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Hyposaline effect on polyamine accumulation in *Ulva fasciata* (Ulvales, Chlorophyta)

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Abstract. This study was conducted to investigate changes of free polyamine levels in response to hyposaline stress in *Ulva fasciata* Delile. Free putrescine, spermidine, and spermine are present in this alga. As salinity decreased from 30‰ to 5‰, specific growth rate (%/d), TTC reduction activity, net photosynthetic O_2 evolution rate and chlorophyll levels decreased. Plants grown at 5‰ bleached except the basal part near rhizoidal portion. Free putrescine maintained at a constant level at salinity over the range of 10~30‰ and increased three fold following 5‰ treatment. Free spermidine also increased when salinity fell below 15‰. In plants grown at 5‰, free putrescine and spermidine accumulation in the basal part was less than in the remaining part. Free spermine levels increased as salinity decreased from 30‰ to 10‰, but dropped sharply at 5‰. Both α-difluoromethylarginine (0.2 mM) and D-arginine (1 mM), inhibitors of arginine decarboxylase (EC 4.1.1.19), and also both α-difluoromethylornithine (0.2 mM) and α-methylornithine (0.2 mM), inhibitors of ornithine decarboxylase (EC 4.1.1.17), inhibited the 5‰-induced free putrescine accumulation. Overall, the present results suggest that an extreme hyposaline condition (5‰) induced a significant accumulation of free putrescine and spermidine in *U. fasciata* and that ADC and ODC each contributed to free putrescine accumulation. The relationships between accumulated putrescine and hyposaline injury are discussed.

Keywords: Hyposaline stress; Putrescine; Spermidine; Spermine; *Ulva fasciata*.

Introduction

Polyamines are involved in the regulation of plant growth and development (Evans and Malmberg, 1989; Galston and Kaur-Sawhney, 1987). In plants, putrescine is synthesized from arginine or ornithine through either arginine decarboxylase (ADC; EC 4.1.1.19) or ornithine decarboxylase (ODC; EC 4.1.1.17), and is then converted to spermidine/spermine by the addition of propylamine group from decarboxylated *S*-adenosylmethionine that is derived from *S*-adenosylmethionine (SAM) by the action of *S*-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50) (Evans and Malmberg, 1989). A variety of studies have shown that polyamines, especially putrescine, accumulate under stresses, and these accumulated polyamines are closely associated with plants' responses to stress (Evans and Malmberg, 1989). It is suggested that, under stresses, polyamines acts as polycations in maintaining membrane and nucleic acid integrity at cellular pH (Galston and Kaur-Sawhney, 1987).

The effects of salinity stress on polyamine biosynthesis were analyzed in several plant systems (Basu et al., 1988; Krishnamurthy and Bhagwat, 1989; Lin and Kao, 1995; Priebe and Jager, 1978), and the pattern of polyamine metabolism in response to salinity stress seems to be dependent on the plant systems and/or duration of exposure to salinity stress. In pea (Anderson and Martin, 1973) and barley (Smith, 1973), neither putrescine nor spermidine accumulate when exposed to increasing salinity. A similar trend was also found in *Vicia faba* (Priebe and Jager, 1978). In the case of *Brassica campestris*, exposure to short-term hypersaline stress increased levels of polyamines and activities of both ADC and ODC, while long term exposure exhibited little effect (Das et al., 1995). Krishnamurthy and Bhagwat (1989) showed that hypersaline conditions elicited an accumulation of putrescine in salt-sensitive rice cultivars and a significant accumulation of spermidine and spermine in salt-tolerant ones. Lin and Kao (1995) showed a contrasting result, finding that increasing NaCl levels lead to a decrease in free putrescine levels but an increase in spermidine levels in a salt-sensitive rice cultivar, cv. Taichung Native 1.

It is well documented that polyamines, especially putrescine and spermidine, exist in prokaryotic and eukaryotic algae (Badini et al., 1994; Hamana et al., 1983, 1990; Hamana and Matsuzaki, 1982, 1985). Uncommon polyamines (norspermidine and norspermine) are also widespread in eukaryotic algae (Hamana and Matsuzaki, 1982), playing a vital role in cell growth and development. In both *Chlorella vulgaris* Beijernck var *vulgaris* fa *vulgaris* (Cohen et al., 1984) and *Euglena gracilis* Z (Villanueva et al., 1980a), a rise of polyamines, especially putrescine derived from ODC, before cell division, is believed to be correlated with DNA replication. Environmental changes also influence the biosynthesis of polyamines in algae.

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Under high light intensity, the polyamine biosynthesis in *Chlorella* was enhanced (Cohen et al., 1984). Exposure to mercury results in an increase of putrescine and 1,3-diaminopropane but a decrease of spermidine, spermine and norspermidine in *Chlorogonium elongatum* (Dang) France, a green microalga (Agrawal et al., 1992). In addition, a rise of culture temperature caused an increase of spermine and/or norspermine in *Cyanidium caldarium*, a unicellular thermo-acidophilic red alga (Hamana et al., 1990).

Salinity fluctuates in intertidal regions, and marine macroalgae from intertidal habitats are believed to develop an ability to resist salinity change (Biebl, 1962; Kirst, 1989). The physiological and biochemical responses to altered salinity have been extensively studied in a large amount of marine algae (Kirst, 1989). Growth rate, photosynthesis, and respiration are influenced by exposure to salinity stress (Kirst, 1989). The control of constant cell turgor by regulating osmotic potentials through the adjustment of ion and/or organic osmolyte levels is a typical tolerance mechanism observed in marine algae (Ben-Amotz and Avron, 1983; Kirst, 1989). A difference in salinity tolerance exists among Chlorophyta, Rhodophyta, and Phaeophyta; Rhodophyta is more tolerant to hyposaline and hypersaline conditions while Chlorophyta and Phaeophyta are sensitive to hyposaline conditions (Kirst, 1989). *Ulva* is a macroscopic marine alga belonging to Chlorophyta and inhabits in the intertidal regions of rocky shores and estuaries. It suffers frequent fluctuations of salinity due to the interaction tides, rainfall, and evaporation. As we know, the responses of polyamine biosynthesis to salinity stress (hyposaline and/or hypersaline conditions) have not been determined in *Ulva.* We have observed that *Ulva fasciata* Delile dominates the intertidal regions at Hsitzu Bay, Kaohsiung, Taiwan, Republic of China (ROC), where its salinity will decrease to a level of 6~11‰ in heavy rain. Therefore, this study was conducted to investigate the hyposaline effects on polyamine biosynthesis in *U. fasciata* Delile. Specific growth rate, net photosynthetic O_2 evolution rate, and 2,3,5-triphenytetrazolium chloride (TTC) reduction activity were used to evaluate the tolerance to decreasing salinity from 30‰ to 5‰. To know the route of putrescine synthesis in *U. fasciata* exposed to hyposaline conditions, both α-difluoromethylarginine (DFMA, 0.2 mM) and D-arginine (1 mM), inhibitors of arginine decarboxylase (ADC, EC 4.1.1.19), and also both α-difluoromethylornithine (DFMO, 0.2 mM) and α-methylornithine (0.2 mM), inhibitors of ornithine decarboxylase (ODC, EC 4.1.1.17), were used.

Materials and Methods

Plant Materials and Treatments

Ulva fasciata was collected in May 1997 from the high intertidal region at Hsitzu Bay, Kaohsiung, Taiwan, Republic of China (ROC), where salinity levels range from 32 to 46‰, temperature from 21 to 26°C, and pH from 7.8 to 8.4. After harvesting, whole plants with the rhizoidal portion were extensively washed with autoclaved natural seawater. Plants with a similar height ranging from 18–23 cm (the highest thallus) and approximately 1.0 gram fresh weight (about 0.2 gram dry weight) per plant were used in this study. For salinity treatment, plants were preincubated in autoclaved natural seawater of 30‰ salinity (adjusted by NaCl and distilled water, pH 7.8) overnight (14–20 h) in darkness at 25°C. We found that this preincubation did not affect TTC reduction activity. Besides, as evaluated by the net photosynthetic O_2 evolution rate, the algae could maintain a healthy status for 4 days. On exposure to various salinity ranging from 5‰ to 30‰, a plant was cultured in a polycarbonate vessel (Magenta GA-7 vessel, Sigma, St. Louis, MO., USA) containing 300 mL natural seawater (pH 7.8) enriched with full strength Provasoli nutrient solution (Provasoli, 1968) in a growth chamber (25±1°C) for 4 days. An inorganic carbon source was provided by applying $NaHCO_3$ to a final concentration of 3 mM. Salinity, ranging from 5‰ to 30‰, was adjusted by adding distilled water or NaCl (Sigma, St. Louis, MO., USA) in natural seawater also taken from Hsitzu Bay, Kaohsiung. The photoperiod was 12 h : 12 h and the photon irradiance (400–700 nm) was 300 μmol photon m^{-2} s^{-1} in the absence of algae, achieved by a mixture of cool-fluorescent (FL40D, 40W, China Electric Apparatus Ltd., Tao-yuan, Taiwan, ROC) and incandescent (I60, 60W, China Electric Apparatus Ltd., Tao-yuan, Taiwan, ROC) light. Seawater was changed every day.

2,3,5-Triphenyltetrazolium Chloride (TTC) Viability Assay

TTC assay, a viability test for plants, was modified according to Steponkus and Lanphear (1967). The thallus tip, a distal end from the rhizoidal portion, of about 2.5 cm long was cut into strips of about 2 mm wide, immersed in TTC incubation solution (0.8% TTC, 50 mM sodium phosphate, pH 7.4), and incubated in darkness at 25°C. Because TTC reduction is sensitive to excessive oxygen (Altman, 1970), the incubation of TTC by *Ulva in vivo* was carried out without shaking. The ratio of thallus fresh weight to incubation solution volume was 0.1 gram fresh weight: 2.0 mL. The reduced TTC, formazan, was extracted twice by 95% ethanol (5 mL each time), and extracts were combined to make up 10 mL. It is known that, if green tissues are used, formazan is measured at 530 nm instead of 485 nm to avoid pigment interference (Steponkus and Lanphear, 1967). In *Ulva* protoplasts, the ethanol-extracted formazan was detected at 520 nm (Badini et al., 1994). Since our unpublished data showed that the ethanol extract from *Ulva* without TTC incubation had a high absorbency at 485 nm, formazan extracted from *Ulva* was measured at 530 nm in this study.

Net Photosynthetic O_2 Evolution Rate Determination

Net photosynthetic O_2 evolution rate was measured by detecting the amounts of released oxygen by *Ulva* strips

using a Clark-type oxygen electrode fitted with a DW3 chamber (Hansatech, Kings Lynn, Norflok, England) thermostated at 25°C. An *Ulva* strip of 50 mg fresh weight (about 2.5 cm long) was cut from a tip at the distal end of the rhizoidal portion and held with a nylon hook in a 20 mL square section reaction vessel filled with 15 mL artificial seawater (pH 7.8). The strip was incubated in darkness till no O_2 evolution and then photosynthetic O_2 evolution was started by illumination. The photon irradiance was 300 μmol m^{-2} s^{-1} provided by low voltage Tungsten halogen lamps (12V, 50W, Sylvania, Japan). Three replicates per salinity treatment were measured.

Specific Growth Rate Determination

After lyophilization, the initial dry weight of each plant was determined as W_0. Plants employed in the determination of growth rate had an average dry weight (DW) of 0.33 g DW per plant (0.31–0.35 g DW per plant). When exposed to various salinity levels for 4 days, each plant was lyophilized and weighed as a value of W_4. The specific growth rate was expressed as a percentage of dry weight increase per day (%/d = $100\times((\ln(W_4/W_0))/4\ d)$). The growth rate was the average of three replicates.

Free Polyamine Determination

Levels of free polyamines were determined by a BIO-RAD HPLC system (BIO-RAD Laboratories, Hercules, CA, USA) according to Lee and Chu (1992). The polyamine levels were the average of three replicates.

Statistical Analysis

The statistical analysis was performed by using the 95% confidence interval and Duncan's multiple range test from significant ANOVA test (SAS for DOS, Version 6.03, SAS Ltd., NC, USA).

Chemicals

Putrescine, spermidine, spermine, D-arginine and α-methylornithine were purchased from Sigma (St. Louis, MO, USA) as a hydrochloride form. Other chemicals were obtained from Sigma (St. Louis, MO, USA) or Merck (Darmstadt, Germany). DFMA and DFMO were kindly provided by Dr P.P. McCann (Merrill-Dow Research Center, Cincinnati, OH, USA).

Results

Specific Growth Rate, TTC Reduction Activity, and Net Photosynthetic O_2 Evolution Rate

Changes in specific growth rate, TTC reduction activity, and net photosynthetic O_2 evolution rate in response to decreasing salinity are shown in Figure 1. Specific growth rate decreased with decreasing salinity (ANOVA, F=68.47, p=0.0001) and even fell below zero when exposed to 5‰ for 4 days. Both TTC reduction activity (F=24.40, p=0.0001) and net photosynthetic O_2 evolution rate (F=4.60, p=0.0389) decreased as salinity decreased.

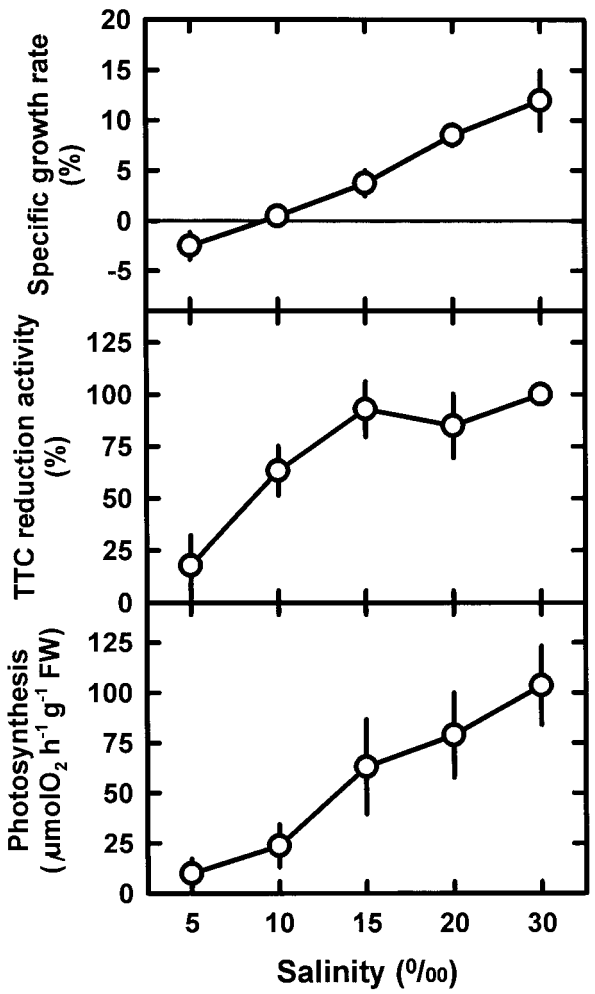

Figure 1. Effects of decreasing salinity on specific growth rate, TTC reduction activity and net photosynthetic O_2 evolution rate. Means ± 95% confidence interval (n=5×(3–1)) are indicated.

As shown in Figure 2A, thalli exposed to 10‰ appeared slightly bleached and those exposed to 5‰ appeared bleached except for the basal parts near the rhizoidal portions. The base of 5‰-grown plants, about 3 cm long near the rhizoidal portion, was green after 4 days of treatment and remained healthy after recovery to 30‰. Figure 2B shows that the decline in salinity decreased total chlorophyll levels (F=30.82, p=0.0001) while a significant decline occurred at 5‰ (Figure 2B).

Free Polyamine Levels

The HPLC profile of polyamines is shown in Figure 3. Putrescine, spermidine and spermine existed in both in 5‰ extract. This identification was also repeated in 30‰ extract as well (data not shown). Compared to 30‰ extract, the putrescine peak was relatively high in 5‰ extract.

Figure 4 shows that a decrease in salinity affected levels of free putrescine (F=11.37, p=0.0069), spermidine (F=11.20, p=0.017), and spermine (F=10.05, p=0.0062). When exposed to decreasing salinity, levels of free putrescine were similar among 10, 15, 20 and 30‰ and sig-

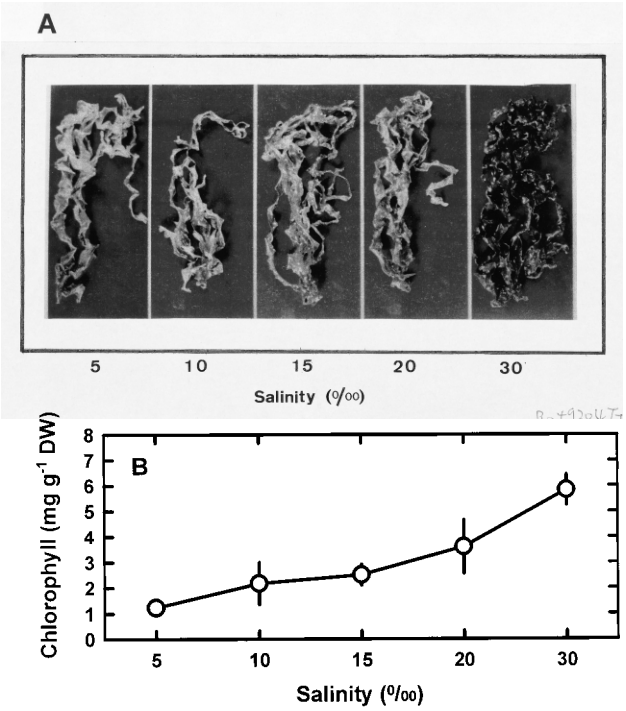

Figure 2. Appearance (A) and chlorophyll levels (Means ± 95% confidence interval (n=5×(3–1)) (B) in *Ulva fasciata* exposed to various salinity for 4 days.

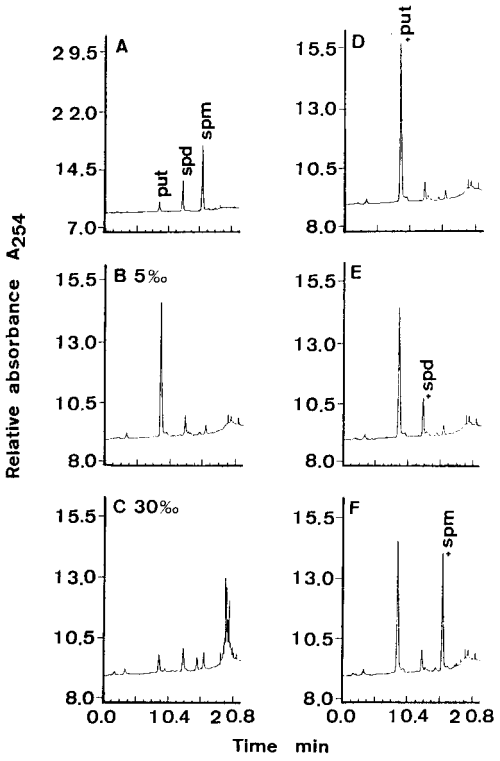

Figure 3. HPLC profile of polyamines in *Ulva* exposed to 5‰ and 30‰. A, standard (1 nmole each); B, 30‰ sample; C, 5‰ sample; D, 5‰ sample + 2 nmole putrescine; E, 5‰ sample + 1 nmole spermidine; F, 5‰ sample + 1 nmole spermine. Putrescine, spermidine and spermine in 15 µL injected were 0.55, 0.26 and 0.08 nmole, respectively, for 30‰ sample, and 4.66, 0.23 and 0.05 nmole, respectively, for 5‰ sample.

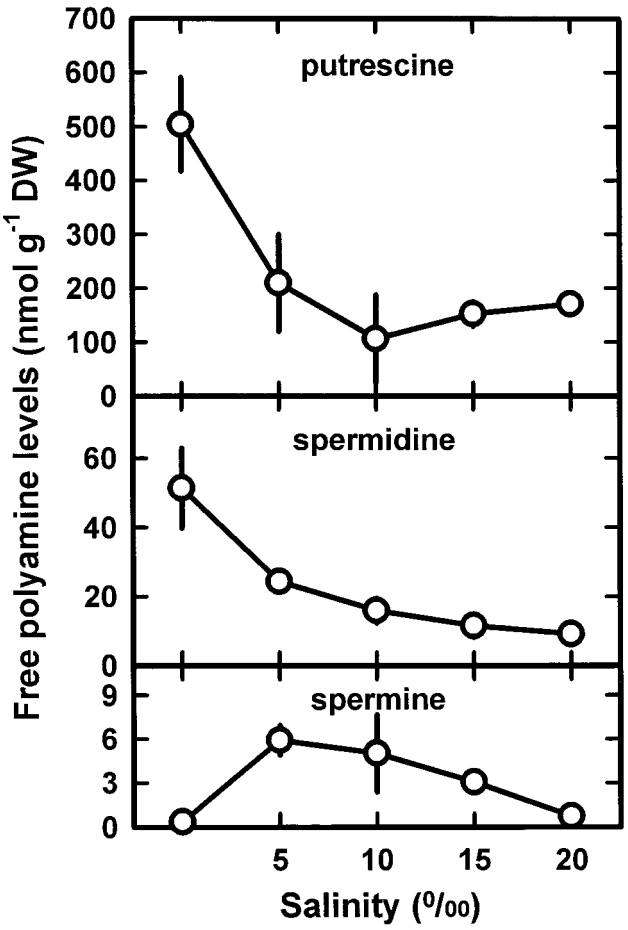

Figure 4. Levels of free polyamines in response to various salinity. Means ± 95% confidence interval (n=5×(3–1)) are indicated.

nificantly increased to three times the level of the 30‰ control at 5‰ (Figure 4). Free spermidine was similar in the 30 and 20‰ treatments and rose when salinity decreased below 15‰, with the extent of spermidine accumulation was in proportion to the extent of salinity decrease (Figure 4). Free spermine levels increased gradually with decreasing salinity from 30 to 10‰, but a sudden decrease was observed at 5‰ (Figure 4).

Responses of Basal and Remaining Parts to 5‰

Since the basal part near the rhizoidal portion is more resistant to hyposaline conditions than the remaining part, the physiological status between the basal and the remaining parts of a single thallus was compared. It was found that at 30‰ the basal part exhibited less TTC reduction activity than the remaining part while total chlorophyll levels were similar between the two parts (Figure 5). When exposed to 5‰, both TTC reduction activity and total chlorophyll levels in both parts significantly decreased; a tremendous decline was observed in the remaining part (Figure 5).

As shown in Figure 6, levels of both free putrescine and spermidine at 30‰ were lower in the basal part than

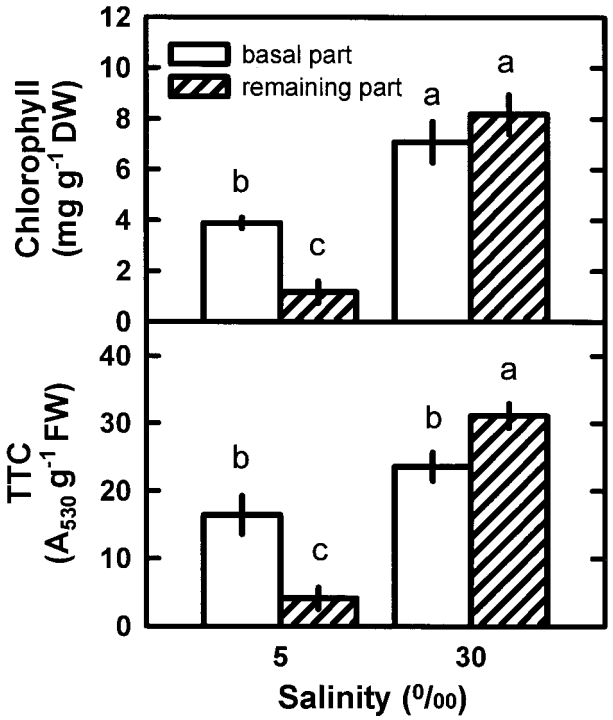

Figure 5. Changes of TTC reduction activity (A) and total chlorophyll levels (B) in the basal and the remaining parts of a single thallus in response to 5‰ and 30‰. Means ± 95% confidence interval (n=5×(3–1)) are indicated and different symbols indicate a significant difference at $p<0.05$.

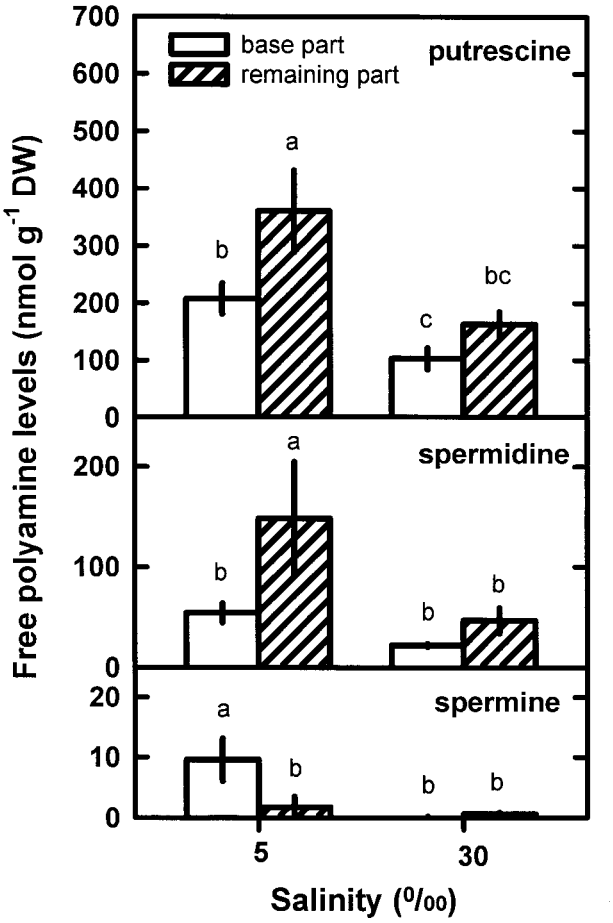

Figure 6. Changes of free polyamines in the basal and the remaining parts of a single thallus in response to 5‰ and 30‰. Means ± 95% confidence interval (n=5×(3–1)) are indicated and different symbols indicate a significant difference at $p<0.05$.

in the remaining part, and the magnitude of 5‰-induced accumulation of free putrescine or spermidine was also. Although a smaller difference was found in free spermine levels between the basal and the remaining parts, they were higher in the basal part than in the remaining part (Figure 6).

Effects of DFMA, D-Arginine, DFMO, α-Methylornithine on Free Polyamine Levels

The effects of putrescine biosynthetic inhibitors on free putrescine levels are shown in Figure 7. At 30‰, DFMO or α-methylornithine exhibited a more effective inhibition on free putrescine synthesis than DFMA or D-arginine, although all of them exerted an inhibitory effect on putrescine synthesis. At 5‰, both DFMA (0.2 mM) and D-arginine (D-Arg, 1 mM), as well as both DFMO (0.2 mM) and α-methylornithine (α-MO, 0.2 mM), inhibited free putrescine accumulation.

Discussion

This study shows that free putrescine, spermidine, and spermine exist in *U. fasciata*; putrescine and spermidine are predominant. It is coincident with the results shown by Hamana and Matsuzaki (1982, 1985) that putrescine and spermidine are abundant in eukaryotic algae while spermine is found in trace amounts. Badini et al. (1994) also showed that putrescine, sermidine and spermine are

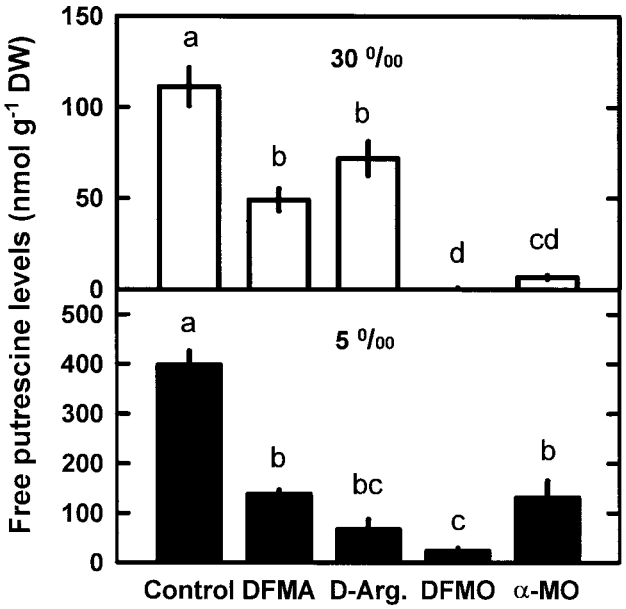

Figure 7. Effects of DFMA (0.2 mM), D-arginine (1 mM), DFMO (0.2 mM) and α-methylornithine (0.2 mM) on free polyamine levels at 5‰ or 30‰. Means ± 95% confidence interval (n=5×(3–1)) are indicated and different symbols indicate a significant difference at $p<0.05$.

present in *Ulva rigida*, with putrescine being the most abundant.

To our knowledge, this is the first report on changes in levels of free polyamines in *U. fasciata* exposed to hyposaline stress. In this macroalga, there was a profound accumulation of free putrescine and spermidine in response to an extreme hyposaline condition (5‰). Levels of both free putrescine and spermidine were not statistically significant at salinities of 10, 15, 20 and 30‰. However, the tetra amine, spermine, showed a contrasting trend, increasing as salinity decreased but leveled off sharply at 5‰. Terrestrial plants have diverse responses in polyamine biosynthesis to high saline conditions; some systems show an increase of polyamine levels while some show less response or even a decrease of polyamine levels (Basu et al., 1988; Krishnamurthy and Bhagwat, 1989; Lin and Kao, 1995; Priebe and Jager, 1978).

In algae, putrescine could be synthesized through ADC and ODC pathways (Cohen et al., 1983; Villanueva et al., 1980a). ADC is generally associated with stress-induced putrescine increase while ODC links to cell division (Evans and Malmberg, 1989). In this study, the inhibition of free putrescine synthesis by biosynthetic inhibitors (DFMA, D-arginine, DFMO and α-methylornithine) shows that ADC and ODC are operating in *U. fasciata* exposed to either 5‰ or 30‰. However, a more effective inhibition on putrescine synthesis by ODC inhibitors (DFMO and α-methylornithine) than ADC inhibitors (DFMA and D-arginine) at 30‰ indicates a relatively important role of ODC in putrescine synthesis under normal saline conditions. An equal effect of ADC and ODC inhibitors on the 5‰-induced putrescine accumulation suggests that both ADC and ODC contribute to putrescine synthesis at 5‰. In the case of *Chlorella*, the cell division-associated putrescine is considered to be derived from ODC (Cohen et al., 1984). In heterotrophically grown *Euglena*, ODC activity instead of ADC activity was detected (Aleksijevic et al., 1979).

A sudden increase of both free putrescine and spermidine and a drop off in free spermine at 5‰ suggest that an extreme hyposaline condition inhibits the conversion from putrescine/spermidine to spermine. This may be also a factor leading to the accumulation of both free putrescine and spermidine in response to 5‰.

This study suggests that putrescine might be related to hyposaline injury in *U. fasciata*. The basal part, which was alive after 4 days at 5‰, accumulated free putrescine to a lesser extent compared with the remaining part, which had already died. In the case of rice, the excessive accumulated putrescine in response to hypersaline conditions has been considered to be a toxic response to salt stress (Krishnamurthy and Bhagwat, 1989). However, although current data show an adverse relationship between free putrescine accumulation and hyposaline tolerance, it can not be concluded that putrescine and spermidine are negative factors in the control of tolerance of *U. fasciata* to hyposaline conditions. The rationale of relationships between accumulated putrescine and hyposaline injury has now been undertaken by employing biosynthetic inhibitors in combination with exogenously applied polyamines in hopes of unveiling the mechanisms conferring salinity tolerance on *U. fasciata*.

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Literature Cited

Agrawal, S.B., M. Agrawal, E.H. Lee, G.F. Kramer, and P. Pillai. 1992. Changes in polyamine and glutathione contents of a green alga, *Chlorogonium elongatum* (Dang) France exposed to mercury. Environ. Exp. Bot. **32:** 145–151.

Aleksijevic, A., J. Grove, and F. Schuber. 1979. Studies on polyamine biosynthesis in *Euglena gracilis*. Biochim. Biophys. Acta **565:** 199–207.

Altman, F.P. 1970. On the oxygen-sensitivity of various tetrazolium salts. Histochemie **22:** 256–261.

Anderson, J.N. and R.O. Martin. 1973. Identification of cadaverine in *Pisum sativum*. Phytochemistry **12:** 443–446.

Badini, L., R. Pistocchi, and N. Bagni. 1994. Polyamine transport in the seaweed *Ulva rigida* (Chlorophyta). J. Phycol. **30:** 599–605.

Basu, R., N. Maritra, and B. Ghosh. 1988. Salinity results in polyamine accumulation in early rice (*Oryza sativa* L.) seedlings. Aust. J. Plant Physiol. **15:** 777–786.

Ben-Amotz, A. and M. Avron. 1983. Accumulation of metabolites by halotolerant algae and its industrial potential. Annu. Rev. Microbiol. **37:** 95–119.

Biebl, R. 1962. Seaweeds. *In* R.A. Lewin (ed.), Physiology and Biochemistry of Algae. Academic Press, New York, pp. 799–815.

Cohen, E., S. Arad, Y.H. Heimer, and Y. Mizrahi. 1983. Polyamine biosynthetic enzymes in *Chlorella*: characterization of ornithine and arginine decarboxylase. Plant Cell Physiol. **24:** 1003–1010.

Cohen, E., S. Arad, Y.H. Heimer, and Y. Mizrahi. 1984. Polyamine biosynthetic enzymes in the cell cycle of *Chlorella*. Plant Physiol. **74:** 385–388.

Das, S., A. Bose, and B. Ghosh. 1995. Effects of salt stress on polyamine metabloism in *Brassica campestris*. Phytochemistry **39:** 283–285.

Evans, P.T. and R.L. Malmberg. 1989. Do polyamines have roles in plant development? Annu. Rev. Plant Physiol. Plant Mol. Biol. **40:** 235–269.

Galston, A.W. and R. Kaur-Sawhney. 1987. Polyamines as endogenous growth regulator. *In* P. J. Davies (ed.), Plant Hormones and Their Role in Plant Growth and Development, Martinus Nijhoff, Boston, pp. 208–295.

Hamana, K. and S. Matsuzaki. 1982. Widespread occurrence of norspermidine and norspermine in eukaryotic algae. J. Biochem. **91:** 1321–1328.

Hamana, K. and S. Matsuzaki. 1985. Further study on polyamines in primitive unicellular eukaryotic algae. J. Biochem. **97:** 1311–1315.

Hamana, K., K. Miyagawa, and S. Matsuzaki. 1983. Occurrence of sym-homospermidine as the major polyamine in nitrogen-fixing cyanobacteria. Biochem. Biophys. Res. Commun. **112:** 606–613.

Hamana, K., S. Matsuzaki, M. Niitsu, K. Samejima, and H. Nagashima. 1990. Polyamines of unicellular thermoacidophilic red alga *Cyanidium caldarium*. Phytochemistry **29:** 377–380.

Kirst, G.O. 1989. Salinity tolerance of eukaryotic marine algae. Annu. Rev. Plant Physiol. Plant Mol. Biol. **40:** 21–53.

Krishnamurthy, R. 1991. Amelioration of salinity effect in salt tolerant rice (*Oryza sativa* L.) by foliar application of putrescine. Plant Cell Physiol. **32:** 699–703.

Krishnamurthy, R. and K.A. Bhagwat. 1989. Polyamines as modulator of salt tolerance in rice cultivars. Plant Physiol. **91:** 500–504.

Lee, T.M. and C. Chu. 1992. Ethylene-induced polyamine accumulation in rice (*Oryza sativa* L.) coleoptiles. Plant Physiol. **100:** 238–245.

Lin, C.C. and C.H. Kao. 1995. Levels of endogenous polyamines and NaCl inhibited growth of rice seedlings. Plant Growth Regul. **17:** 15–20.

Priebe, A. and H.-J. Jager. 1978. Effect of NaCl on the levels of putrescine and related polyamines in plants differing in salt tolerance. Plant Sci. Lett. **12:** 365–369.

Provasoli, L. 1968. Media and prospects for the cultivation of marine algae. *In* A. Watanable and A. Hattori (eds.), Cultures and Collections of Algae. Proceeding of the U.S., Japan Conference, Hakone, Japanese Society of Plant Physiology 1968, pp. 63–75.

Smith, T.A. 1973. Amine levels in mineral deficient *Hordeum vulgaris* leaves. Phytochemistry **12:** 2093–2100.

Steponkus, P.L. and O.F. Lanphear. 1967. Refinement of the triphenyl tetrazolium chloride method of determining cold injury. Plant Physiol. **42:** 1423–1426.

Villanueva, V.R., R.C. Adlakha, and R. Calvayrac. 1980a. Biosynthesis of polyamines in *Euglena gracilis*. Phychemistry **19:** 787–790.

Villanueva, V.R., R.C. Adlakha, and R. Calvayrac. 1980b. Cell cycle related changes in polyamine content in *Euglena*. Phychemistry **19:** 962–964.