Effects of auxin derivatives on phenotypic plasticity and stress tolerance in five species of the green alga *Desmodesmus* (Chlorophyceae, Chlorophyta)

Wei-Jiun Lin [1], Han-Chen Ho [2], Sheng-Chang Chu [1], Jui-Yu Chou [Corresp. 1]

[1] Department of Biology, National Changhua University of Education, Changhua, Taiwan

[2] Department of Anatomy, Tzu Chi University, Hualien, Taiwan

Corresponding Author: Jui-Yu Chou
Email address: jackyjau@cc.ncue.edu.tw

Green microalge of the genus *Desmodesmus* are characterized by a high degree of phenotypic plasticity, allowing them to be truly cosmopolitan and withstand environmental fluctuations. This flexibility enables *Desmodesmus* to produce a phenotype–environment match across a range of environments broader compared to algae with more fixed phenotypes. Indoles and their derivatives are a well-known crucial class of heterocyclic compounds and are widespread in different species of plants, animals, and microorganisms. Indole-3-acetic acid (IAA) is the most common, naturally occurring, plant hormone of the auxin class. IAA may behave as a signaling molecule in microorganisms, and the physiological cues of IAA may also trigger phenotypic plasticity responses in *Desmodesmus*. In this study, we demonstrated that the changes in colonial morphs of five species of the green alga *Desmodesmus* were specific to IAA but not to the chemically more stable synthetic auxins, naphthalene-1-acetic acid and 2,4-dichlorophenoxyacetic acid. Moreover, inhibitors of auxin biosynthesis and polar auxin transport inhibited cell division. Notably, different algal species (even different intraspecific strains) exhibited phenotypic plasticity different to that correlated to IAA. Thus, the plasticity involving individual-level heterogeneity in morphological characteristics may be crucial for microalgae to adapt to changing or novel conditions, and IAA treatment potentially increases the tolerance of *Desmodesmus* algae to several stress conditions. In summary, our results provide circumstantial evidence for the hypothesized role of IAA as a diffusible signal in the communication between the microalga and microorganisms. This information is crucial for elucidation of the role of plant hormones in planktonecology.

1 **Effects of auxin derivatives on phenotypic plasticity and stress tolerance in five species of**

2 **the green alga *Desmodesmus* (Chlorophyceae, Chlorophyta)**

4 Wei-Jiun Lin[1], Han-Chen Ho[2], Sheng-Chang Chu[1] & Jui-Yu Chou[1]*

5 [1]Department of Biology, National Changhua University of Education, Changhua 50007, Taiwan

6 [2]Department of Anatomy, Tzu Chi University, Hualien 97004, Taiwan

8 Corresponding author.

9 Correspondence to Jui-Yu Chou

10 Tel: +886-4-7232105 Ext. 3426; Fax: 886-4-7211156; Email: jackyjau@cc.ncue.edu.tw

12 **Abstract**

13 Green microalgae of the genus *Desmodesmus* are characterized by a high degree of phenotypic

14 plasticity (i.e. colony morphology), allowing them to be truly cosmopolitan and withstand

15 environmental fluctuations. This flexibility enables *Desmodesmus* to produce a phenotype–

16 environment match across a range of environments broader compared to algae with more fixed

17 phenotypes. Indoles and their derivatives are a well-known crucial class of heterocyclic

18 compounds and are widespread in different species of plants, animals, and microorganisms.

19 Indole-3-acetic acid (IAA) is the most common, naturally occurring, plant hormone of the auxin

20 class. IAA may behave as a signaling molecule in microorganisms, and the physiological cues of

21 IAA may also trigger phenotypic plasticity responses in *Desmodesmus*. In this study, we

22 demonstrated that the changes in colonial morphs (cells per coenobium) of five species of the

23 green alga *Desmodesmus* were specific to IAA but not to the chemically more stable synthetic

24 auxins, naphthalene-1-acetic acid and 2,4-dichlorophenoxyacetic acid. Moreover, inhibitors of

25 auxin biosynthesis and polar auxin transport inhibited cell division. Notably, different algal

26 species (even different intraspecific strains) exhibited phenotypic plasticity different to that

27 correlated to IAA. Thus, the plasticity involving individual-level heterogeneity in morphological

28 characteristics may be crucial for microalgae to adapt to changing or novel conditions, and IAA

29 treatment potentially increases the tolerance of *Desmodesmus* algae to several stress conditions.

30 In summary, our results provide circumstantial evidence for the hypothesized role of IAA as a

31 diffusible signal in the communication between the microalga and microorganisms. This

32 information is crucial for elucidation of the role of plant hormones in plankton ecology.

34 **Keywords** Coenobial algae · *Desmodesmus* · Indole derivatives · Microalgae · Phenotypic

35 plasticity

37 **Introduction**

38 Phenotypic plasticity can be broadly defined as the capacity of a single genotype to exhibit
39 variable phenotypes in different environments and implies that a species can conquer diverse
40 environments. Phenotypic plasticity refers to some of the changes in an organism's behavior,
41 morphology and physiology in response to a unique environment. A well-known example of
42 phenotypic plasticity is changes in multicelled structures in coenobial algae. In these algae,
43 colonies reproduce asexually by successive divisions of the protoplast within the parent cell wall,
44 and when progeny are released, the parent wall remains. Daughter colony may be
45 morphologically identical to the parent, or they may exhibit remarkable phenotypic plasticity.
46 Most studies on phenotypic plasticity in coenobial algae have been conducted considering
47 morphological responses to an abiotic factor. Neustupa and Hodač (2005) demonstrated that
48 morphological plasticity of *Pediastrum duplex* var. *duplex* is related to the pH dynamics of
49 freshwater lakes. Peña-Castro et al. (2004) also reported the phenotypic plasticity in
50 *Scenedesmus incrassatulus* in response to heavy metal stress. However, microalgae are typically
51 associated with other microorganisms, such as zooplankton, fungi, and bacteria. Thus, studies on
52 phenotypic plasticity of the coenobial algae have increased in number and broadened their scope
53 from the focus on abiotic factors to biotic ones. Hessen and Van Donk (1993) first indicated that
54 the presence of the grazing pressure from water flea (*Daphnia magna*) can induce colony
55 formation in *Scenedesmus* algae. Furthermore, Lurling and his colleague proved that the induced
56 colony formation in the presence of herbivores is considered a strategy more efficient than
57 constitutive defenses under variable grazing risk (Lürling & Van Donk, 1996; Lürling, 2003).
58 Wu et al. (2013) further revealed that the number of cells per coenobium of *Scenedesmus*
59 increased with the population density of *Daphnia* growth, thus indicating a grazer density–
60 dependent response.

61   Auxins, which constitute a class of plant hormones, have previously been suggested to
62 regulate physiological responses and gene expression in microorganisms (Spaepen et al., 2007).
63 Indole-3-acetic acid (IAA) is one of the most physiologically active auxins that can be produced
64 by numerous microbial species (Spaepen et al., 2007; Fu et al., 2015). Furthermore, phylogenetic
65 analyses have revealed that IAA biosynthetic pathways evolved independently in plants, bacteria,
66 algae, and fungi (Fu et al., 2015). The convergent evolution of IAA production leads to the
67 hypothesis that natural selection might have favored IAA as a widespread physiological code in
68 these microorganisms and their interactions. In natural water bodies, the crucial physical
69 associations and biochemical interactions between microalgae and other microorganisms are
70 generally well recognized (Natrah et al., 2014). Piotrowska-Niczyporuk and Bajguz (2014) found
71 that IAA plays a crucial role in the growth and metabolism of *Chlorella vulgaris* during a 72-
72 hour culture period. Jusoh et al. (2015) indicated that IAA can induce changes in oil content,

73 fatty acid profiles, and expression of four genes responsible for fatty acid biosynthesis in *Ch.*
74 *vulgaris* at early stationary growth phase. In addition, the significance of these interactions in
75 algal phenotypic plasticity has attracted considerable scientific attention (Lürling & Van Donk,
76 1996; Lürling & Van Donk, 2000; Lürling 2003). Furthermore, IAA has been detected in some
77 species of Scenedesmaceae microalgae (Mazur et al., 2001; Prieto et al., 2011). We previously
78 used IAA as a signal molecule in microorganisms to simulate a selection pressure caused by
79 interspecific competition. The results indicated that the mean number of cells per particle of
80 *Desmodesmus opoliensis* and *D. komarekii* decreased gradually as the IAA concentration
81 increased gradually. The proportion of *Desmodesmus* unicells in monocultures increased with
82 IAA concentration. We also demonstrated that these unicells exhibited a lower tendency to
83 sedimentation than did large cells and that shrinkage may facilitate nutrient uptake and light
84 capture (Chung et al., 2018). However, whether other coenobial algal species of *Desmodesmus*
85 use the same strategy to overcome stress remains unknown. Hence, the objective of the present
86 study was to compare the effects of IAA at different concentrations on phenotypic responses in
87 different *Desmodesmus* species. Moreover, to address the auxin specificity of these processes
88 and obtain an insight into the complex auxin-related regulatory mechanism(s) in algal physiology,
89 we have selected a group of compounds called "auxin analogs," such as synthetically produced
90 naphthalene-1-acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D), which are
91 structurally related to IAA. We thus aim to determine the differential effects of auxins and auxin-
92 like compounds on the morphological responses of these coenobial algae. In addition, we
93 investigated the effects of inhibitors of auxin biosynthesis and auxin transport in *Desmodesmus*.
94 Here, 4-biphenylboronic acid (BBo), a potent YUCCA enzyme inhibitor and an *Arabidopsis*
95 growth inhibitor, and 2,3,5-triiodobenzoic acid (TIBA), a polar auxin transport inhibitor, were
96 used (Dhonukshe et al. 2008; Kakei et al. 2015). To elucidate the physiological changes induced
97 by phytohormone treatment, we also investigated whether IAA pretreatment promotes an
98 enhanced stress-tolerant phenotype. The obtained results are crucial for elucidating the role of
99 plant hormones in microalgal physiology.
100
101 **Materials and Methods**
102 *Isolation and Culture of Microalgae*
103 The algal strains used here were isolated from natural water bodies in Central Taiwan. Water
104 samples with visible microalgal population were centrifuged at 3000 ×*g* for 10 minutes at room
105 temperature to concentrate the cells and spread onto CA agar plates (with 0.8% w/v agar) (for
106 more details see Supplementary Materials). For isolating an axenic single colony from field
107 water samples, the streak plate method was used. The algae were cultured in CA medium.
108 Isolated algal cells were stored at −80°C in 15%–20% glycerol. For each experiment, the alga

109   was cultured axenically in liquid CA medium at 125 rpm in a tube rotator and grown at 25°C
110   under cool white fluorescent light (approximately 46.30 μmol m^{-2} s^{-1}) with a 14:10-h light–dark
111   period. Each algal culture sample was observed for cellular growth rates by measuring the
112   optical density at 680 nm. The regression equation between cell density (y × 10^5/mL) and OD$_{685}$
113   (x) was derived as y = 162.1x + 1.3463 (r^2 = 99.34%) (Qian et al., 2009).

114

115   *Algae Identification*
116   The algal cells were harvested by centrifugation at 3000×*g* at 25°C for 10 minutes. The genomic
117   DNA used for analysis was isolated using AccuPrep GMO DNA Extraction Kit (Bioneer, Korea).
118   The 18S rDNA was amplified through PCR by using the following primers: 18S forward-
119   TTTCTGCCCTATCAACTTTCGATG and 18S reverse-TACAAAGGGCAGGGACGTAAT,
120   which yielded a fragment of approximately 1200 bp (Pan et al., 2011). The PCR conditions were
121   as follows: initial denaturation at 96°C for 4 minutes; 36 cycles of denaturation at 96°C for 30 s,
122   annealing at 50°C for 30 s, and extension at 72°C for 1 minutes; and final extension at 72°C for 6
123   minutes. The ITS1-5.8S-ITS2 rDNA was amplified using the primers ITS forward1
124   (ACCTAGAGGAAGGAGAAGTCGTAA)   and   ITS   reverse1
125   (TTCCTCCGCTTATTGATATGC), which yielded a fragment of approximately 1200 bp (Pan et
126   al., 2011). The PCR conditions were as follows: initial denaturation at 96°C for 4 minutes; 36
127   cycles of denaturation at 96°C for 30 s, annealing at 48°C for 30 s, and extension at 72°C for 1
128   minutes; and final extension at 72°C for 6 minutes. DNA sequencing was performed by Tri-I
129   Biotech, Inc. (Taipei, Taiwan). The Basic Local Alignment Search Tool was used to find regions
130   of local similarity between sequences on the website of the National Center for Biotechnology
131   Information (http://www.ncbi.nlm.nih.gov). The voucher specimens of algal strains used in this
132   study are deposited in the Freshwater Algae Culture Collection at the Institute of Hydrobiology,
133   Hubei Province, China (http://algae.ihb.ac.cn/). The detailed information of each strain is
134   provided in Supplementary Table 1. Any requests can be addressed to the corresponding author.

135

136   *Experimental Design*
137   Solutions containing different concentrations of phytohormones (IAA, NAA, and 2,4-D) and
138   auxin-related compounds (4-biphenylboronic acid and 2,3,5-triiodobenzoic acid) were prepared
139   to investigate their influence on the growth and morphological plasticity of *Desmodesmus* strains.
140   The concentrations of each phytohormone and compounds used in each experiment depended on
141   the sensitivity of each species. The initial algal density in each culture was approximately 8.24 ×
142   10^3 cells·mL^{-1}. Algae were harvested after each experiment, and the proportions of different-
143   celled populations were calculated under an optical microscope (DMRB, Leica, Germany). The
144   proportion of different algal populations (including unicellular; two-, four-, and eight-celled; and

145   other colonial morphs) were calculated, and the mean numbers of cells in different morphotypes

146   were calculated. The numbers of cells per coenobium were counted by dividing the total cell

147   number by the number of coenobia.

148

149   *Transmission Electron Microscopy*

150   All specimens were prefixed in 2.5% glutaraldehyde/0.1 M sodium cacodylate buffer (pH 7.3)

151   containing 1% tannic acid at 4°C overnight. After washing in 0.1 M sodium cacodylate buffer

152   with 5% sucrose for 15 minutes three times, specimens were postfixed with 1% osmium

153   tetroxide in 0.1 M sodium cacodylate buffer at 4°C overnight. Specimens were then washed in

154   buffer, *en bloc* stained with 2% aqueous uranyl acetate, dehydrated through a graded series of

155   ethanol and two times with 100% acetone. Specimens were infiltrated with Spurr resin overnight

156   and embedded in fresh Spurr resin the next day. Serial ultrathin sections of approximately 70 nm

157   were cut with a diamond knife on a Leica Ultracut R ultramicrotome (Leica, Heerbrugg,

158   Switzerland). Ultrathin sections were collected on Formvar-coated copper slot grids (type:

159   GS2x1, Cat. #: G2010-Cu, Electron Microscopy Sciences) and examined with a Hitachi H-7500

160   transmission electron microscope (Hitachi, Tokyo, Japan) at 80 kV. Images were recorded using

161   a 2048 × 2048 Macrofire monochrome CCD camera (Optronics, Goleta, CA, USA).

162

163   *Stress Tests*

164   The log-phase algal cells were treated with 300μM IAA for 24h. The culture samples were

165   harvested; the cells were then washed with CA medium and resuspended in the CA medium with

166   different treatments. The initial algal density in each culture was approximately 9.86×10^6

167   cells·mL^{-1}. For osmotic shock test, the cells were incubated in the CA medium with 0.5 M NaCl.

168   For the effects of pH value, the culture samples were resuspended in CA medium at pH 3.0

169   (adjusted with HCl) or at pH 8.0 (adjusted with NaOH). For oxidative stress, the cells were

170   exposed to hydrogen peroxide at final concentration of 5 mM. The cell suspensions subjected to

171   the aforementioned treatments were shaken at 25°C for 15 or 30 minutes. For inducing heat

172   shock, the cells were exposed to 40°C for 10, 15, or 20 minutes by immersing the cultures in a

173   shaking water bath. For cold treatment, the cultures were exposed to 4°C for 24 hours. Fractions

174   of viable cells of each experiment were determined by plating appropriate dilutions of the

175   cultures on CA agar plates before and after treatments. There were six replicates of each

176   treatment. The controls (without IAA treatment) received the same treatments used throughout

177   the procedure.

178

179   *Statistical Analysis.*

180   In the experiment of effect of auxin analogs and inhibitors of auxin biosynthesis or transport on

181 algal growth, the statistical differences between different groups were analyzed with Kruskal-
182 Wallis one-way analysis of variance on ranks with post hoc Dunn's test. In the experiment of
183 plastic phenotypic changes in response to auxin analogs, the proportions of colonies with
184 different numbers of cells and mean number of cells per coenobium were compared using a one-
185 way analysis of variance with least significant difference post hoc test. In stress tests, the
186 significance of differences between the groups was determined using the Mann-Whitney U test.
187 A p of <0.05 was considered statistically significant. In these abovementioned experiments, data
188 are presented as means of three replicates ± their standard deviations (SDs).

189

190 **Results**
191 *Effect of Auxin Analogs and Inhibitors of Auxin Biosynthesis or Transport on Algal Growth and*
192 *Phenotypic Plasticity Induction*
193 In a previous study, we performed a dose–response analysis to determine the fitness effects of
194 IAA on the coenobial alga *D. komarekii* (Chung et al., 2018). The results revealed that different
195 concentrations of IAA had different effects on the growth and morphological changes of *D.*
196 *komarekii*. Thus, we concluded that *Desmodesmus* can respond to the external phytohormone
197 IAA signal and then integrate the information to initiate physiological changes. In this study, our
198 aim was to determine whether the physiological cues of IAA-related compounds also trigger the
199 growth and phenotypic plasticity responses in *Desmodesmus*. With respect to *D. komarekii*
200 growth, we examined the effects of the natural auxin IAA as well as those of the synthetic auxins
201 NAA and 2,4-D. At 300 μM, IAA, NAA, and 2,4-D clearly inhibited growth; however, IAA
202 caused lower inhibition than did NAA and 2,4-D (Fig. 1a). This inhibitory effect was also
203 observed at 100 and 200 μM NAA and 2,4-D, but not in the cells treated with 100 μM IAA (Fig.
204 1b, c). These observations indicated that these auxin-related compounds inhibit *D. komarekii*
205 growth. We next tested the effects of an auxin biosynthesis inhibitor and a polar auxin transport
206 inhibitor. BBo strongly inhibited growth even at 100 μM and its inhibitory effect increased with
207 its concentration (Fig. 1c). At 200 and 300 μM, both of TIBA and BBo inhibited *D. komarekii*
208 growth (Fig. 1a, b). These results suggested that inhibition of auxin transport and inhibition of
209 YUCCA function both inhibit cell growth.

210   To measure phenotypic plasticity responses in algal populations, monocultures of *D.*
211 *komarekii* were used. After 1 week of culturing, the monocultures of *D. komarekii* in the groups
212 with exogenous 300 μM IAA and synthetic auxins were compared with those in the control
213 environment (without treatment). We found that the monocultures of *D. komarekii* in the control
214 groups (without IAA treatment) were dominated by one- and four-celled coenobia (Fig. 2a–c).
215 The morphology of *D. komarekii* monocultures changed drastically compared with the control
216 after exposure to IAA (Fig. 2b, c). The proportion of unicells increased rapidly from day 3, and

217   the proportion of four-celled coenobia decreased (Fig. 2a, c). The mean number of cells per
218   particle reached its minimum on approximately day 7 (Fig. 2a), the proportion of unicells
219   increased from 37% to approximately 73%, and the proportion of four-celled coenobia decreased
220   from 49% to approximately 16% on day 9. The proportion of two-celled coenobia changed only
221   slightly from approximately 13% to approximately 7%. The mean number of cells per particle in
222   the control groups remained at >2.5 during the 9-day period. By contrast, we found that the
223   auxin-related compounds NAA and 2,4-D both inhibited the growth of *D. komarekii* in a dose-
224   dependent manner, but they did not influence their number of cells per coenobium/individual
225   colony (Fig. 2d, e). In this experiment, the *D. komarekii* population of each culture was
226   composed of unicells and two-, four-, and eight-celled colonies; a few three-, five-, six-, and
227   seven-celled colonies were also present, but coenobia with more than eight cells were not
228   observed.
229   Through transmission electron microscopy (TEM) analysis, we confirmed that the
230   morphological changes in coenobia were not caused by cell aggregation but by the vegetative
231   growth of a mother cell (Fig. 3a-b). No extracellular matrix was seen on or around the cells, and
232   the connecting strands between cells were highly visible (Fig. 3c). Electron-dense kitting
233   material (warty layer) can be seen at each corner of coenobial junction between two neighboring
234   cells (yellow circle; Fig. 3c). Notably, we observed that specific large unicells were formed in
235   the monocultures of *D. komarekii* after day 5 under IAA treatment. Thus, the samples collected
236   at day 7 after IAA treatment and the cells in the control groups were used for observation of
237   morphology through TEM. The accumulation of many starch granules and lipid bodies was
238   observed in the large unicells compared with the cells in control groups (Fig. 3d).
239   The auxin-like physiological competence of selected compounds was analyzed in
240   *Desmodesmus* based on the inhibition of growth in liquid cultures and morphological changes.
241   Thus, we performed a dose–response analysis to determine the fitness effects of IAA and other
242   analogs on eight other *Desmodesmus* strains. The results revealed that different concentrations of
243   indole derivatives had divergent effects on the growth of different *Desmodesmus* species (Figs.
244   4–5, Supplementary Figures 1–2). In general, high concentrations (>300 μM) of IAA and other
245   analogs inhibited the growth of the algal population. Thus, *Desmodesmus* can respond to the
246   external phytohormone signal of IAA and other analogs and then integrate the information to
247   initiate physiological changes. In the subsequent experiment, our aim was to determine whether
248   the physiological cues of IAA and other analogs in these cultures also trigger phenotypic
249   plasticity responses.
250
251   *Plastic Phenotypic Changes in Response to IAA Are Strain-Dependent Behaviors*
252   To measure the phenotypic plasticity responses to indole derivatives, four strains of *D. armatus*,

253   two strains of *D. communis*, and one strain of *D. intermedius* and *D. opoliensis* were used in this
254   study. After 1 week of treatment, the monocultures of *D. armatus* in the control groups (without
255   indole derivative treatment) were compared with those with indole derivatives. We found that the
256   changes in colonial morphs in *D. armatus* are specific to IAA but not to chemically synthesized
257   auxins, NAA and 2,4-D, which are chemically more stable than IAA (Figs. 4, Supplementary
258   Figure 1). Moreover, we found the different algal strains of *D. armatus* demonstrated phenotypic
259   plasticity to different IAA concentrations. In *D. armatus* JYCA037, the monocultures in the
260   control groups were dominated by two- and four-celled coenobia, with <2% unicells (Fig. 4a,
261   Supplementary Figure 1). The morphology of *D. armatus* JYCA037 populations considerably
262   changed under high concentration of IAA treatment compared with that in the control
263   environment (without IAA addition). When the IAA concentration increased, the proportion of
264   four-celled coenobia declined from >90% to approximately 21%, and the number of unicells
265   increased from <2% to approximately 12% and two-celled coenobia increased from
266   approximately 7% to 66%. The mean number of cells per particle of *D. armatus* JYCA037
267   decreased gradually as the IAA concentration gradually increased, and the cell number reached
268   its minimum level at an IAA concentration of 400 μM (Fig. 4a). Similar results were observed in
269   the monocultures of *D. communis* JYCA040; it was dominated by two- and four-celled coenobia
270   in the control groups (Fig. 5a, Supplementary Figure 2). When IAA concentration increased, the
271   proportion four-celled coenobia decreased, and the number of unicells increased. The mean
272   number of cells per coenobium particle in the control groups of these two strains remained for >3
273   days after 7-day culturing. By contrast, in *D. armatus* JYCA041, the monocultures in the control
274   groups were dominated by unicells (47%), with 47% two- and <7% four-celled individuals (Fig.
275   4b, Supplementary Figure 1). The morphology of *D. armatus* JYCA041 populations changed
276   considerably under high concentration of IAA treatment compared with that in the control
277   environment (without IAA addition). When IAA concentration increased, the proportion of two-
278   celled coenobia increased from approximately 47% to approximately 69% and the number of
279   unicells declined from approximately 47% to approximately 28%. The proportion of four-celled
280   coenobia only slightly changed from approximately 6% to approximately 3%. The mean number
281   of cells per particle of *D. armatus* JYCA041 increased gradually as the IAA concentration
282   gradually increased and reached its maximum level at an IAA concentration of 400 μM (Fig. 4b,
283   Supplementary Figure 1) Similarly, the mean number of cells per particle of *D. armatus*
284   JYCA039 increased gradually as the IAA concentration gradually increased and reached its
285   maximum level when the IAA concentration was approximately 200 μM (Fig. 4d,
286   Supplementary Figure 1). Notably, the aforementioned morphological changes were not observed
287   in *D. armatus* JYCA045 even under treatment with high concentrations with IAA (Fig. 4c,
288   Supplementary Figure 1). By contrast, we found that the auxin-related compounds NAA and 2,4-

289   D both inhibit *Desmodesmus* growth in a dose-dependent manner, but the treatment did not
290   influence their number of cells of individuals in these four *D. armatus* strains (Fig. 4). The
291   strain-dependent response to IAA but not to NAA and 2,4-D also occurred in one strain of *D.*
292   *communis* (JYCA040; Fig. 5a, e, i, Supplementary Figure 2) and *D. opoliensis* (JYCA043; Fig.
293   5b, f, j, Supplementary Figure 2). However, the phenotypic plasticity caused by auxin analogs
294   was not obviously shown in one strain of *D. communis* (JYCA044; Fig. 5c, g, k, Supplementary
295   Figure 2) and *D. intermedius* (JYCA042; Fig. 5d, h, l, Supplementary Figure 2).
296
297   *Effect of IAA Treatment on Stress Resistance*
298   In this study, we found that starch granules and lipid bodies accumulated in algal cells grown at a
299   high IAA concentration. In this environment, algal cells also demonstrated slow growth. Thus,
300   algae contain storage in the form of natural oils, such as neutral lipids or triglycerides, and algal
301   growth diminishes when exposed to stresses. The data reported in Table 1 showed that IAA-
302   treated cells could withstand sudden changes in the environment, demonstrating significantly
303   longer survival rates in the media subjected to temperature shock (40°C, 15 minutes and 4°C, 24
304   hours), osmotic shock (0.5 M NaCl, 15 and 30 minutes), oxidative stress (2 mM H_2O_2, 30
305   minutes), acid treatment (pH 3.0, 15 minutes) and alkaline shock (pH 8.0, 30 minutes).
306
307   **Discussion**
308   A central question in biodiversity theory and ecology is the "paradox of the plankton," which
309   indicates that the number of coexisting planktonic species far exceeds the expected and
310   explicable number based on competition theory (Hutchinson, 1961). Ecologists have provided
311   multiple solutions to the paradox by applying game theory, chaos, tradeoffs, and many other
312   concepts in the past five decades (Tilman, 1994; Huisman & Weissing, 1999; Károlyi et al., 2000;
313   Kerr et al., 2002; Goyal & Maslov, 2018). A leading theory to explain the paradox is that
314   individual variability maintains high biodiversity in planktonic microorganisms (Menden-Deuer
315   & Rowlett, 2014). In aquatic ecosystems, significant evidence supports individual variability, in
316   individual behaviors or physiology, among planktonic microorganisms. This phenotypic
317   plasticity has played a central role in studies on the evolution of diversity. Ecologically,
318   phenotypic plasticity has been considered particularly crucial when environmental changes occur
319   and different phenotypes have different fitness values across environments. This plasticity
320   decides the survival of an individual in the face of environmental changes (West-Eberhard, 1989).
321   The plasticity even can potentiate evolvability of microorganisms by opening up new regions of
322   the adaptive landscape (Yi & Dean, 2016).
323   In our study, we revealed that the morphological characteristics of *Desmodesmus* changed
324   considerably when exposed to IAA compared with the algal cells in the control environment. We

325   found that the algal strains we assayed here have different response patterns to the external IAA.
326   In this study and our previous study, we found that when IAA concentration increased, the mean
327   number of cells per particle of some *Desmodesmus* species decreased (Chung et al., 2018). The
328   surface-to-volume ratios of the unicells was larger than the colony cells in microalgae. Changes
329   in colony size influence algal surface-to-volume ratios, and the surface-to-volume ratio can
330   affect light capture and nutrient uptake (Reynolds, 2006; Steele et al., 2009). Notably, in this
331   study, we found that in some algal strains, this trend was reversed: the mean number of cells per
332   particle of some *Desmodesmus* strains was increased when IAA concentration increased. These
333   colonial populations have higher sinking velocities than the unicells and two-celled coenobia;
334   consequently, their competitive ability might be altered (Lürling, 2003). Thus, plasticity
335   involving individual-level heterogeneity in behaviors and physiological characteristics is crucial
336   for planktonic microorganisms to adapt to changing or novel conditions. This may suggest that
337   individual variability is perhaps the key mechanism supporting planktonic biodiversity.
338   In this study, two widely used auxins in plant tissue culture, NAA and 2,4-D, were also used
339   to investigate their effect on algal growth and physiological responses. These synthetic auxins
340   show varying degrees of auxin-like activity in different bioassays (Abebie et al., 2007; Savaldi-
341   Goldstein et al., 2008). For instance, the seedlings of *Arabidopsis thaliana* and
342   suspension-cultured cells of *Nicotiana tabacum* BY-2 were used to investigate the physiological
343   activity of several auxin analogs, along with their capacity to induce auxin-dependent gene
344   expression, to inhibit endocytosis and to be transported across the plasma membrane (Simon et
345   al., 2013). The authors concluded that the major determinants for the auxin-like physiological
346   potential of a particular compound are highly complex and involve its chemical and metabolic
347   stability, its ability to distribute in tissues in a polar manner, and its activity toward auxin-
348   signaling machinery. Thus, the distinct behavior of some synthetic auxin analogs suggests that
349   they might be useful tools in investigations of the molecular mechanism of auxin action. Ohtaka
350   et al. (2017) also examined the responses of the natural auxin (indole-3-butylic acid; IBA) as
351   well as the synthetic auxins (NAA and 2,4-D) on the charophyte alga *Klebsormidium nitens*.
352   Consistent with our results, the authors indicated that these auxin-related compounds all inhibit *K.*
353   *nitens* growth in a dose-dependent manner. In their study, it was also indicated that TIBA and
354   BBo both inhibited *K. nitens* growth. Notably, the IAA was detected in cultures of *K. nitens*, but
355   *K. nitens* lacks the central regulators of the canonical auxin-signaling pathway found in land
356   plants. However, the authors found that the exogenous IAA inhibited cell division and elongation,
357   and this treatment rapidly induced expression of a LATERAL ORGAN BOUNDARIES-
358   DOMAIN transcription factor. During evolution, *K. nitens* may have acquired a primitive auxin-
359   response pathway to regulate transcription and cell growth. Here, we found that the natural auxin
360   IAA and the synthetic auxins NAA and 2,4-D can all influence *Desmodesmus* growth rate.

361 However, the changes in the colonial morphs in *Desmodesmus* are specific to IAA, but not to
362 chemically more stable synthetic auxins. These studies have suggested that structure–activity
363 relationships determined precisely at the level of a particular protein (e.g., receptor or carrier)
364 may not correspond completely to the final auxin-like physiological activity of a particular
365 compound in the streptophytes and their sister group, the chlorophytes. Thus, the comparison of
366 the structure–activity relationships for the aforementioned phenotypic changes highlights
367 differences in the structural requirements of these auxin-related physiological processes, thus
368 making the differential (or the same) phenotypic outcome of the same (or different) compound a
369 very crucial aspect of auxin biology. However, in the use of auxin-inhibitors and other
370 experiments derived from the *Arabidopsis* methodology, we should be more careful in making
371 conclusions, because it has not been carefully tested whether the mechanisms in *Arabidopsis* are
372 really the same as in the green alga. In future studies, a more comprehensive examination of
373 auxin systems of green algae, including charophyte algae, will help elucidate the origin and
374 evolution of the plant auxin system.

375 Microalgae are unicellular photosynthetic microorganisms, typically found in freshwater
376 and marine systems. The high flexibility and adaptability of this extremely diverse group of
377 eukaryotic organisms enable it to grow in diverse environments, including fresh water, blackish,
378 marine, and soil environments. Microalgae coexist with heterotrophic microorganisms, and the
379 exchange of chemical compounds is central to the interactions of microalgae with other
380 microorganisms. How microalgal–microbial interactions and participating chemical compounds
381 shape their communities and considerably affect their fitness remains unknown (Hom et al.,
382 2015). Notably, not only plants but also bacteria, fungi (including yeast), and even some
383 microalgae produce or respond to IAA (Fu et al., 2015). Researchers have hypothesized that the
384 microbes sense environmental IAA concentrations to determine the cell density of its
385 competitors (Spaepen et al., 2007; Fu et al., 2015; Chung et al., 2018). Thus, IAA has been
386 speculated to be a signal that coordinates microbial behavior to enhance protection against
387 damage by adverse conditions (Bianco et al., 2006; Chung et al., 2018). We proposed that the
388 physiological changes in response to IAA confers a fitness advantage by promoting the ability of
389 *Desmodesmus* strains to survive in their niches that often undergo fluctuations in environmental
390 factors, such as temperature, osmotic pressure, reactive oxygen species, and pH changes. Under
391 unfavorable stress conditions, such as nutritional starvation, salinity stress and high light
392 intensity, lipid production is usually enhanced in algal cells, due to shifts in lipid biosynthetic
393 pathways toward neutral lipid accumulation (Sun et al., 2018). Microalgae generally accumulate
394 neutral lipids, mainly in the form of triacylglycerols (TAG) under environmental stress
395 conditions. The accumulation of TAG likely occurs as a means of creating an energy deposit that
396 can be readily used in response to a more favorable environment allowing for rapid growth (Tan

397 & Lee, 2016). In green algae, stress conditions also trigger the accumulation of starch granules in
398 the cells, with starch accumulation preceding the accumulation of lipid bodies following stress
399 onset (Siaut et al., 2011). It is generally assumed that the starch and TAG serve as electron sinks
400 under conditions where photosynthesis or metabolism of an exogenous carbon source remains
401 active but the growth is limited (Hu et al., 2008). This phenomenon suggests that carbon sources
402 in algal cells during stress conditions were allocated not only to storage lipid production but also
403 starch biosynthesis, and this finding demonstrates the possibility of partitioning manipulation in
404 the cells. To link physiological changes to phenotype, we performed various cell viability assays
405 in response to heat, cold, osmotic stress, oxidative stress, reactive oxygen species, and pH
406 changes. We found an increased ability to tolerate these stresses, thereby confirming the inferred
407 enhanced stress-tolerant phenotypes when exposed to IAA. The results are consistent with earlier
408 research on bacteria that found enhanced stress tolerance when the bacteria were pretreated with
409 IAA across various stress conditions (Bianco et al., 2006; Imperlini et al. 2009; Donati et al.,
410 2013). Here we verified that IAA increased the cell viability under many other stress conditions,
411 but to varying extents for the different stresses. Understanding the mechanisms underlying the
412 phenomenon, it is necessary to further investigate the effect of IAA treatment on some of the
413 structural components of the envelope that may be involved in cellular response to stresses.

414   In natural water bodies, the importance of physical associations and biochemical
415 interactions between microalgae and microorganisms is generally well appreciated, but the
416 significance of these interactions to microbial ecology has not been investigated. In our previous
417 study, we found that a low concentration of IAA promoted the growth of algal cells, but high
418 concentrations of IAA inhibited cell growth (Chung et al., 2018). Herein, we further proved that
419 the effects of exogenous IAA and on algal growth and phenotypic changes is species- and even
420 strain-dependent. IAA can exert stimulatory and inhibitory effects not only on algae, fungi, and
421 yeast but also bacteria (Prusty et al., 2004; De-Bashan et al., 2008; Hu et al., 2010; Kerkar et al.
422 2012; Kulkarni et al., 2013; Sun et al., 2014; Liu et al., 2016; Fu et al., 2017). Bagwell et al.
423 (2014) reported the frequency of co-occurrence between IAA-producing bacteria and green algae
424 in natural and engineered ecosystems and revealed that the chlorophyll content and dry weight of
425 algal cells were IAA concentration-dependent. A recent study also indicated that IAA produced
426 by associated bacteria was transferred to diatoms and influenced their growth in exchange for
427 organosulfur compounds (Amin et al., 2015). Thus, exposure to IAA could be likely to affect the
428 outcome of competition among these coexisting organisms. We finally suggested that both algae
429 and other microorganisms altered their metabolism to defend themselves form their competitors
430 (or suit each other's needs), and this interaction is potentially very prevalent in the aquatic
431 ecosystems. These findings indicated that IAA is a major factor determining the competition (or
432 mutualistic interactions) between microbial species occupying the same niche. In this study,

433   different amounts of IAA (up to 500 μM) were used to assay the effects of exogenous IAA on
434   algal physiology. Some concerns exist that this concentration does not reflect all actual
435   concentrations found in the wild or those produced by microbes. Many studies have shown that
436   the amount of IAA that many bacteria and fungi can secrete was similar to or even more than
437   500 μM which is the highest dose we used here (Mohite, 2013; Limtong et al., 2014; Nutaratat et
438   al., 2015, 2017). According to our previous study, some fungi (e.g. *Aureobasidium pullulans*)
439   can even can secrete more than 200 μg/mL IAA (~1000 μM) (Fu et al., 2016). Furthermore, the
440   IAA measured in this study was secreted by microbes into liquid medium. We believe that the
441   amount of IAA produced by these microbes in the microniche under wild conditions should be
442   higher than that we used in this study.
443

444   **Conclusions**
445   In this study, we indicated that the changes in colonial morphs of five species of the green alga
446   *Desmodesmus* were specific to IAA but not to the chemically more stable synthetic auxins,
447   naphthalene-1-acetic acid and 2,4-dichlorophenoxyacetic acid. Our results also proved that IAA
448   treatment potentially increases the tolerance of *Desmodesmus* algae to several stress conditions.
449   Our results suggest that IAA could be used as a diffusible signal to elicit interspecific
450   communication among different organisms. Furthermore, the plasticity involving individual-
451   level heterogeneity in behaviors and physiological characteristics is crucial for planktonic
452   microorganisms to adapt to changing or novel conditions.
453

454   **Acknowledgments** We thank the members of the Chou Laboratory for their helpful discussions
455   and comments on the manuscript. We are grateful to the staff of the Electron Microscopy
456   Laboratory, Tzu Chi University, for technical support. This manuscript was edited by Wallace
457   Academic Editing. This work was supported by grants from the Ministry of Science and
458   Technology (MOST 105-2311-B-018-001-MY3 and MOST 108-2621-B-018-002-MY3 to Jui-
459   Yu Chou).

460

462   **References**

463   Abebie B, Lers A, Philosoph-Hadas S, Goren R, Riov J, Meir S. 2007. Differential effects of
464   NAA and 2, 4-D in reducing floret abscission in Cestrum (*Cestrum elegans*) cut flowers
465   are associated with their differential activation of Aux/IAA homologous genes. *Annals of*
466   *Botany* 101:249-259.

467   Amin S, Hmelo L, Van Tol H, Durham B, Carlson L, Heal K, Morales R, Berthiaume C, Parker
468   M, Djunaedi B. 2015. Interaction and signalling between a cosmopolitan phytoplankton
469   and associated bacteria. *Nature* 522:98.

470   Bagwell CE, Piskorska M, Soule T, Petelos A, Yeager CM. 2014. A diverse assemblage of
471   indole-3-acetic acid producing bacteria associate with unicellular green algae. *Applied*
472   *Biochemistry and Biotechnology* 173:1977-1984.

473   Bianco C, Imperlini E, Calogero R, Senatore B, Amoresano A, Carpentieri A, Pucci P, Defez R.
474   2006. Indole-3-acetic acid improves *Escherichia coli*'s defences to stress. *Archives of*
475   *Microbiology* 185:373-382. Chung TY, Kuo CY, Lin WJ, Wang WL, Chou JY. 2018.
476   Indole-3-acetic-acid-induced phenotypic plasticity in *Desmodesmus* algae. *Scientific*
477   *Reports* 8:10270.

478   De-Bashan LE, Antoun H, Bashan Y. 2008. Involvement of indole-3-acetic acid produced by the
479   growth-promoting bacterium *Azospirillum* spp. In promoting growth of *Chlorella*
480   *vulgaris*. *Journal of Phycology* 44:938-947.

481   Dhonukshe P, Grigoriev I, Fischer R, Tominaga M, Robinson DG, Hašek J, Paciorek T, Petrášek
482   J, Seifertová D, Tejos R. 2008. Auxin transport inhibitors impair vesicle motility and
483   actin cytoskeleton dynamics in diverse eukaryotes. *Proceedings of the National Academy*
484   *of Sciences* 105:4489-4494.

485   Donati AJ, Lee HI, Leveau JH, Chang WS. 2013. Effects of indole-3-acetic acid on the
486   transcriptional activities and stress tolerance of *Bradyrhizobium japonicum*. *PLOS ONE*
487   8:e76559.

488   Fu SF, Chen HW, Wei JY, Lee YI, Chou J-Y. 2017. Yeast-produced IAA is not only involved in
489   the competition among yeasts but also promotes plant growth and development. *Nova*
490   *Hedwigia* 105:135-150.

491   Fu SF, Sun PF, Lu HY, Wei JY, Xiao HS, Fang WT, Cheng BY, Chou JY. 2016. Plant growth-
492   promoting traits of yeasts isolated from the phyllosphere and rhizosphere of *Drosera*
493   *spatulata* Lab. *Fungal Biology* 120:433-448.

494   Fu SF, Wei JY, Chen HW, Liu YY, Lu HY, Chou JY. 2015. Indole-3-acetic acid: A widespread
495   physiological code in interactions of fungi with other organisms. *Plant Signaling &*
496   *Behavior* 10:e1048052.

497   Goyal A, Maslov S. 2018. Diversity, stability, and reproducibility in stochastically assembled

498   microbial ecosystems. *Physical Review Letters* 120:158102.

499 Hessen DO, Van Donk E. 1993. Morphological changes in *Scenedesmus* induced by substances
500   released from Daphnia. *Archiv für Hydrobiologie* 127:129-129.

501 Hom EF, Aiyar P, Schaeme D, Mittag M, Sasso S. 2015. A chemical perspective on microalgal–
502   microbial interactions. *Trends in Plant Science* 20:689-693.

503 Hu M, Zhang C, Mu Y, Shen Q, Feng Y. 2010. Indole affects biofilm formation in bacteria.
504   *Indian Journal of Microbiology* 50:362-368.

505 Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A. 2008.
506   Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and
507   advances. *The Plant Journal* 54:621-639.

508 Huisman J, Weissing FJ. 1999. Biodiversity of plankton by species oscillations and chaos.
509   *Nature* 402:407.

510 Hutchinson GE. 1961. The paradox of the plankton. *The American Naturalist* 95:137-145.

511 Imperlini E, Bianco C, Lonardo E, Camerini S, Cermola M, Moschetti G, Defez R. 2009. Effects
512   of indole-3-acetic acid on *Sinorhizobium meliloti* survival and on symbiotic nitrogen
513   fixation and stem dry weight production. *Appl Microbiol Biotechnol* 83:727-738.Jusoh M,
514   Loh SH, Chuah TS, Aziz A, Cha TS. 2015. Indole-3-acetic acid (IAA) induced changes
515   in oil content, fatty acid profiles and expression of four fatty acid biosynthetic genes in
516   *Chlorella vulgaris* at early stationary growth phase. *Phytochemistry* 111:65-71.

517 Kakei Y, Yamazaki C, Suzuki M, Nakamura A, Sato A, Ishida Y, Kikuchi R, Higashi S, Kokudo
518   Y, Ishii T. 2015. Small-molecule auxin inhibitors that target YUCCA are powerful tools
519   for studying auxin function. *The Plant Journal* 84:827-837.

520 Kerkar S, Raiker L, Tiwari A, Mayilraj S, Dastager S. 2012. Biofilm-associated indole acetic
521   acid producing bacteria and their impact in the proliferation of biofilm mats in solar
522   salterns. *Biologia* 67:454-460.

523 Kerr B, Riley MA, Feldman MW, Bohannan BJ. 2002. Local dispersal promotes biodiversity in
524   a real-life game of rock–paper–scissors. *Nature* 418:171.

525 Károlyi G, Péntek Á, Scheuring I, Tél T, Toroczkai Z. 2000. Chaotic flow: the physics of species
526   coexistence. *Proceedings of the National Academy of Sciences* 97:13661-13665.

527 Kulkarni GB, Sanjeevkumar S, Kirankumar B, Santoshkumar M, Karegoudar T. 2013. Indole-3-
528   acetic acid biosynthesis in Fusarium delphinoides strain GPK, a causal agent of Wilt in
529   Chickpea. *Applied Biochemistry and Biotechnology* 169:1292-1305.

530 Limtong S, Kaewwichian R, Yongmanitchai W, Kawasaki H. 2014. Diversity of culturable
531   yeasts in phylloplane of sugarcane in Thailand and their capability to produce indole-3-
532   acetic acid. *World Journal of Microbiology and Biotechnology* 30:1785-1796.

533 Lürling M. 2003. Phenotypic plasticity in the green algae *Desmodesmus* and *Scenedesmus* with

534   special reference to the induction of defensive morphology. *Annales de Limnologie -*
535   *International Journal of Limnology* 39:85-101.

536   Lürling M, Van Donk E. 1996. Zooplankton-induced unicell-colony transformation in
537   *Scenedesmus acutus* and its effect on growth of herbivore Daphnia. *Oecologia* 108:432-
538   437.

539   Lürling M, Van Donk E. 2000. Grazer-induced colony formation in *Scenedesmus*: are there costs
540   to being colonial? *Oikos* 88:111-118.

541   Liu YY, Chen HW, Chou JY. 2016. Variation in indole-3-acetic acid production by wild
542   *Saccharomyces cerevisiae* and *S. paradoxus* strains from diverse ecological sources and
543   its effect on growth. *PLOS ONE* 11:e0160524.

544   Mazur H, Konop A, Synak R. 2001. Indole-3-acetic acid in the culture medium of two axenic
545   green microalgae. *Journal of Applied Phycology* 13:35-42.

546   Menden-Deuer S, Rowlett J. 2014. Many ways to stay in the game: individual variability
547   maintains high biodiversity in planktonic microorganisms. *Journal of The Royal Society*
548   *Interface* 11:20140031.

549   Mohite B. 2013. Isolation and characterization of indole acetic acid (IAA) producing bacteria
550   from rhizospheric soil and its effect on plant growth. *Journal of Soil Science and Plant*
551   *Nutrition* 13: 638-649.

552   Nutaratat P, Amsri W, Srisuk N, Arunrattiyakorn P, Limtong S. 2015. Indole-3-acetic acid
553   production by newly isolated red yeast *Rhodosporidium paludigenum*. *The Journal of*
554   *General and Applied Microbiology* 61:1-9.

555   Nutaratat P, Monprasit A, Srisuk N. 2017. High-yield production of indole-3-acetic acid by
556   *Enterobacter* sp. DMKU-RP206, a rice phyllosphere bacterium that possesses plant
557   growth-promoting traits. *3 Biotech* 7:305.

558   Natrah FM, Bossier P, Sorgeloos P, Yusoff FM, Defoirdt T. 2014. Significance of microalgal–
559   bacterial interactions for aquaculture. *Reviews in Aquaculture* 6:48-61.

560   Neustupa J, Hodac L. 2005. Changes in shape of the coenobial cells of an experimental strain of
561   *Pediastrum duplex* var. *duplex* (Chlorophyta) reared at different pHs. *Preslia* 77:439-452.

562   Ohtaka K, Hori K, Kanno Y, Seo M, Ohta H. 2017. Primitive auxin response without TIR1 and
563   Aux/IAA in the charophyte alga *Klebsormidium nitens*. *Plant Physiology* 174:1621-1632.

564   Pan YY, Wang ST, Chuang LT, Chang YW, Chen CN. 2011. Isolation of thermo-tolerant and
565   high lipid content green microalgae: oil accumulation is predominantly controlled by
566   photosystem efficiency during stress treatments in *Desmodesmus*. *Bioresource*
567   *technology* 102:10510-10517.

568   Pena-Castro JM, Martinez-Jeronimo F, Esparza-Garcia F, Canizares-Villanueva RO. 2004.
569   Phenotypic plasticity in *Scenedesmus incrassatulus* (Chlorophyceae) in response to heavy

570   metals stress. *Chemosphere* 57:1629-1636.

571   Piotrowska-Niczyporuk A, Bajguz A. 2014. The effect of natural and synthetic auxins on the

572   growth, metabolite content and antioxidant response of green alga *Chlorella vulgaris*

573   (Trebouxiophyceae). *Plant Growth Regulation* 73:57-66.

574   Prieto C, Rosa E, Cordoba C, Nancy M, Montenegro J, Andres M, González-Mariño GE. 2011.

575   Production of indole-3-acetic acid in the culture medium of microalga *Scenedesmus*

576   *obliquus* (UTEX 393). *Journal of the Brazilian Chemical Society* 22:2355-2361.

577   Prusty R, Grisafi P, Fink GR. 2004. The plant hormone indoleacetic acid induces invasive

578   growth in *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences*

579   101:4153-4157.

580   Qian H, Chen W, Li J, Wang J, Zhou Z, Liu W, Fu Z. 2009. The effect of exogenous nitric oxide

581   on alleviating herbicide damage in *Chlorella vulgaris*. *Aquatic Toxicology* 92:250-257.

582   Reynolds CS. 2006. *The ecology of phytoplankton*: Cambridge University Press.

583   Savaldi-Goldstein S, Baiga TJ, Pojer F, Dabi T, Butterfield C, Parry G, Santner A, Dharmasiri N,

584   Tao Y, Estelle M. 2008. New auxin analogs with growth-promoting effects in intact

585   plants reveal a chemical strategy to improve hormone delivery. *Proceedings of the*

586   *National Academy of Sciences* 105:15190-15195.

587   Siaut M, Cuiné S, Cagnon C, Fessler B, Nguyen M, Carrier P, Beyly A, Beisson F,

588   Triantaphylidès C, Li-Beisson Y. 2011. Oil accumulation in the model green alga

589   *Chlamydomonas reinhardtii*: characterization, variability between common laboratory

590   strains and relationship with starch reserves. *BMC Biotechnology* 11:7.

591   Simon S, Kubeš M, Baster P, Robert S, Dobrev PI, Friml J, Petrášek J, Zažímalová E. 2013.

592   Defining the selectivity of processes along the auxin response chain: a study using auxin

593   analogues. *New Phytologist* 200:1034-1048.

594   Spaepen S, Vanderleyden J, Remans R. 2007. Indole-3-acetic acid in microbial and

595   microorganism-plant signaling. *FEMS Microbiology Reviews* 31:425-448.

596   Steele JH, Thorpe SA, Turekian KK. 2009. *Elements of physical oceanography: a derivative of*

597   *the encyclopedia of ocean sciences*: Academic Press.

598   Sun PF, Fang WT, Shin LY, Wei JY, Fu SF, Chou JY. 2014. Indole-3-acetic acid-producing

599   yeasts in the phyllosphere of the carnivorous plant *Drosera indica* L. *PLOS ONE*

600   9:e114196.

601   Sun XM, Ren LJ, Zhao QY, Ji XJ, Huang H. 2018. Microalgae for the production of lipid and

602   carotenoids: a review with focus on stress regulation and adaptation. *Biotechnol Biofuels*

603   11:272.

604   Tan KWM, Lee YK. 2016. The dilemma for lipid productivity in green microalgae: importance

605   of substrate provision in improving oil yield without sacrificing growth. *Biotechnology*

606   *for Biofuels* 9:255.

607   Tilman D. 1994. Competition and biodiversity in spatially structured habitats. *Ecology* 75:2-16.

608   West-Eberhard MJ. 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of*

609   *Ecology, Evolution, and Systematics* 20:249-278.

610   Wu X, Zhang J, Qin B, Cui G, Yang Z. 2013. Grazer density-dependent response of induced

611   colony formation of *Scenedesmus obliquus* to grazing-associated infochemicals.

612   *Biochemical Systematics and Ecology* 50:286-292.

613   Yi X, Dean AM. 2016. Phenotypic plasticity as an adaptation to a functional trade-off. *eLife*

614   5:e19307.

615

617 **Figure legends**

618 **Fig. 1** Growth of coenobial algae *Desmodesmus komarekii* in the presence of several auxins and
619 inhibitors of auxin biosynthesis and auxin transport. *D. komarekii* was cultured in the presence of
620 **a** 300, **b** 200, and **c** 100 μM auxin derivatives, including indole-3-acetic acid (IAA), 2,4-
621 dichlorophenoxyacetic acid (2,4-D), naphthalene-1-acetic acid (NAA), a polar auxin transport
622 inhibitor, 2,3,5-triiodobenzoic acid (TIBA), and a potent YUCCA enzyme inhibitor and
623 *Arabidopsis* growth inhibitor, 4-biphenylboronic acid (BBo). Growth curves of *D. komarekii* for
624 each compound were measured at 1, 3, 5, 7 and 9 days. Error bars represent standard deviation of
625 values for three replicates. Data were evaluated with Kruskal-Wallis one-way analysis of
626 variance on ranks with post hoc Dunn's test. Different lower case letters indicate significant
627 differences ($p < 0.05$).

628

629 **Fig. 2** The monocultures of *Desmodesmus komarekii* in the groups with exogenous IAA and
630 synthetic auxins were compared with those in the control environment (without treatment). **a**
631 Mean number of cells per coenobium of *Desmodesmus komarekii* cultured at 300 μM indole-3-
632 acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA)
633 concentrations and cells without treatment. Data are presented as means (n = 3) for each group,
634 and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means
635 with the same letter are not significantly different from each other according to a one-way
636 analysis of variance and least significant difference post hoc test. Different lower case letters
637 indicate significant differences ($p < 0.05$). **b-e** The proportions of unicells and of two- and four-
638 celled coenobia of *Desmodesmus komarekii* cultured at 300 μM IAA, 2,4-D, and NAA
639 concentrations and cells without treatment.

640

641 **Fig. 3** Transmission electron micrographs of *Desmodesmus komarekii* cells under indole-3-acetic
642 acid (IAA) treatment. **a-b** Through transmission electron microscopy, we confirmed that the
643 morphological changes in coenobia were not caused by cell aggregation but by the vegetative
644 growth of a mother cell. **c** No extracellular matrix was seen on or around the cells, and the
645 connecting strands between cells were highly visible. Electron-dense kitting material (warty
646 layer) can be seen at each corner of coenobial junction between two neighboring cells (yellow
647 circle). **d** The accumulation of many starch granules (S) and lipid bodies (L) was observed in the
648 large unicells at day 7 after IAA treatment compared with the cells in control groups.

649

650 **Fig. 4** Mean number of cells per coenobium in three strains of *Desmodesmus armatus* cultured at
651 different indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-
652 acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and

653   morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with
654   the same letter are not significantly different from each other according to the results of a one-
655   way analysis of variance and least significant difference post hoc test. **a**, **e, i** *D. armatus*
656   JYCA037. **b, f, j** *D. armatus* JYCA041. **c, g, k** *D. armatus* JYCA045. **d, h, l** *D. armatus*
657   JYCA039. Data were evaluated with a one-way analysis of variance with least significant
658   difference post hoc test. Different lower case letters indicate significant differences ($p < 0.05$).
659

660   **Fig. 5** Mean number of cells per coenobium of different *Desmodesmus* strains cultured at
661   different indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-
662   acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and
663   morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with
664   the same letter are not significantly different from each other according to a one-way analysis of
665   variance and least significant difference post hoc test. **a, e, i** *D. communis* JYCA040. **b, f, j** *D.*
666   *opoliensis* JYCA043. **c, g, k** *D. communis* JYCA044. **d, h, l** *D. intermedius* JYCA042. Data were
667   evaluated with a one-way analysis of variance with least significant difference post hoc test.
668   Different lower case letters indicate significant differences ($p < 0.05$).
669
670

Figure 1

Figure 1

Fig. 1 Growth of coenobial algae *Desmodesmus komarekii* in the presence of several auxins and inhibitor of auxin biosynthesis and auxin transport. *D. komarekii* was cultured in the presence of **a** 300, **b** 200, and **c** 100 µM auxin derivatives, including indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), naphthalene-1-acetic acid (NAA), a polar auxin transport inhibitor, 2,3,5-triiodobenzoic acid (TIBA), or a potent YUCCA enzyme inhibitor and *Arabidopsis* growth inhibitor, 4-biphenylboronic acid (BBo). Growth curves of *D. komarekii* for each compound were measured at 1, 3, 5, 7 and 9 days. Error bars represent standard deviation of values for three replicates. Data were evaluated with Kruskal-Wallis one-way analysis of variance on ranks with post hoc Dunn's test. Different lower case letters indicate significant differences ($p < 0.05$)

PeerJ
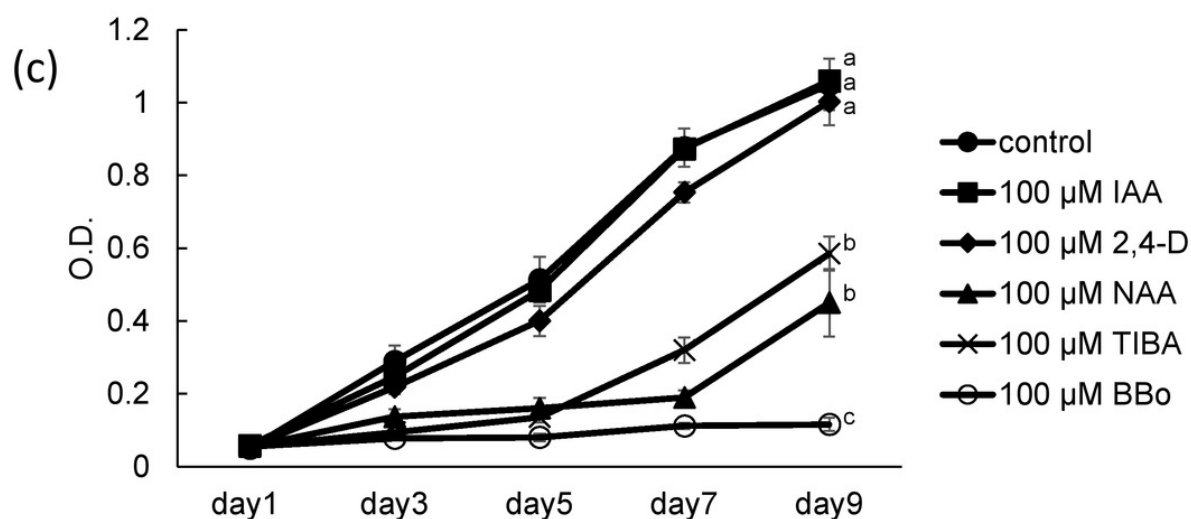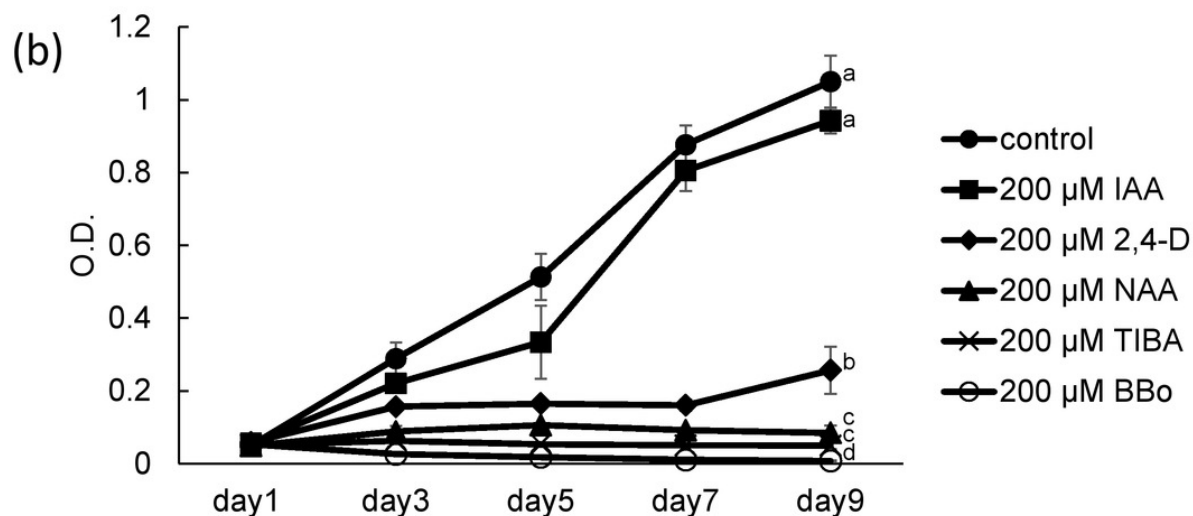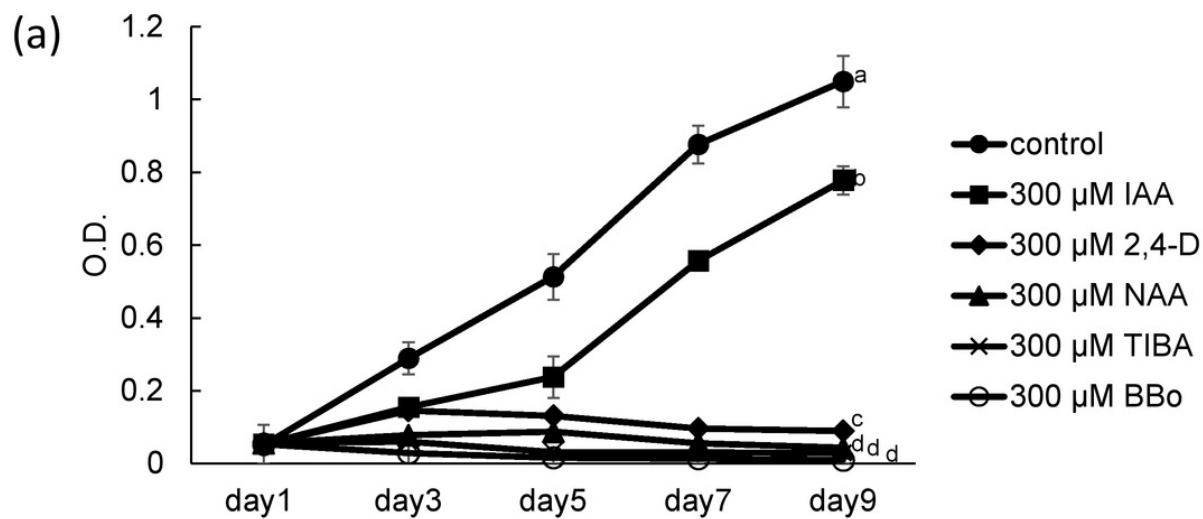
Figure 2

Figure 2

Fig. 2 Mean number of cells per coenobium and proportions of unicells and of two- and four-celled coenobia of *Desmodesmus komarekii* cultured at 300 µM indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA) concentrations and cells without treatment. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test.
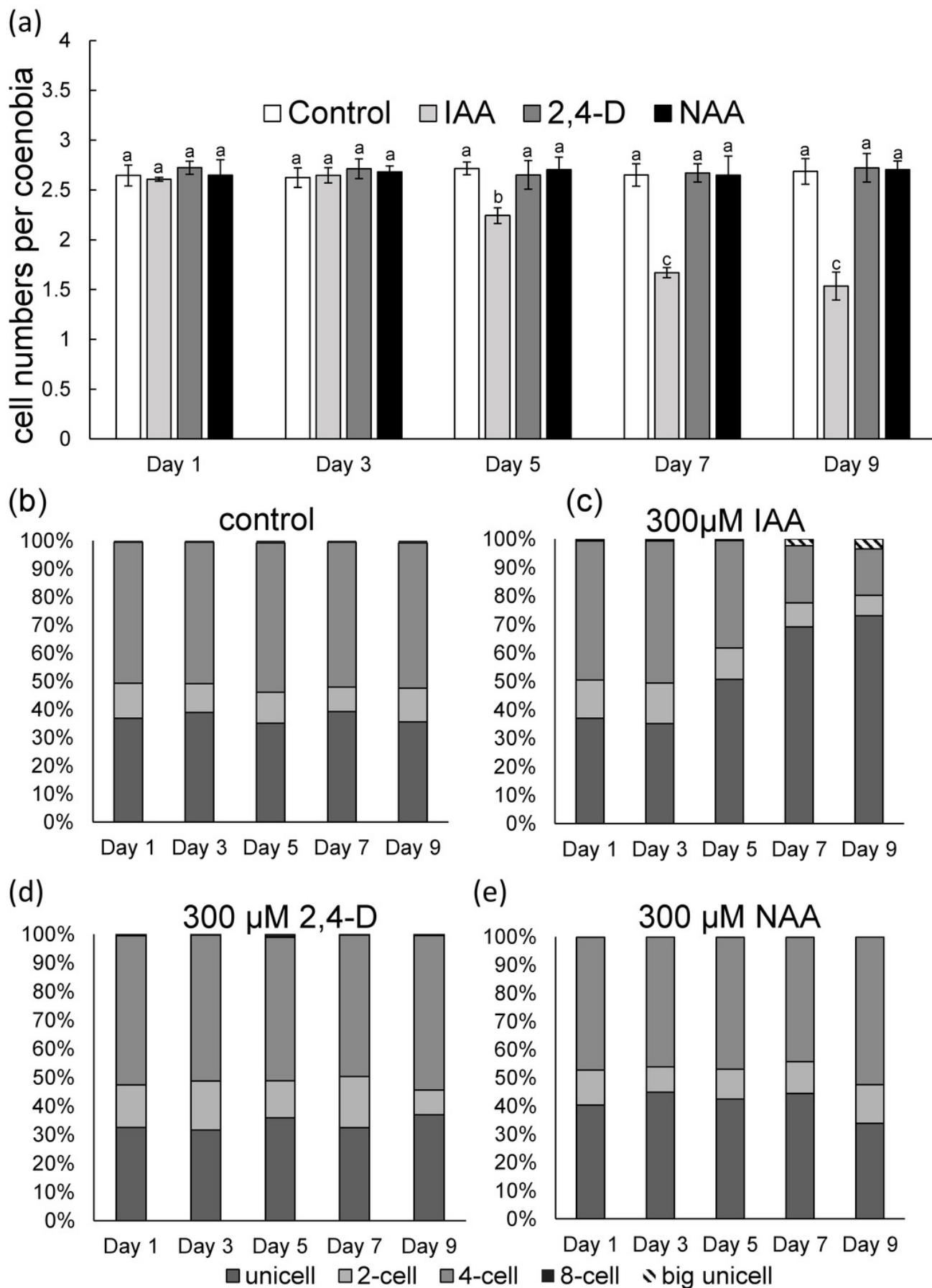
Figure 3

Figure 3

Fig. 3 Transmission electron micrographs of *Desmodesmus komarekii* cells under indole-3-acetic acid (IAA) treatment. **a-b** Through transmission electron microscopy, we confirmed that the morphological changes in coenobia were not caused by cell aggregation but by the vegetative growth of a mother cell. **c** No extracellular matrix was seen on or around the cells, and the connecting strands between cells were highly visible. Electron-dense kitting material (warty layer) can be seen at each corner of coenobial junction between two neighboring cells (yellow circle). **d** The accumulation of many starch granules (S) and lipid bodies (L) was observed in the large unicells at day 7 after IAA treatment compared with the cells in control groups.
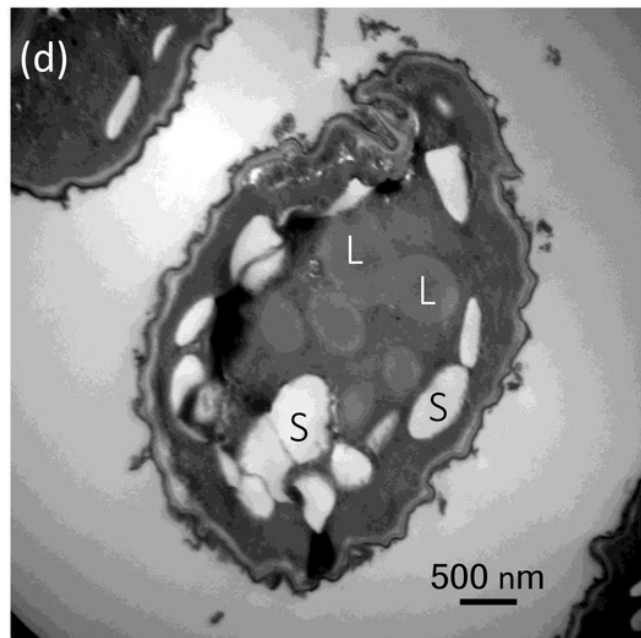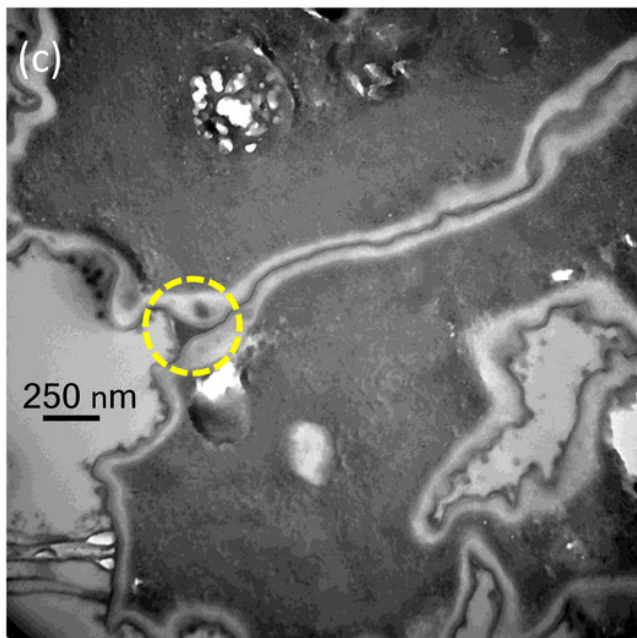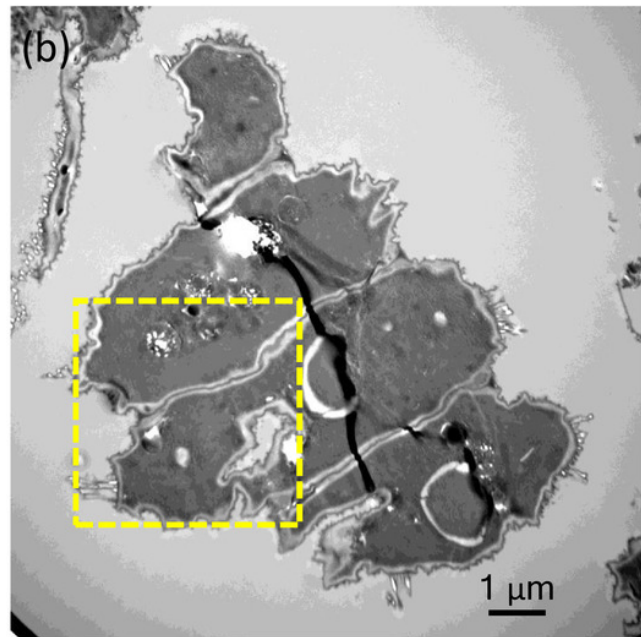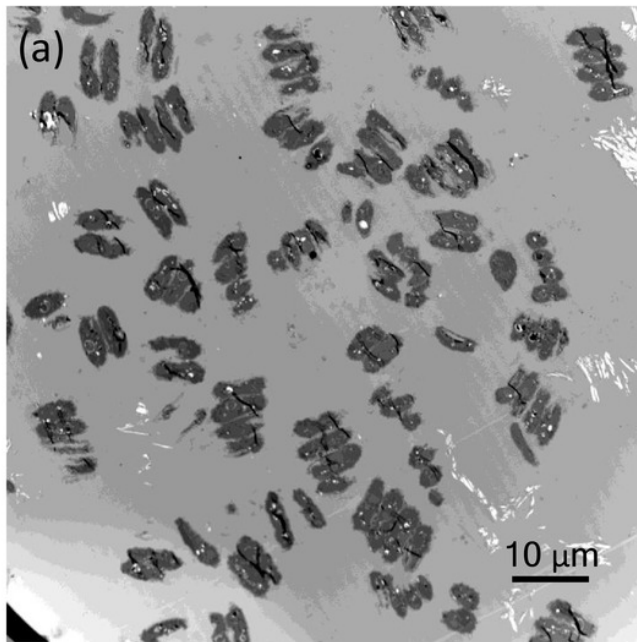
Figure 4

Figure 4

Fig. 4 Mean number of cells per coenobium in three strains of *Desmodesmus armatus* cultured at different indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to the results of a one-way analysis of variance and least significant difference post hoc test. **a**, **e, i** *D*. *armatus* JYCA037. **b, f, j** *D*. *armatus* JYCA041. **c, g, k** *D*. *armatus* JYCA045. **d, h, l** *D*. *armatus* JYCA039. Data were evaluated with a one-way analysis of variance with least significant difference post hoc test. Different lower case letters indicate significant differences (p < 0.05).
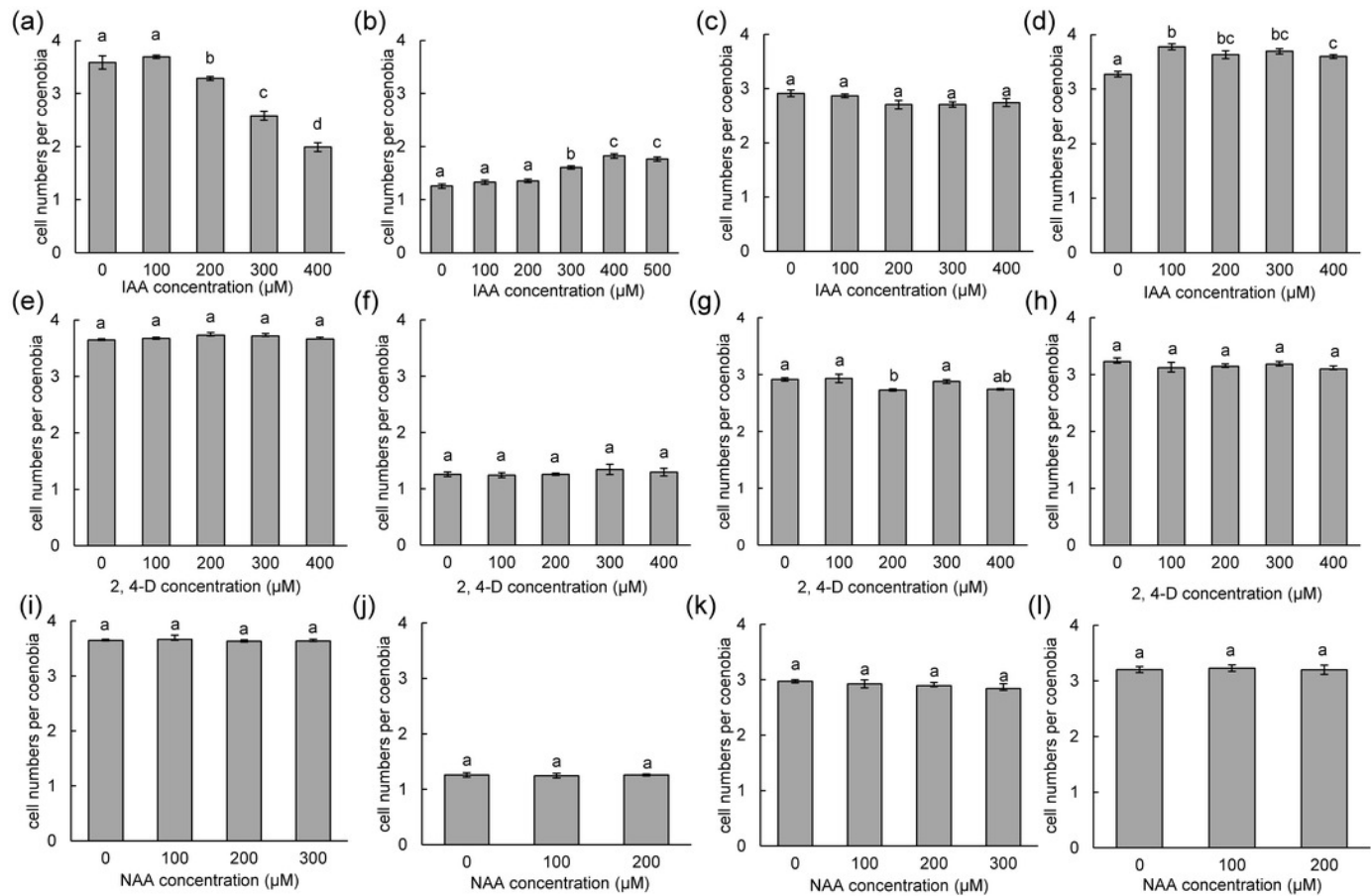
Figure 5

Figure 5

Fig. 5 Mean number of cells per coenobium of different *Desmodesmus* strains cultured at different indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test. **a, e, i** *D. communis* JYCA040. **b, f, j** *D. opoliensis* JYCA043. **c, g, k** *D. communis* JYCA044. **d, h, l** *D. intermedius* JYCA042. Data were evaluated with a one-way analysis of variance with least significant difference post hoc test. Different lower case letters indicate significant differences (p < 0.05).
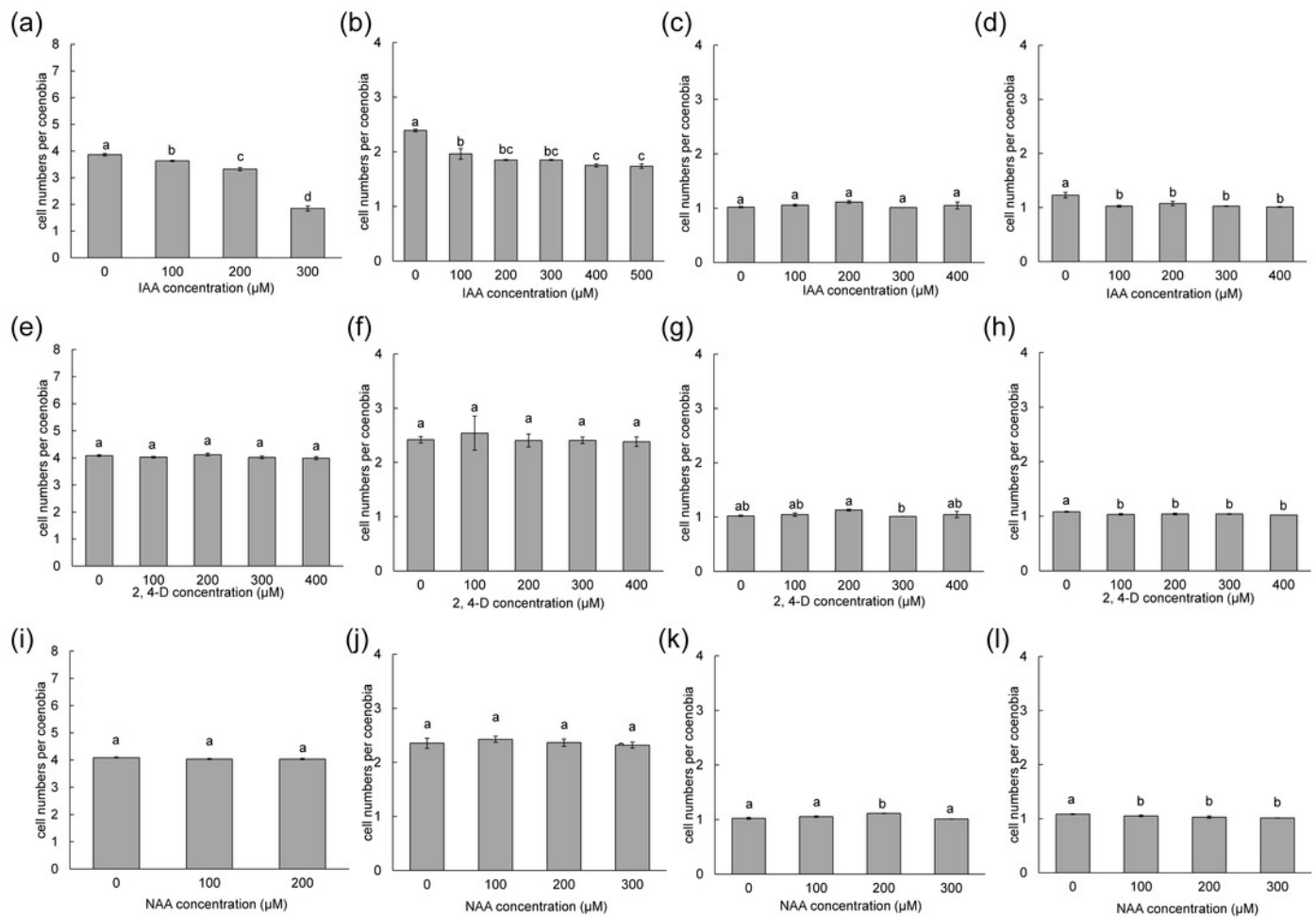
Table 1**(on next page)**

Table 1

Table 1 Increased resistance of *D. komarekii* cells to various stress conditions after exposure to IAA

1 **Table 1** Increased resistance of *D. komarekii* cells to various stress conditions after exposure to
2 IAA

	Survival (%)	
	Control	IAA-treated
Heat-shock (40°C, 10 mins)	76.0±15.4	87.3±9.9
Heat-shock (40°C, 15 mins)	66.6±9.3	85.4±3.5**
Heat-shock (40°C, 20 mins)	52.8±15.8	62.0±19.3
Cold-shock (4°C, 24 hrs)	24.8±10.7	50.0±2.0**
Osmotic shock (0.5 M NaCl, 15 mins)	40.7±8.8	50.0±3.6**
Osmotic shock (0.5 M NaCl, 30 mins)	24.2±12.4	44.5±7.3*
Oxidative stress (2 mM H_2O_2, 15 mins)	71.9±16.4	73.4±8.2
Oxidative stress (2 mM H_2O_2, 30 mins)	45.7±14.7	65.7±9.5*
Acid shock (pH 3.0, 15 mins)	71.8±7.3	86.3±3.9**
Acid shock (pH 3.0, 30 mins)	44.7±19.1	61.0±8.6
Alkaline shock (pH 8.0, 15 mins)	73.7±14.7	81.4±7.0
Alkaline shock (pH 8.0, 30 mins)	60.8±11.7	80.3±5.3**

3 The values reported in the table are the averages ± standard deviation of three measurements. The
4 significance of differences between groups was determined using the Mann-Whitney U test. *$p <$
5 0.05 was considered statistically significant. **$p < 0.01$