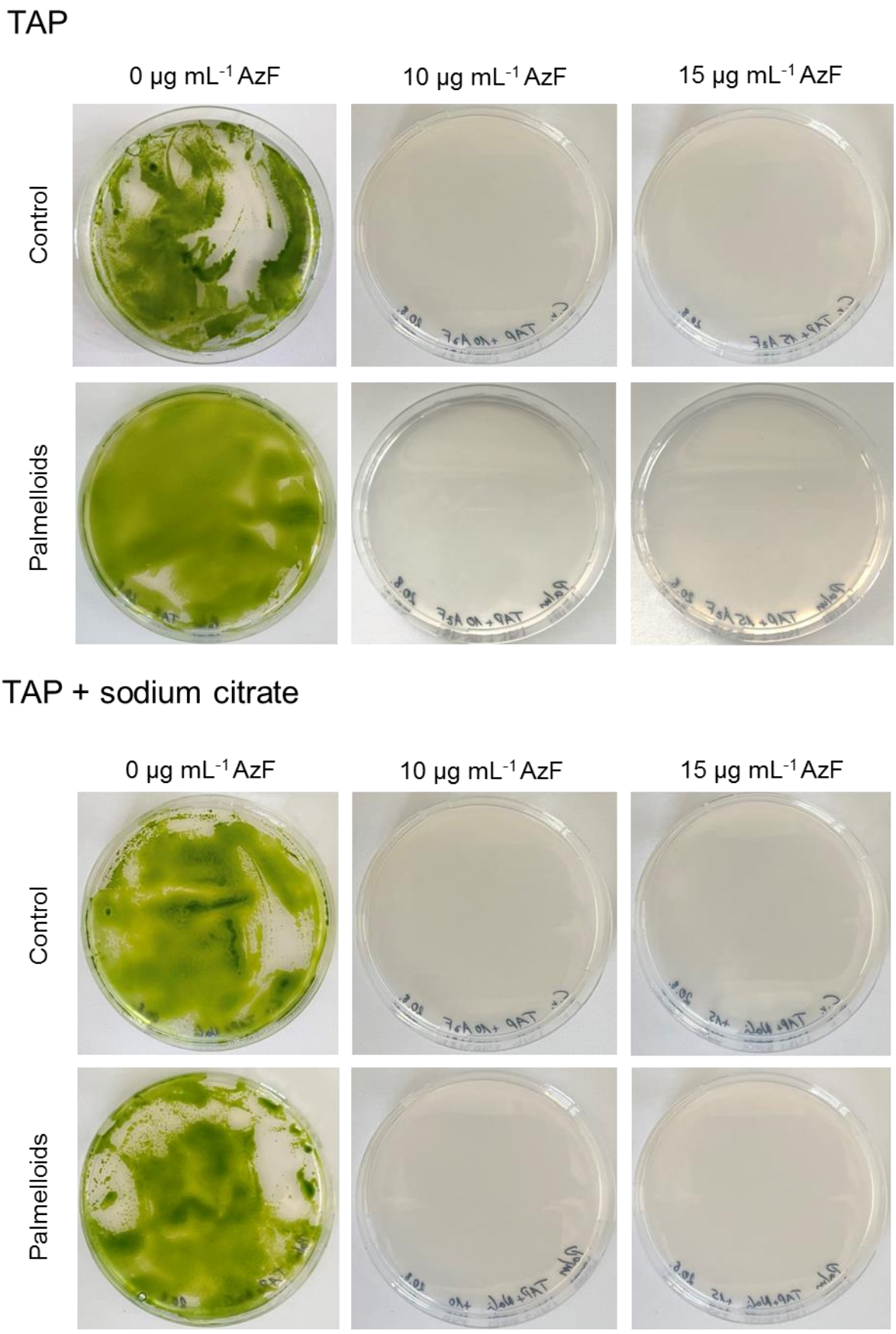
Supplementary Figure 11: Palmelloids induced by 100 mM NaCl confer no resistance against azalomycin F. NaCl-induced palmelloids are shown in Supplementary Figure 4. (**A**) Control cells and palmelloids of *C. reinhardtii* were inoculated into TAP medium, treated with 0, 10 or 15 µg mL^{-1} azalomycin F and their autofluorescence was measured daily. (**B**) Autofluorescence at day seven of *C. reinhardtii* control cells and palmelloids incubated in TAP medium. (**C**) Control cells and palmelloids of *C. reinhardtii* were inoculated into TAP medium + 100 mM NaCl, treated with 0, 10 or 15 µg mL^{-1} azalomycin F and their autofluorescence was measured daily. (**D**) Autofluorescence at day seven of *C. reinhardtii* control cells and palmelloids incubated in TAP medium + 100 mM NaCl and 0, 10 or 15 µg mL^{-1} azalomycin F. For each time point, n = 3; SEMs are shown. ns = P > 0.05 calculated using 1way ANOVA. Azalomycin F-induced gloeocapsoids protect *C. reinhardtii* against azalomycin F (see Figure 4), which is not observed for NaCl-induced palmelloids.



Supplementary Figure 12: Palmelloids induced by 100 mM NaCl confer little resistance against azalomycin F. NaCl-induced palmelloids are shown in Supplementary Figure 4. Control cells and palmelloids of *C. reinhardtii* were inoculated into TAP medium (top pictures) or TAP + 100 mM NaCl (bottom pictures) and treated with 0, 10 or 15 µg mL^{-1} azalomycin F. After seven days of incubation, the cells were streaked on TAP agar and incubated under constant light for five days.



Supplementary Figure 13: Palmelloids induced by 15 mM sodium citrate confer little resistance against azalomycin F. Sodium citrate-induced palmelloids are shown in Supplementary Figure 4. (**A**) Control cells and palmelloids of *C. reinhardtii* were inoculated into TAP medium, treated with 0, 10 or 15 µg mL^{-1} azalomycin F and their autofluorescence was measured daily. (**B**) Autofluorescence at day seven of *C. reinhardtii* control cells and palmelloids incubated in TAP medium. (**C**) Control cells and palmelloids of *C. reinhardtii* were inoculated into TAP medium + 15 mM sodium citrate, treated with 0, 10 or 15 µg mL^{-1} azalomycin F and their autofluorescence was measured daily. (**D**) Autofluorescence at day seven of *C. reinhardtii* control cells and palmelloids incubated in TAP medium + 15 mM sodium citrate and 0, 10 or 15 µg mL^{-1} azalomycin F. For each time point, n = 3; SEMs are shown. ns = $P > 0.05$ calculated using 1way ANOVA.



Supplementary Figure 14: Palmelloids induced by 15 mM sodium citrate confer little resistance against azalomycin F. Sodium citrate-induced palmelloids are shown in Supplementary Figure 4. Control cells and palmelloids of *C. reinhardtii* were inoculated into TAP medium (top pictures) or TAP + 15 mM sodium citrate (bottom pictures) and treated with 0, 10 or 15 µg mL^{-1} azalomycin F. After seven days of incubation, the cells were streaked on TAP agar and incubated under constant light for five days.

References

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