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Performance of plant growth-promoting bacterium of duckweed under different kinds of abiotic stress factors

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ABSTRACT

The effectiveness of a plant growth-promoting bacterium (PGPB) of duckweed, *Aquitalea magnusonii* H3, was studied under treatment with various abiotic stress factors repressing host plant growth (copper, zinc, salinity, weak light, low temperature, and nutrient scarcity). In the absence of stress factors, strain H3 stably improved the weekly yield of duckweed (*Lemna minor*) by 5.2–8.1% relative to aseptic control plants. However, in the presence of stress factors that reduced the duckweed weekly yield by approximately 40%, the performance of strain H3 changed both positively and negatively depending on the stress factor. Specifically, the growth promotion of strain H3 increased to 17.2% and 14.3% when the plant was subjected to copper and zinc stresses, respectively. Further evidence of increased plant accumulation of copper and zinc in the presence of strain H3 suggested that strain H3 enhanced the plant's capacity to both accumulate and tolerate these heavy metals. Conversely, the effects of strain H3 were inhibitory for duckweed growth under high salinity and nutrient-poor conditions. Additionally, other stress factors (weak light and low temperature) had no significant influence on PGPB performance. These results suggest that notably higher or lower performance of PGPB can be expressed under different kinds of plant stress factors.

1. Introduction

Duckweeds (family Lemnaceae) are free-floating aquatic plants characterized by rapid clonal multiplication on the surface of eutrophic waters (Landolt, 1986). Since duckweeds can synthesize starch- and protein-rich biomass and absorb nutrient minerals from wastewater, they are emerging as eco-friendly biomass crops that do not compete for land and fertilizer use with food production (Cui and Cheng, 2015). In fact, Toyama et al. (2018) reported that duckweeds can grow in various types of wastewater, including municipal wastewater, swine wastewater, and anaerobic digestion effluent, and they generate valuable biomass that can be readily converted to ethanol and methane gas through simple fermentation processes. Large-scale cultivations have also shown that duckweed can produce 39–110 metric tons of dry biomass per hectare annually when grown in wastewater, which is appreciably higher than the biomass production of conventional bioenergy crops (Xu et al., 2012).

As has been noted for terrestrial plants, it is known that the coexisting bacterial community of duckweed consists of both beneficial and deleterious ones for plant growth, which are called plant growth-promoting bacteria (PGPB) and plant growth-inhibiting bacteria (PGIB), respectively (Ishizawa et al., 2017a; Yamakawa et al., 2018). The first PGPB strain of duckweed to be discovered, *Acinetobacter calcoaceticus* P23, has been shown to improve duckweed growth in both synthetic media and environmental waters (Yamaga et al., 2010; Suzuki et al., 2014; Toyama et al., 2017), which offers the possibility of utilizing this PGPB as a bioinoculant for duckweed cultivation. Recently, *Aquitalea magnusonii* H3 was isolated from duckweed (*Lemna minor*) co-cultured with pond water-derived bacterial community, and characterized as another PGPB strain that can robustly improve duckweed growth even in the presence of other bacterial strains, including PGIB (Ishizawa et al., 2017a). This robust growth promoting effect is associated to the efficient competitive plant colonization ability of strain H3 (Ishizawa et al., 2019). As the presence of indigenous microbes is one of the major obstacles for PGPB application, this trait makes it a promising strain for use in nonsterile environments.

However, information on the PGPB performance in duckweed cultivations, especially how it is affected by environmental conditions, is extremely scarce. In full-scale duckweed cultivation systems, full growth speed of plants cannot be realized due to the presence of abiotic stress factors such as heavy metals, salinity, and unfavorable light and thermal conditions. Moreover, the kind of stress factor that principally

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repress plant growth differs depending on the climate and type of wastewater used for cultivation. Therefore, questions remain as to whether the PGPB of duckweed can retain its growth-promoting effects under the presence of a variety of stress factors.

Thus, this study was conducted to evaluate the growth promotion of duckweed by PGPB under various stress conditions, hypothesizing that the performance of PGPB depends largely on the kind of stress factor that restricts host plant growth. To this end, *L. minor* (common duckweed) was subjected to six stress factors (copper, zinc, salinity, weak light, low temperature, and nutrient scarcity) at similar severity, and the improvement in growth owing to inoculation of a PGPB strain, *A. magnusonii* H3, was examined. This study is the first to address the variation in PGPB performance induced by different kinds of plant stress factors.

2. Materials and methods

2.1. Plant and bacterial strains

The laboratory stock of common duckweed (*L. minor*, RDSC 5512) collected from the botanical garden of Hokkaido University (Hokkaido, Japan) was used in this study. Prior to experiments, plants were aseptically precultured for 7–10 days in 200 mL of half-strength Schenk and Hildebrandt medium (SH medium; Apollo Scientific Ltd., Stockport, UK; 1250 mg L^{-1} KNO_3, 150 mg L^{-1} $NH_4H_2PO_4$, 97.7 mg L^{-1} $MgSO_4$, 75.5 mg L^{-1} $CaCl_2$, 7.5 mg L^{-1} $FeSO_4{\cdot}7H_2O$, 5 mg L^{-1} $MnSO_4{\cdot}H_2O$, 2.5 mg L^{-1} $B(OH)_3$, 0.5 mg L^{-1} $ZnSO_4{\cdot}7H_2O$, 0.5 mg L^{-1} KI, 0.1 mg L^{-1} $CuSO_4$, 0.05 mg L^{-1} $CoCl_2{\cdot}6H_2O$, 0.05 mg L^{-1} $Na_2MoO_4{\cdot}2H_2O$, 10 mg L^{-1} EDTA·2Na) supplemented with 1.0 g L^{-1} of 2-(N-morpholino)ethanesulfonic acid (MES) as a pH buffer in a growth chamber (28 °C, light intensity of 80 μmol m^{-2} s^{-1}, photoperiod of 16 h/8 h-day/night cycle).

A. magnusonii H3 is a PGPB strain that was originally isolated from the same *L. minor* strain (Ishizawa et al., 2017a). For bacterial cultivation, a loop of an *A. magnusonii* H3 colony was inoculated in 20 mL of liquid LB medium and grown to the late exponential phase with shaking at 120 rpm at 28 °C. Cells were harvested and washed twice with sterile half-strength SH medium prior to plant inoculations.

2.2. Duckweed cultivation and growth evaluation

We developed a compact and high-throughput cultivation system for duckweed that uses polystyrene 6-well plates (VTC-P6, As One, Aichi, Japan). Each well of the 6-well plate was filled with 10 mL of sterile half-strength SH medium supplemented with 1.0 g L^{-1} of MES. Subsequently, four fronds of sterile *L. minor* were transplanted to each well, and the lid of the plate was fixed at 5 mm above the original position to secure air ventilation. Surgical tape (3M, St. Paul, MN) was used to wrap around the gap between the plates and lids. The plates were then incubated in a growth chamber at 28 °C for 7 days. Irradiance was supplied by an LED panel (3LH-256, NKsystem, Osaka, Japan) at an intensity of 70 μmol m^{-2} s^{-1} with a photoperiod of 16 h/8 h-day/night cycle, unless otherwise indicated.

This study evaluated the growth speed of plants by monitoring the frond area of *L. minor* (Fig. 1). For frond area measurement, photographs of the 6-well plates were periodically taken from a static distance and angle (Fig. 1a). Next, the image (.jpeg format) was converted into a Lab color field using ImageJ software v. 1.51n (https://imagej.nih.gov/ij/) (Fig. 1b). As shown in Fig. 1c, the frequency distribution of the a* value, representing green-red color components, exhibited two major peaks representing the pixels of plant and non-plant parts. Thus, selection of plant parts was performed by setting the threshold value to the least frequent point between the peaks (Fig. 1d). Particles smaller than the size of one frond were filtered, and the area (in pixel) of *L. minor* fronds was measured and tallied for each respective well. The growth speed of *L. minor* during the 7-day cultivation was expressed as relative yield (RY; $week^{-1}$) based on frond area by equation (1), in accordance with Ziegler et al. (2014).

$$RY = \text{Frond area at day 7/ Frond area at day 0} \quad (1)$$

To assess the accuracy of the frond area measurement, a linear regression between the frond area and frond number of *L. minor* was assessed for 12 sterile *L. minor* cultures (two 6-well plates) with the above-described procedure. The frond area was analyzed every 24 h during the 7-day cultivations, and the frond number was manually counted from photographs used for the frond area measurements.

2.3. Experimental treatments

Cultivation experiments were performed with and without inoculation of *A. magnusonii* H3 under the presence of six stress factors (copper, zinc, salinity, weak light, low temperature, and nutrient scarcity) using the cultivation system as described above. These six stress factors were selected based on their prevalence and significance in duckweed cultivation (Basiglini et al., 2018; Zhou et al., 2018). The strength of each stress factor was determined by preliminary experiments to decrease the RY of *L. minor* by approximately 40%. Namely, copper, zinc, and salinity stresses were produced by adding 25 μM $CuSO_4$, 75 μM $ZnSO_4$, and 40 mM NaCl to the medium, respectively. For weak light stress, the light intensity was decreased to 20 μmol m^{-2} s^{-1}, and the temperature was adjusted to 22 °C to confer low temperature stress. Poor nutrient conditions were created by using 25-fold diluted half-strength SH medium. The inoculation of *A. magnusonii* H3 was performed by suspending *A. magnusonii* H3 cells in the media at a density of 1×10^6 colony forming units per milliliter. Each treatment was performed with six replicates using one 6-well plate, and three separate experiments were conducted to test all six stress factors. Cultivations without stress treatments were also conducted for each time period as control (nonstress) experiments. After 7 days of cultivation, plant chlorophyll and hydrogen peroxide content were assayed as described below.

2.4. Extraction and quantification of total chlorophyll and hydrogen peroxide

Hydrogen peroxide (H_2O_2) content was assayed as described by Velikova et al. (2000) with modifications made by Ishizawa et al. (2017b). Briefly, 20–30 mg fresh weight of plants were homogenized for 30 s with 1.0 mL of 0.1% trichloroacetic acid using a high-power homogenizer (ASG50, As One, Aichi, Japan), followed by centrifugation (10,000 × *g*, 4 °C, 15 min). The 500 μL of supernatant was mixed with 500 μL of 10 mmol L^{-1} potassium phosphate buffer and 1 mL of 1 mol L^{-1} KI. After incubation at room temperature for more than 60 min, the absorbance at 390 nm was measured with a spectrophotometer (UV-1850, Shimadzu, Kyoto, Japan) and compared with that of known concentrations of H_2O_2.

Extraction of total chlorophyll was performed by soaking 20–30 mg fresh weight of plants in 2 mL of 99.5% methanol. After at least 90 min of extraction, the absorbance at 650 nm (A_{650}) and 665 nm (A_{665}) was measured with a spectrophotometer. The chlorophyll content was determined by the following equation (2) proposed by Grimme and Boardman (1972).

$$\text{Chlorophyll content } (\mu g\ ml^{-1}) = 4.0 \times A_{665} + 25.5 \times A_{650} \quad (2)$$

2.5. Determination of copper and zinc distributions

For the plant cultures treated with copper or zinc stress, contents of the metals (copper or zinc) in plants, culture media, and suspended bacterial cells were analyzed using inductively coupled plasma-atomic emission spectroscopy (ICP-AES; SPS7800, SII NanoTechnology Inc., Chiba, Japan). Plant samples were taken, and the fresh weight was

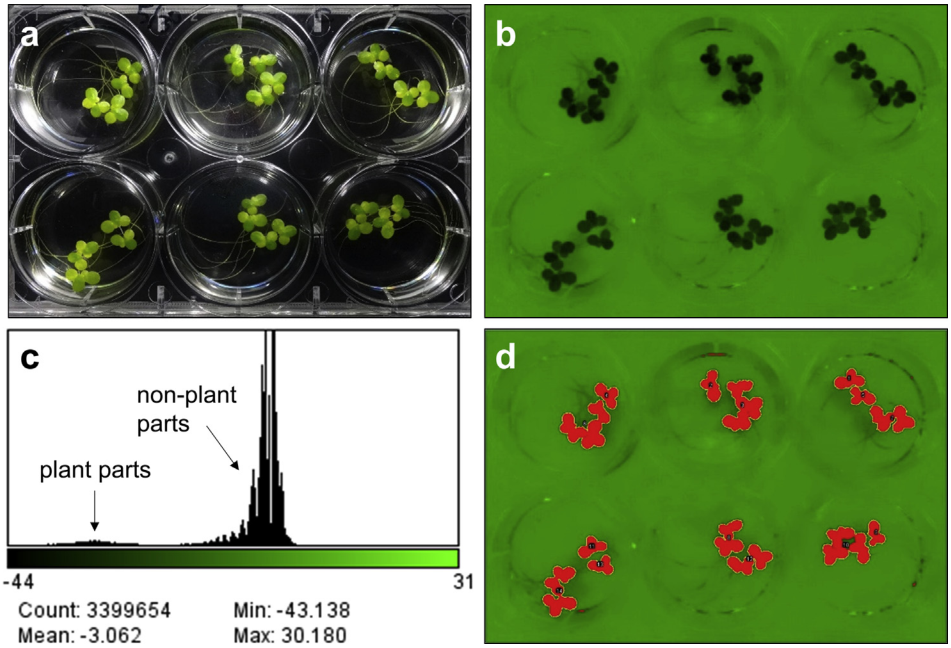

Fig. 1. An example of image processing for duckweed frond area measurement. (a) Raw image. (b) Converted image showing a* value (green-red color component) of Lab color field. (c) Histogram of a* value. (d) Selection of plant parts based on a* thresholding. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

measured after the 7-day cultivations. The remaining culture media were centrifuged at 8500 × *g* for 10 min, and the supernatant was directly analyzed with ICP-AES as media samples. The precipitates were washed twice with sterile 5 mg L^{-1} tripolyphosphate solution and served as bacterial cell samples. Plant and bacterial samples were dried for 24 h in an oven at 100 °C, solubilized in 4 mL of mixed acid (69% HNO_3 and 95% H_2SO_4 (1:1)) at 100 °C for 1 h, and then analyzed with ICP-AES.

2.6. Bacterial growth assays

The effects of each stress factor on the *A. magnusonii* H3 activity were assayed by monitoring cellular growth in LB medium. Precultured cells of *A. magnusonii* H3 were inoculated in 20 mL of LB medium at an optical density of 0.1 at 600 nm (OD_{600}) and incubated at 28 °C with shaking at 120 rpm for 12 h under the presence and absence of the same stress factors as those imposed during plant cultivation (copper, 25 μM $CuSO_4$; zinc, 75 μM $ZnSO_4$; salinity, 40 mM NaCl; and low temperature, 22 °C). The growth curve was generated by measuring the OD_{600} of duplicate cultures at 1-h intervals. The effect of low light intensity was not included due to equipment limitations, and poor nutrient stress was not applicable in this assay.

2.7. Statistical analyses

Student's *t*-test was performed to test the statistical significance of the effects of *A. magnusonii* H3 on *L. minor* growth (n = 6) and heavy metal accumulation (n = 3). For the results of plant chlorophyll content (n = 3) and hydrogen peroxide content (n = 3), significant differences among treatments were confirmed by one-way analysis of variance (ANOVA). Duncan's multiple-range test was further performed to separate the means. Pearson correlation analysis was used to verify the correlation between frond area and frond number of the plants. A significance level of $p < 0.05$ was adopted for all statistical analyses. All statistical analyses were conducted using R software v3.2.3 (http://www.r-project.org).

3. Results

3.1. Validation of culturing and growth evaluation methods

To best take advantage of the small size of duckweeds, we first developed a compact and high-throughput duckweed culturing system combined with a frond area-based growth evaluation method. As depicted in Fig. 1, the image analysis protocol could correctly recognize plant parts and reproducibly calculate plant area in respective wells. In fact, the frond area measured with the protocol linearly correlated to the frond number (Fig. 2). The high correlation coefficient (R^2 = 0.992, $p < 0.001$) indicates that the growth of *L. minor* can be comprehensively monitored by the frond area, as measured using our image analysis protocol. Although frond number and fresh/dry weights are the most common indicators of duckweed biomass, in our small experimental system, those indicators may lead to low accuracy because the amount of plant material in each culture is too low to accurately

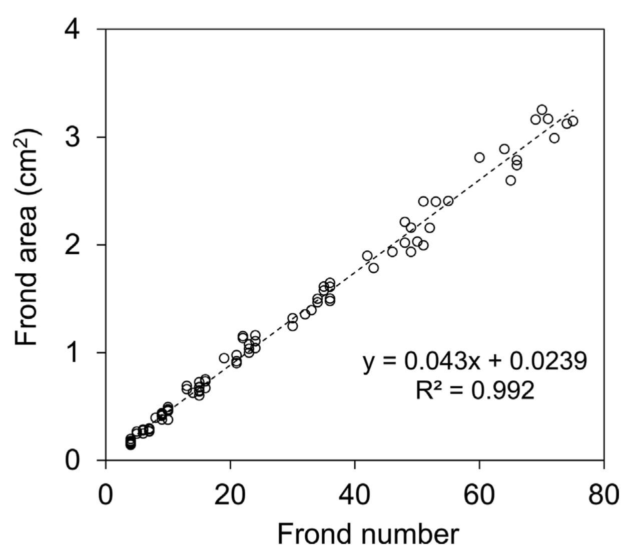

Fig. 2. Linear regression among frond number and frond area of *Lemna minor* during 7-day cultivations (n = 84). Frond area of 1 cm^2 was equivalent to 51205 pixels.

measure fresh/dry weights or to express exact biomass with an integer frond number. However, the frond area can properly estimate the biomass regardless of the small experimental scale and change in individual frond size (Wang et al., 2014). Therefore, we consider the frond area to be an adequate growth indicator for the following experiments.

The cultivation system enabled rapid exponential growth of *L. minor* for a week, even with the small volume of culture media (10 mL). The RY of sterile *L. minor* cultures showed an average value of 16.7, which corresponds to a relative growth rate of 0.402 and a doubling time of 1.72 days. Since these growth rates are similar to or higher than those in previous studies adopting optimized growth conditions for *L. minor* (Ziegler et al., 2014), this cultivation method would have realized a nearly maximum growth speed of *L. minor*. This indicates that there is no specific stress factor under this culture condition without stress treatments.

3.2. Performance of A. magnusonii H3 under various stress conditions

Six stress factors (copper, zinc, salinity, weak light, low temperature, and nutrient scarcity) were applied to the above-mentioned culturing system at moderate strength, and *L. minor* plants were cultured with and without inoculation of strain H3. In sterile cultures, each stress factor reduced duckweed growth to a similar extent (31.2–46.7% in RY) compared to nonstress cultures (Table 1). Under this extent of growth inhibition, only slight chlorotic and necrotic fronds were observed, and plants could still grow continuously. Fig. 3a–c shows the change of *L. minor* growth by inoculation with strain H3 compared with the growth of non-inoculated plants under each of those stress conditions. When inoculated in nonstress cultures, strain H3 consistently promoted the growth of the host by 5.2–8.1%. The extents of growth promotion were smaller than those of previous studies using the same strain (Ishizawa et al., 2017a, b), which is probably due to the higher growth rates of the control plants. A similar extent of growth promotion was also observed under weak light and low temperature conditions. Furthermore, the growth-promoting effects of strain H3 increased to 17.2% and 14.3% when the host plants were subjected to copper and zinc stresses, respectively. However, the effects of strain H3 were inhibitory to *L. minor* growth under high salinity and nutrient-deficient conditions, in which the RY decreased by 6.8% and 12.3%, respectively, compared with nonstress conditions. These results confirmed that the effects of strain H3 on duckweed growth differed depending on the growth-restricting plant stress factor.

3.3. Change in plant H_2O_2 and chlorophyll levels

The plant oxidative stress level is often used to describe the extent of plant damage caused by stress factors. We assayed H_2O_2 content of the plant, one of the most common indicators of oxidative stress (Apel and Hirt, 2004), after 7 days of cultivation. As expected, stress treatments generally led to higher levels of plant H_2O_2 content than those observed in nonstress conditions (Fig. 3d–f). In particular, nutrient-poor and low temperature conditions led to the greatest increases in H_2O_2 content, while weak light stress led to a slight decrease in H_2O_2 content. Although the influence of strain H3 during nonstress conditions was similar to that found in a previous study (Ishizawa et al., 2017b), it seemed to alleviate the increase in H_2O_2 in nutrient-poor and low temperature conditions, under which the H_2O_2 content of control plants (sterile plants) was particularly high. However, a similar effect was not observed under copper and zinc stress conditions, where strain H3 showed greater growth-promoting effects (Fig. 3a). Therefore, the results indicate that the effects of strain H3 on plant H_2O_2 levels are not simply correlated with effects on plant growth.

Chlorophyll content was also determined as an indicator of plant nutritional properties and photosynthetic capacity. Among the six stress factors, poor nutrient stress notably decreased the chlorophyll content of *L. minor*, most likely due to the scarcity of nutrient salts (Fig. 3g–i). However, high salinity and weak light stresses tended to induce higher chlorophyll content compared to nonstress conditions, while the other stress factors did not show significant effects. Although the difference could not be statistically confirmed, strain H3 seemed to improve the plant chlorophyll content under copper and zinc stress conditions where the strain showed greater growth-promoting effects.

Table 1
Effects of six stress factors on the growth of *Lemna minor* in sterile conditions. Relative yield (RY; week^{-1}) and its percent decrease from nonstress cultures are shown.

		RY	% decrease
Nonstress	-	16.7 ± 0.5	–
Copper	25 μM	9.2 ± 0.2	46.7 ± 4.5
Zinc	75 μM	11.0 ± 0.4	36.8 ± 5.1
Salinity	40 mM	9.7 ± 0.7	46.4 ± 4.4
Week light	20 μmol m^{-2} s^{-1}	9.9 ± 0.3	45.1 ± 2.8
Poor nutr.	1/25 dilution	10.1 ± 0.2	31.2 ± 4.3
Low temp.	22°C	8.9 ± 0.3	39.3 ± 4.6

3.4. Copper and zinc contents in plant, culture media, and suspended bacteria

Fig. 4 shows the distribution of copper and zinc after the 7-day cultivations. We found that suspended bacterial cells contained negligible amounts (< 0.03 μg) of these metals, and the majority of copper and zinc was distributed in the plants and culture media. Therefore, bacterial sequestration of heavy metals may not be the reason for improved growth shown in Fig. 3a. Interestingly, inoculation of strain H3 led to a significant ($p < 0.01$) increase in the amounts of copper or zinc accumulated in plants by approximately 1.7 and 1.2 times, respectively. Additionally, the metal concentration per unit plant weights increased in the presence of strain H3 as follows: copper from 0.053 (± 0.003) μg mg^{-1} to 0.071 (± 0.001) μg mg^{-1} ($p < 0.05$) and zinc from 0.388 (± 0.013) μg mg^{-1} to 0.420 (± 0.005) μg mg^{-1} ($p > 0.05$).

3.5. Growth rate of A. magnusonii H3 under stress conditions

The effects of each stress factor on the activity of *A. magnusonii* H3 were assessed by monitoring the OD_{600} in the LB medium. Fig. 5 shows the observed growth curve of strain H3 under the same stress factors as those imposed during plant cultivation. It was found that growth of strain H3 was delayed under high salinity and low temperature stresses, although it reached its highest cell density within 12 h. The growth of strain H3 was not significantly affected by the presence of copper and zinc at the concentrations added to the plant cultures.

4. Discussion

Abiotic stress factors are the principal cause of diminished plant growth in the environment, and they account for more than 50% of worldwide crop loss (Boyer, 1982). Although such stress factors are likely to influence PGPB performance in each environment, studies regarding the tripartite interactions between plant, PGPB, and abiotic stress have only recently begun (Vimal et al., 2017; Kumar and Verma, 2018). In this study, we evaluated the efficacy of *A. magnusonii* H3 in simplified culture conditions, in which plant growth was repressed solely by one of six stress factors. By adjusting the harshness of stress factors to equally repress duckweed growth (approximately −40% in RY; Table 1), our experiments reasonably compared the effects of different kinds of plant stress factors on PGPB performance.

We found that the performance of strain H3 was affected both positively and negatively by these different stress factors. It has long been pointed out that the positive results for PGPB performance obtained

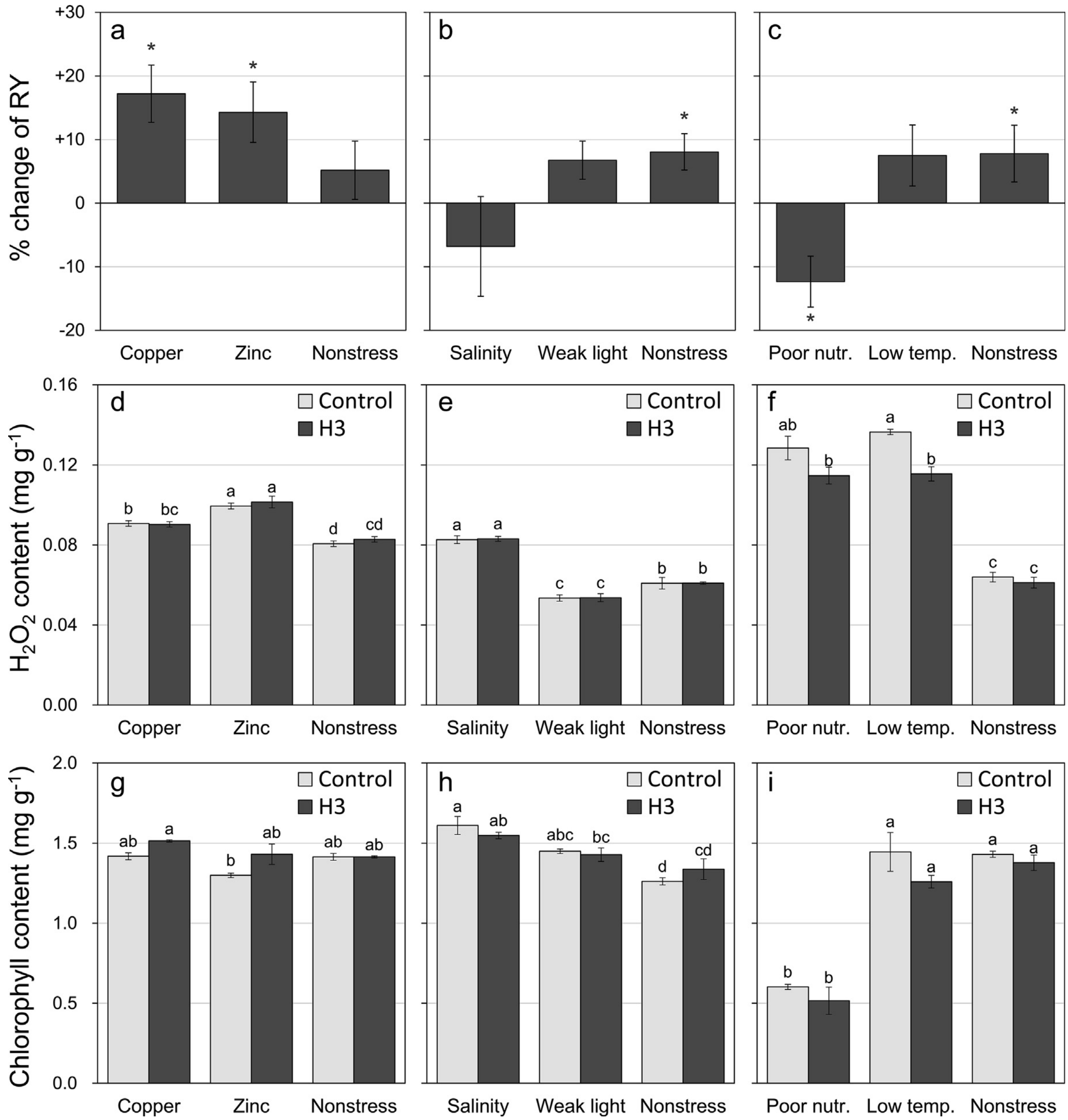

Fig. 3. (a–c) Effects of the inoculation of *Aquitalea magnusonii* H3 on duckweed relative yield (RY; week^{-1}). Percent changes from non-inoculated plants under the indicated stress treatments are shown. (d–f) Changes in duckweed hydrogen peroxide (H_2O_2) content. (g–i) Changes in chlorophyll content of *Lemna minor*. Error bars show the standard errors (n = 6). The values significantly larger or smaller than 0 (*t*-test, $p < 0.05$) are marked with asterisks in (a–c). The values share the same letter indicates no significant differences (ANOVA, $p < 0.05$) in (d–i).

under laboratory conditions are not always reproduced under field conditions (Chanway and Holl, 1994; Bacilio et al., 2017). Here, the presence of an abiotic stress factor is suggested to be one important explanation for such instability in PGPB performance. Moreover, the results also indicate that improved performance of PGPB can be achieved by utilizing the proper PGPB strain corresponding to principal stress factors present in culture conditions.

The PGPB strain H3 showed the greatest growth-promoting effects under heavy metal (copper and zinc) stress (Fig. 3a). Further, it was unexpectedly found that strain H3 increased plant heavy metal accumulation and did not decrease plant oxidative stress levels (Figs. 3d and 4). Therefore, bacterial alleviation of plant heavy metal stress *via* prevention of plant metal uptake, as reported in previous studies (Stout et al., 2010; Tang et al., 2015; Kumar and Verma, 2018), would not explain the observed growth promotion. However, Zhao et al. (2018) recently reported that metabolic activity of duckweed-associated microbial communities positively correlates with host growth and antioxidant enzyme activities, and weakly with plant metal accumulation under heavy metal stress conditions. This can be interpreted as implying that bacterial activity increased the ability of duckweed to both accumulate and tolerate heavy metals, so that plants can rapidly improve their environment. While this study did not directly evaluate plant antioxidant activity, the observed increase in plant metal uptake, as well as improved growth and chlorophyll content, and constant hydrogen peroxide levels, might be based on similar mechanism to Zhao et al. (2018). Further investigation is required to confirm the possibility and mechanism of bacterial function to increase plant heavy metal uptake and tolerance. Considerable efforts have been dedicated to cultivating duckweed in metal-contaminated wastewaters for water remediation and/or biomass production (Uysal and Taner, 2010; Mishra et al., 2013; Sasmaz et al., 2016). Here, our results suggest that strain H3 is especially useful in such cultivation systems.

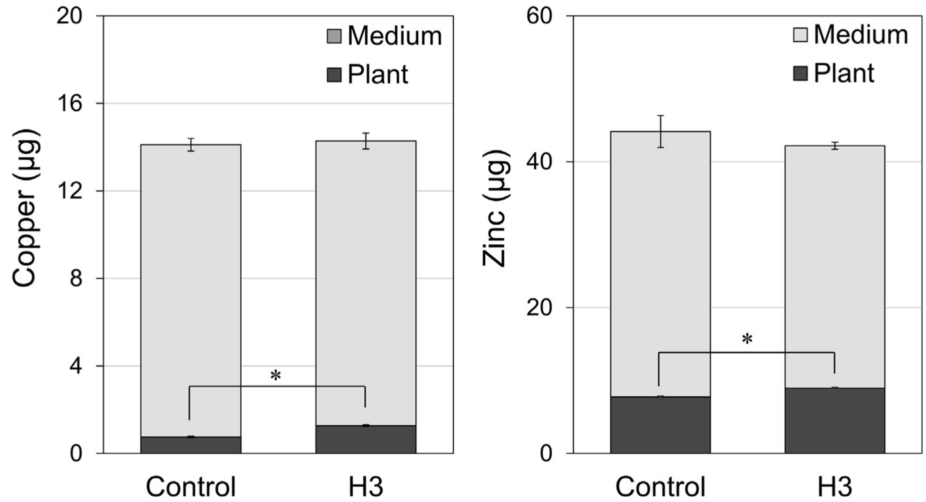

Fig. 4. Distribution of copper and zinc after a 7-day cultivation with and without inoculation of *Aquitalea magnusonii* H3. Error bars show the standard errors (n = 3). Significant differences in the amount of copper or zinc distributed in plant biomass ($p < 0.01$) are labeled with asterisks.

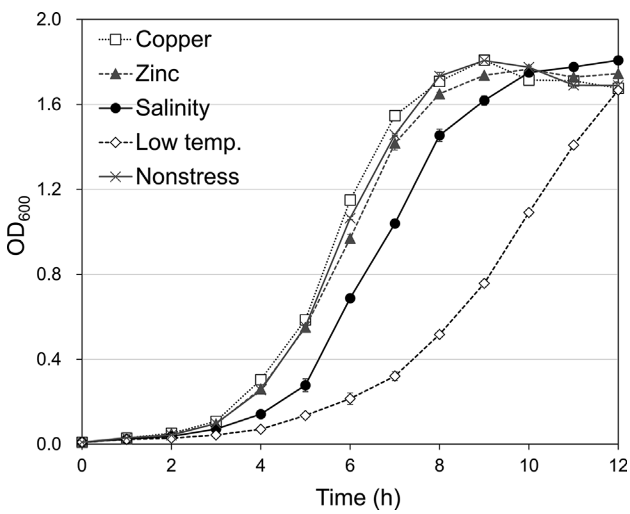

Fig. 5. Growth curves of *Aquitalea magnusonii* H3 in the presence and absence of stress factors. Error bars show the standard errors (n = 2).

However, strain H3 inhibited duckweed growth in high salinity and nutrient-poor conditions (Fig. 3a–c). Since each stress factor had limited adverse effects on strain H3 (Fig. 5), and growth promotion was not impaired under low temperature conditions where the bacterial growth was the lowest (Fig. 3c), diminished activity of strain H3 would not be a key factor in the observed reduction in PGPB performance. Instead, it may be that these stress factors strictly constrained plant growth and consequently masked the beneficial effects of this PGPB. In addition, significant growth inhibition in poor nutrient conditions could be attributed to the nutrient competition between plants and bacteria (Griffiths et al., 1999; De Gregorio et al., 2017).

Although the detailed mechanism of differential PGPB performance under each stress condition is beyond the scope of this research, we sought clues regarding the change in plant H_2O_2 and chlorophyll content. The results showed that strain H3 reduced plant H_2O_2 levels only when the H_2O_2 content increased significantly by stress factors (Fig. 3d–f). This finding partly supports the idea that PGPB functions to alleviate plant oxidative stress in stressful environments (Gururani et al., 2013; Islam et al., 2014). However, growth promotion by strain H3 did not necessarily coincide with a decrease in H_2O_2 content. Therefore, alleviation of oxidative stress could not be the sole mechanism by which PGPB improves plant growth under stressful conditions. Furthermore, no significant difference in chlorophyll content was observed between inoculated and noninoculated plants (Fig. 3g–i). As the majority of these stress factors, other than poor nutrient, reduced plant growth without decreasing chlorophyll content, it might have been difficult to associate chlorophyll content with growth improvements.

In summary, this study is the first to describe how the performance of a PGPB strain varied under a broad spectrum of stress conditions. The newly developed methods of high-throughput duckweed cultivation and growth evaluation can help accelerate further investigations on this topic. Our results demonstrated that notably higher or lower performance of PGPB strains can be induced by different kinds of plant stress factors, which are prevalent in duckweed culture environments. In addition, greater growth promotion by strain H3 under copper and zinc stress conditions indicates the prospect of enhanced PGPB performance in environments accompanied by specific plant stress factors. Future studies will focus on exploring reasonable combinations of PGPB strain and culture environments (e.g., type of wastewaters), toward effective and reliable PGPB application.

Conflict of interest

No conflict of interest declared.

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