

Polyamine analysis of unicellular, colonial,
and multicellular green algae
—Detection of aminobutylcadaverine, N^1 -aminopentylspermidine,
 N^8 -aminopentylspermidine, and penta-amines—

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To obtain the relation between cellular polyamine distribution profiles and multicellular evolution in green algae, we acid-extracted polyamines from 59 additional unicellular, colonial and multicellular green-algal samples (52 species), newly analyzed them with HPLC and HPGC, and compared them with 124 previously analyzed green algal polyamine profiles. Tetra-amines, norspermine and/or spermine, which are distributed as major polyamines predominantly in multicellular, macro green algae, were found also in unicellular photobiontic *Trebouxia* species and endosymbiotic *Chlorella variabilis* (Trebouxiophyceae), respectively. In the class Chlorophyceae, colonial freshwater *Volvox*, *Pleodorina*, *Eudorina*, *Yamagishiella*, *Pandorina*, *Tetrabaena* and *Gonium* (Volvocales) always contain putrescine, norspermidine and spermidine as major polyamines, similar to unicellular *Chlamydomonas* and *Haematococcus*, indicating that there is no correlation between their colony-forming and polyamine profiles. A novel triamine, aminobutylcadaverine, homospermidine and homospermine were all detected as minor polyamines in another colonial alga, *Westella* (Sphaeropleales). Furthermore, the aminobutylcadaverine level increased, and novel tetra-amines N^1 -aminopentylspermidine and N^8 -aminopentylspermidine appeared in a culture supplemented with 1 mM cadaverine. The occurrence of thermospermine was limited in multicellular thallic freshwater *Prasiola* (Trebouxiophyceae) and multicellular thallic marine *Ulva* (Ulvophyceae). Caldopentamine, homocaldopentamine and/or thermopentamine were distributed as minor polyamines in unicellular coenocytic *Codium*, multicellular branched-filamentous *Aegagropila*, thallic *Monostroma* (Ulvophyceae) and filamentous *Spirogyra* (Zygnematophyceae) in addition to *Prasiola* and *Ulva*, suggesting that the occurrence of penta-amines is related to multicellular and macro green-algal evolution.

Key words: aminobutylcadaverine, aminopentylspermidine, green alga, penta-amine, polyamine, thermospermine

INTRODUCTION

We have analyzed various eukaryotic algae to reveal the phylogenetic significance of polyamine distribution profiles in varieties of unicellular algae by secondary and tertiary symbioses and their evolution into multicellular forms (Hamana, 2008; Hamana & Matsuzaki, 1982, 1985; Hamana & Niitsu, 2006; Hamana *et al.*, 1988, 1990, 2004a, 2004b, 2013, 2016b, 2017). Within the 124 previously analyzed

green algal samples belonging to the two phyla Chlorophyta (divided into four classes Trebouxiophyceae, Chlorophyceae, Prasinophyceae, and Ulvophyceae) and Streptophyta (divided into six classes Mesostigmatophyceae, Chlorokybophyceae, Klebsormidiophyceae, Zygnematophyceae, Coleochaetophyceae, and Charophyceae) (Inouye, 2007; Finet *et al.*, 2010; Leliaert *et al.*, 2012), 1,3-diaminopropane (3) (numeric codes of polyamines abbreviated as the number of methylene (CH₂) groups between amino (NH₂) or imino (NH) groups), putrescine (4), cadaverine (5), spermidine (34), norspermidine (33), homospermidine (44), norspermine (333), spermine (343) and thermospermine (334) were wide-

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ly distributed (Hamana & Matsuzaki, 1982; Hamana *et al.*, 1988, 2004a, 2013). Non-photosynthetic achlorophyllous heterotrophic green algae *Polytoma*, *Polytomella*, *Prototheca*, and *Helicospiridium* belonging to the class Trebouxiophyceae or Chlorophyceae contained putrescine, norspermidine and spermidine (Hamana *et al.*, 2004a). In the multicellular terrestrial green algae, such as *Nitella* and *Chara*, belonging to the class Charophyceae, two unusual tetra-amines, aminopropylhomospermidine (344) and canavamine (434), were found in addition to the common diamines, triamines and tetra-amines (Hamana *et al.*, 2013).

Outside of the 124 previously analyzed green algal samples, we selected *Trebouxia* species isolated as a photobiont (photosynthetic symbiont) in lichen (Ahmadjian, 1960; Inouye, 2007) and endosymbiotic *Chlorella variabilis* isolated from the ciliate *Paramecium bursaria* (Hoshina *et al.*, 2004; Minaeva & Ermilova, 2017). Multicellular freshwater *Prasiola japonica* with membranous thalli belongs to the class Trebouxiophyceae. Multicellular seaweeds, such as thallic (foliose) *Monostroma* and *Ulva*, branched filamentous *Chaetomorpha* and *Caulerpa*, and unicellular coenocytic *Codium* and *Halimeda*, belong to the class Ulvophyceae (Lewis & McCourt, 2004). Multicellular branched-filamentous freshwater *Aegagropila* and marine *Valonia aegagropila* forming bubble colonies with large spherical single coenocytic cells are also found in this class. *Gonium*, *Tetrabaena*, *Pandorina*, *Yamagishiella*, *Eudorina*, *Pleodorina* and *Volvox* are the genera of colony (coenobium)-forming microalgae, as the members of the order Volvocales (Chlamydomonadales) of the class Chlorophyceae were evolved from a single primitive *Chlamydomonas*-like alga (Arakaki *et al.*, 2013; Herron *et al.*, 2009; Inouye, 2007; Lewis & McCourt, 2004; Nozaki *et al.*, 2000). *Westella* and *Pediastrum* (Sphaeropleales) are colony-forming (colonial) genera, and *Stigeoclonium* (Chaetophorales) and *Oedogonium* (Oedogoniales) form branched-filament and linear-filament, respectively, in the class Chlorophyceae (Inouye, 2007; Lewis & McCourt, 2004).

To reveal the polyamine components related to multicellular morphological development and colony-forming in addition to their phylogenetic locations, here we newly analyzed polyamines of 59 additional green-algal samples (52 species) including the above-mentioned green algae by employing a high-perfor-

mance gas chromatography (HPGC) technique developed with a long capillary column and a high-performance liquid chromatography (HPLC) method advantageous for minor polyamine analysis (Hamana *et al.*, 2016a, 2016b; Niitsu *et al.*, 2014).

MATERIALS AND METHODS

The green microalgae strains supplied by the Microbial Culture Collection at the National Institute for Environmental Studies (MCC-NIES) and the Biological Resource Center, National Institute of Technology and Evaluation (NBRC) were cultivated phototrophically in the light (10–14 h/24 h) at 20–25°C using 1–10 l of the liquid media designed by MCC-NIES (<http://mcc.nies.go.jp>, 2017) and NBRC (<https://www.nite.go.jp/en/nbrc>, 2017), respectively. DBT microalgal strains were purchased from the Department of Biotechnology, Institute of Environmental Biology Co. (DBT) and cultured phototrophically in the media (1–1.5 l) designed by DBT. In the additional mass culture of three unicellular algae, the medium was supplemented with 1 mM cadaverine (cadaverine 2HCl, Sigma, USA). The dried powders of *Dunaliella* DIC strain (DIC LIFETEC Co.), Yaeyama *Chlorella* strain (Yaeyama Shokusan Co.), Sun *Chlorella* strain (Sun *Chlorella* Co.), nichie strain (nichie Co.), ORIHIRO strain (ORIHIRO Co.) of *Chlorella*, and *Chlorella* Industry (Chikugo) strain (*Chlorella* Industry Co.) of *Parachlorella* are commercially available in Japan as food supplements. The freshwater macroalga “Kawa-nori” (*Prasiola japonica*) was kindly supplied by Kuwaya Co., Minakami (cultured in the Tone River) and Misato-kan of Tange Spa, Nakanojo (collected in the Tange River), and collected by Hamana, K. with the support of Shimonita Shizenshikan in the Aokura River, Shimonita, Gunma, Japan. The marine green macroalgae “Hitoegusa” (*Monostroma nitidum*), “Ana-aosa” (*Ulva perusa*), “Suji-aonori” (*Ulva prolifera*) and “Kubirezuta” (*Caulerpa lentillifera*), and the freshwater green macroalga “Marimo” (*Aegagropila linnaei*) were purchased from the markets in their habitats in Japan (Table 1). “Aomidoro” (*Spirogyra* sp.) were collected from paddy fields in Gunma. Marine “Futo-juzumo” (*Chaetomorpha spirales*), “Tama-baronia” (*Valonia aegagropila*) and “Uchiwa-sabotengusa” (*Halimeda discoidea*), and freshwater “Hime-furasukomo” (*Nitella flexilis*) were purchased from aquarium markets in Chiba, Gunma and Saitama, respectively.

The seaweeds “Miru” (*Codium fragile*) and “Nagami” (*Codium cylindricum*) were supplied by Mie Prefecture Fisheries Research Institute, Mie, Japan.

Microalgae (1–10 g wet weight) harvested in our laboratory in the early stationary phase, dehydrated microalgae and macroalgae powder (5–10 g), and wet macroalgae (50–100 g) were homogenized in 5% perchloric acid (PCA). The PCA extract was subjected to a column containing a cation-exchange resin, Dowex 50WX8 (1 cm I.D. × 3 cm or 3 cm I.D. × 1 cm) and then eluted with 6M HCl. The concentrated polyamines were analyzed by HPLC on a Hitachi L6000 employing a column of cation-exchange resin, Hitachi 2619F (=Hitachi 2720) (4 mm I.D. × 50 mm), using post-labeled fluorometry after heating with *o*-phthalaldehyde (Hamana *et al.*, 2016a, 2016b). After the heptafluorobutyrylation of the concentrated polyamines, we performed HPGC on a SHIMADZU GC-17A and HPGC-mass spectrometry (HPGC-MS) on a JEOL JMS-700, equipped with a capillary column of Inert Cap IMS (0.32 mm I.D. × 30 m, df 0.25 μm) (GL Sciences) at a column temperature of 120°C–(16°C/min)–280°C and 90°C–(16°C/min)–280°C, respectively (Hamana *et al.*, 2016a, 2016b, 2017; Niitsu *et al.*, 2014), as shown in Fig. 1. The final identification of the polyamine peaks in HPGC-MS was accomplished by employing mass spectra using authentic linear diamines, triamines, tetraamines and pentaamines, and tertiary branched tetraamines, chemically synthesized in our laboratory (Niitsu *et al.*, 1992, 1993) as shown in Fig. 2. The mass spectra of the HFB-polyamine derivatives were taken from previous studies (Furuchi *et al.*, 2015a, 2015b; Hamana *et al.*, 2016a, 2016b, 2017; Niitsu *et al.*, 2014). Table 1 shows the molar concentrations of cellular polyamines per gram of wet weight of the starting *wet algae*, estimated from the HPLC and HPGC analyses using the peak high of authentic polyamine standards. From the total of 124 green algal samples previously analyzed, 39 green-algal polyamine data (analyzed by HPLC and/or standard GC equipped with a packed column in 1982–2004) related to the 59 new green algal samples, are cited for comparison in Table 1.

RESULTS AND DISCUSSION

Distribution of norspermidine, spermidine, homospermidine, norspermine, spermine and thermospermine

In our previous analysis of the most primitive uni-

cellular class Prasinophyceae in the phylum Chlorophyta, *Mantoniella*, *Micromonas*, *Prasinococcus* and *Pyramimonas* species contained norspermidine but did not contain norspermine, whereas *Prasinoderma*, *Prasinopapilla* and *Pseudoscourfieldia* species contained neither norspermidine nor norspermine (not cited in Table 1) (Hamana *et al.*, 2004a, 2013). In the present study, freshwater *Tetraselmis cordiformis* and marine *Tetraselmis subcordiformis* contained both norspermine and norspermidine (Table 1). Homospermidine was found in the freshwater species. The polyamine profiles varied in the seven previously analyzed genera and the two *Tetraselmis* species. We cannot exclude the possibility that their growth environments affected their polyamine profiles rather than their phylogenetic positions.

In the class Trebouxiophyceae, the occurrence of homospermidine and norspermine in the four species of terrestrial photobiontic (ectosymbiotic) *Trebouxia* is unique among the polyamine profiles of unicellular green microalgae. Without statistical analysis, various polyamine levels were found within the four species isolated in the USA and Japan, suggesting the possibility of a relationship between their polyamine patterns and their photobiont abilities. The reclassification of the ellipsoidal *Chlorella*-like green algae “*Chlorella saccharophyla*”, “*Chlorella ellipsoidea*” and “*Chlorella pyrenoidosa*” is in progress (Dariencko *et al.*, 2010). The occurrence of spermine as a major polyamine and the absence of norspermidine and norspermine from endosymbiotic, amino acid-required *Chlorella variabilis* NIES-2540 is unique among green unicellular algae. This polyamine profile resembles to that of *Auxenochlorella protothecoides* (Hamana *et al.*, 2004a) (not cited in Table 1). Since *C. variabilis* and *A. protothecoides* were cultured heterotrophically (nonphotosynthetically) in CYT medium containing yeast extract and tryptone and Pro medium containing peptone, respectively, the uptake of spermine from the media containing spermine is possible. Homospermidine was found in “*C. pyrenoidosa*” and *Parachlorella bejerinckii*, however, its level differed within the four commercial *Chlorella* supplements (Table 1).

In the order of Volvocales, polyamines of *Chlamydomonas reinhardtii* NIES-2235 (=IAM C-9) and *Chlamydomonas* sp. DBT newly analyzed in the present study showed a similar polyamine profile for several *Chlamydomonas* species. The normal

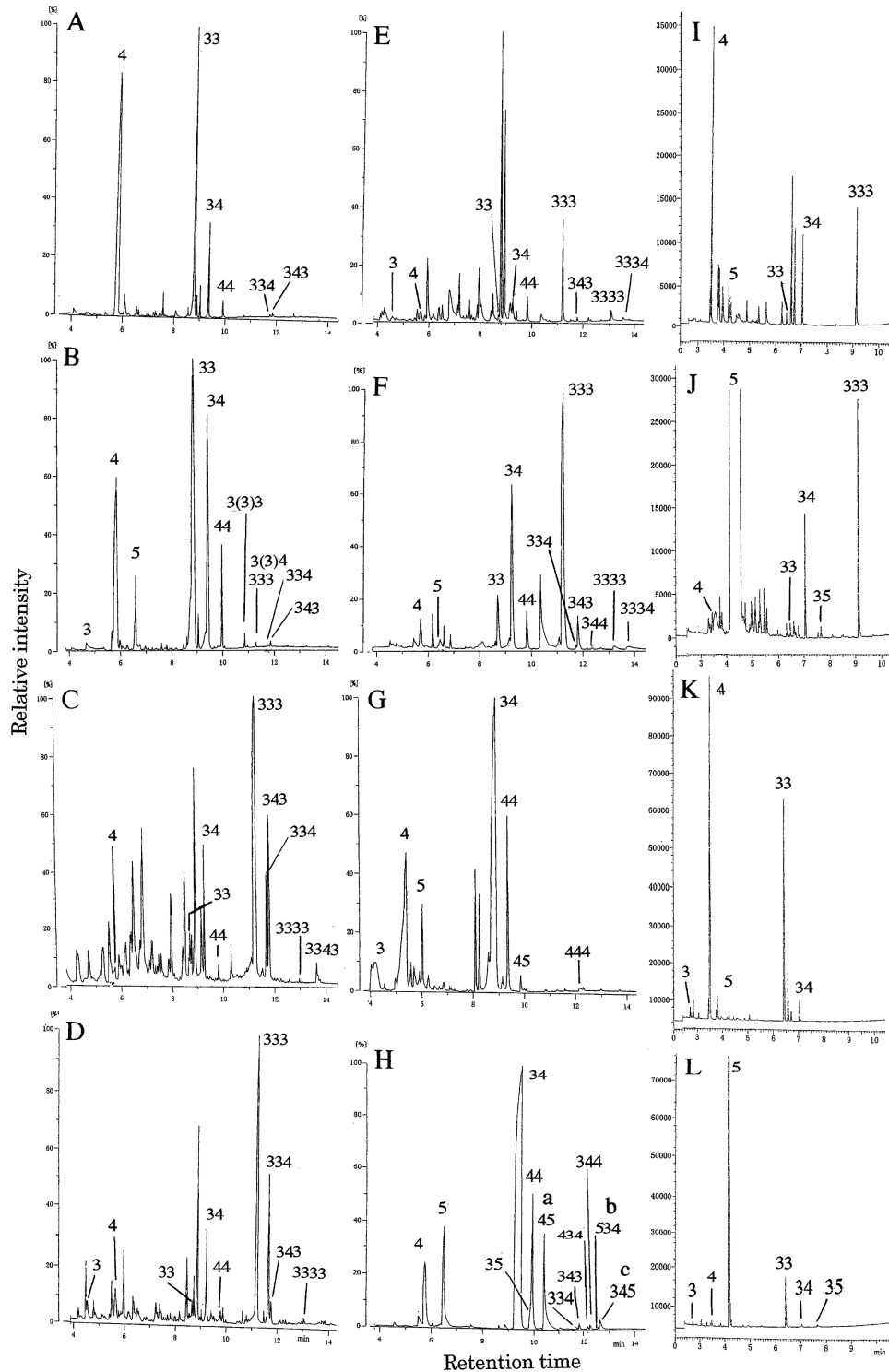


Fig. 1 HPGC spectra of polyamines of (A) *Tetrabaena socialis* NIES-691, (B) *Chlorogonium complexum* NBRC 105662, (C) *Prasiola japonica* No. 1, (D) *Ulva prusa* No. 2, (E) *Aegagropila linnaei* No. 2, (F) *Codium cylindricum*, (G) *Westella* sp. DBT No. 1, (H) *Westella* sp. No. 3 +1 mM cadaverine, (I) *Tetraselmis subcordiformis* NIES-2572, (J) *T. subcordiformis* +1 mM cadaverine, (K) *Raphidocelis subcapitata* NIES-35, and (L) *R. subcapitata* +1 mM cadaverine on a JEOL JMS-700 (A-H) or a SHIMADZU GC-17A (I-L). Numeric codes (abbreviations for polyamines) are explained in Table 1.

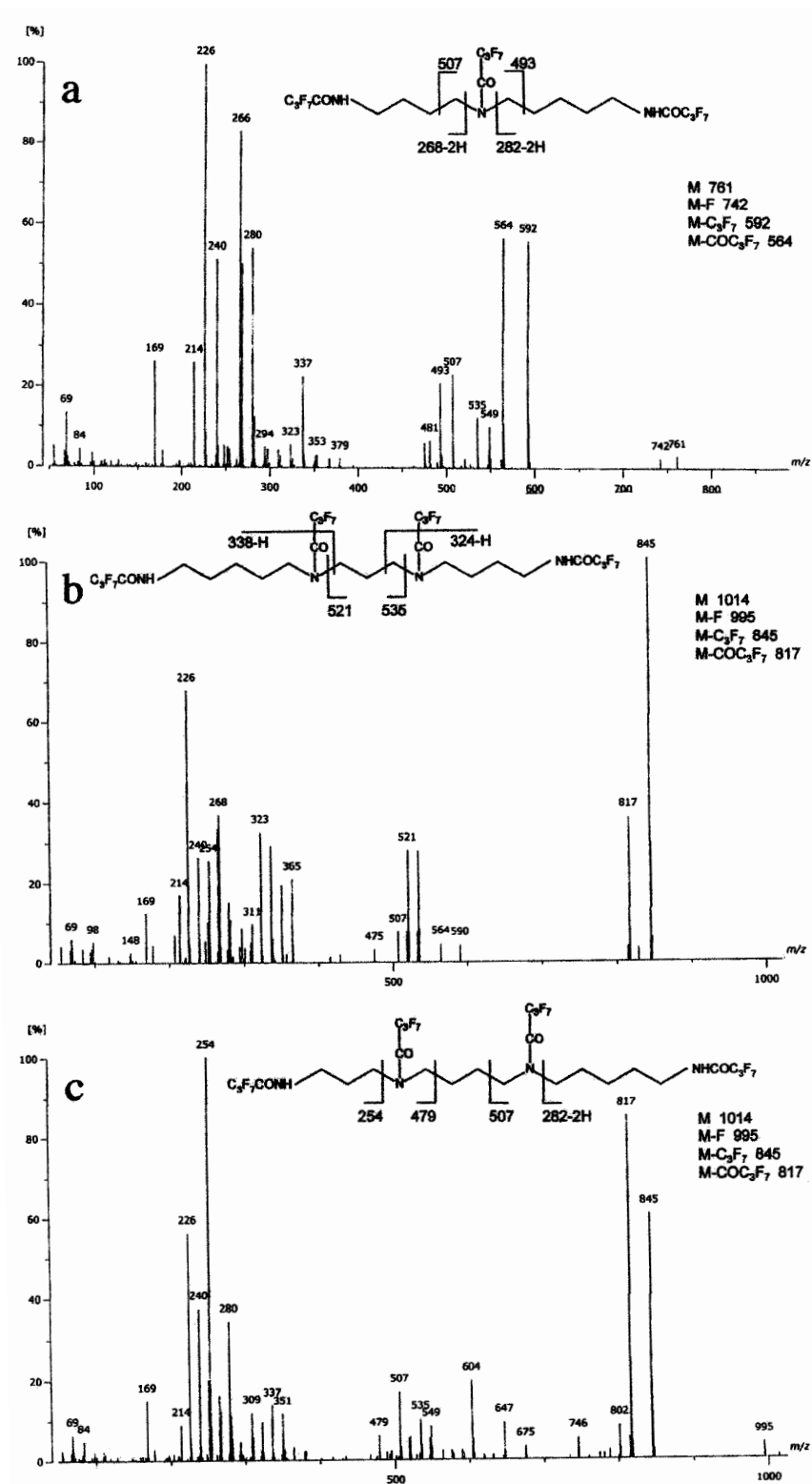


Fig. 2 Mass spectra of the HFB-polyamine peaks (a), (b) and (c) obtained with HPGC on a JEOL JMS-700 (H in Fig. 1). The spectra are identical to that of authentic HFB-45, HFB-534 and HFB-345, respectively. The JMS-700 mass spectrometer was operated in the electron impact mode at an ionization energy of 70 eV. The structure, molecular weight [M] and mass number of the three fragment ions from the HFB-amines are shown.

growth cell (green swarmer) and red cyst of *Haematococcus* sp. DBT gave the same polyamine profile (Table 1). The red cells were collected from an old culture under high light irradiation.

Westella sp. DBT, *Pediastrum biwae*, *Chlamydomonas* sp. DBT, and *Chlorogonium complexum* of the class Chlorophyceae contained homospermidine as shown in the present study. A minor occurrence of spermine and thermospermine was observed in *Botryococcus braunii* and *Tetrabaena socialis* in addition to *Eudorina*, *Chlamydomonas* and *Chlorogonium* species of the class. Although it has been reported that several halophilic *Dunaliella* species contained putrescine and spermidine as major polyamines (Hamana *et al.*, 1988, 2004a) (not cited in Table 1), norspermidine, homospermidine, spermine, and thermospermine were detected in *Dunaliella* sp. DIC as a minor polyamine by the large-scale analysis undertaken in the present study.

As regards streptophytes, thermospermine was not detected in *Mesostigma* (Mesostigmatophyceae), *Chlorokybus* (Chlorokybophyceae), or *Klebsormidium* and *Interfilum* (Klebsormidiophyceae) (Hamana *et al.*, 2004a, 2013) (not cited in Table 1). In the class Zygnematophyceae of the phylum Streptophyta, homospermidine was found as a major polyamine in the newly analyzed *Staurastrum* sp., and spermine, norspermine and thermospermine were detected as minor polyamines in the newly analyzed *Spirogyra* sp. Polyamines of the green algae belonging to the class Coleochaetophyceae of the phylum have never been analyzed.

In the present study, thermospermine was found as a major polyamine to a limited extent in thallic *Prasiola japonica* in the class Trebouxiophyceae, and thallic *Ulva perusa* and *Ulva prolifera* (Ulvales) in the class Ulvophyceae. In contrast, thermospermine was a minor component in *Monostroma* (Ulotrichales), *Aegagropila*, *Chaetomorpha* and *Valonia* (Cladophorales), *Caulerpa*, *Codium* and *Halimeda* (Caulerpales) of the class Ulvophyceae of the phylum Chlorophyta as well as *Nitella* and *Chara* of the class Charophyceae of the phylum Streptophyta. It is suggested that the occurrence of thermospermine is essential for the formation of the thallus structure in green multicellular algae.

Polyamine profiles in colonial green algae

Colonial green algae are distributed within the class Chlorophyceae. Within the order Volvocales

(Chlamydomonadales), it has been shown that unicellular primitive *Chlamydomonas*-like alga evolved into colonial *Gonium* (4–16 cells in a colony), *Pandorina* (16 cells), *Yamagishiella* (16 cells), *Eudorina* (16, 32 or 64 cells), *eodorina* (32, 64 or 128 cells) and then *Volvox* (up to 500 cells) through the simplest integrated green alga, *Tetrabaena* (4 cells), in which four identical cells constitute an individual (Arakaki *et al.*, 2013; Herron *et al.*, 2009). These flat or spherical colony-forming green algae are located in a clade of the class. *Gonium pectonera* NIES-2261 (=IAM C-588) re-cultured and reanalyzed by HPGC in the present study showed no cadaverine (5) content, indicating a misassignment in a previous study that used HPLC alone (Hamana *et al.*, 2004a). Except for minor distributions of homospermidine, spermine and thermospermine, the occurrence of putrescine, norspermidine and spermidine, and the absence of norspermine were observed in the seven colonial genera, indicating that the major cellular polyamine profile did not change during their evolution for colony-forming within the order Volvocales.

Different polyamine profiles were obtained in the four other colonial genera *Westella* (Chlorococcales), *Pediastrum* (Sphaeropleales), *Stigeoclonium* (Chaetophorales), and *Oedogonium* (Oedogoniales) within the class Chlorophyceae, suggesting that the polyamine profiles varied in the order levels within the class.

Occurrence of novel and unusual diamine, triamines and tetra-amines

A minor peak corresponding to the unusual long linear diamine, 1,6-diaminohexane (6) first found in multicellular macro brown-algal seaweeds belonging to the class Phaeophyceae of the phylum Heterokontophyta (Hamana *et al.*, 2017) was unexpectedly detected in the HPGC of *Yamagishiella unicocca* and *Pandorina morum* polyamines and identified by MS (not shown in Figs. 1 and 2). The results show the widespread distribution of 1,6-diaminohexane as a minor diamine in algae.

The natural occurrence of aminopropylcadaverine (35) and aminobutylcadaverine (45) in algae has never been reported, however, their production from cadaverine has been expected in unicellular-algal cultures. Aminopropylcadaverine has been found in some bacteria and aminobutylcadaverine has been detected in a leguminous root nodule. When *Tetraselmis subcordiformis* (Prasinophyceae)

and *Raphidocelis subcapitata* (Chlorophyceae) were cultured in the presence of 1 mM cadaverine (5) in the culture medium, aminopropylcadaverine was found in addition to excess cellular cadaverine but aminobutylcadaverine was not detected (Fig. 1 and Table 1).

Aminobutylcadaverine and homospermine (444) were detected in the colonial green alga *Westella* sp. DBT (culture No. 1) belonging to the class Chlorophyceae. Since an aminobutyl-transfer to cadaverine (5) and homospermidine (44) occurred in the green alga, the culture No. 2 in which the two polyamines were at low levels, was absent from 45 and 444. Although the same mass culture conditions were repeated, culture No. 2 showed the lowest cell density of the four cultures. When the green alga (culture No. 3 in which aminopropylspermidine (344) and canavalmine (434) were detected) was cultured in the presence of 1 mM cadaverine, 35 and 45, furthermore, N^1 -aminopentylspermidine (534, =435) and N^8 -aminopentylspermidine (345, =543) appeared. The ratio of 35:44 in the mix peak in HPGC-MS (Fig. 1H) was estimated to be 1:20 from MS data (not shown in Fig. 2). We think that the two novel tetra-amines 534 and 345 were produced by an aminobutyl-transfer to the aminopropyl moiety of 35 and an aminopropyl-transfer to the aminobutyl moiety of 45, respectively. It has been shown that *Nitella* and *Chara* located in the class Charophyceae contain the two unusual tetra-amines aminopropylhomospermidine (344) and canavalmine (434) (Hamana *et al.*, 2013) (Table 1). Although this is the first report on the production of 45, 534 and 345 in algae, their occurrences in *Westella* sp. DBT were not constant and the biological significance of the polyamines in the green alga is not clear.

The tertiary branched tetra-amines N^1 -aminopropylhomospermidine (3(3)3) and N^1 -aminopropylspermidine (3(3)4) were detected as a minor tetra-amine in unicellular *Chlorogonium complexum* in the order Volvocales of the class Chlorophyceae. This unicellular green alga contained 4, 5, 33, 34, and 44 as major polyamines and 3, 333, 343 and 334 as a minor polyamine in addition to the two branched tetra-amines. The coexistence of 333 and 3(3)4 in an HPGC peak (Fig. 1B) was confirmed by MS analysis of the peak (not shown in Fig. 2). This is the first report of the occurrence of tertiary branched tetra-amine in algae, however, the branched tetra-amines have already been found in

thermophilic bacteria and archaea.

Distribution of penta-amines

Caldopentamine (3333), homocaldopentamine (3334) and/or thermopentamine (3343), a long linear polyamine, were found as minor polyamines in multicellular *Prasiola japonica* belonging to the class Trebouxiophyceae, *Aegagropila linnaei* and *Codium cylindricum* belonging to the class Ulvophyceae, and *Spirogyra* sp. belonging to the class Zygnematophyceae. Caldopentamine was also detected in the three multicellular marine algae (green-algal seaweeds) belonging to the genera *Monostroma* and *Ulva* of the class Ulvophyceae. Therefore, the occurrence of the linear penta-amines seems to be related to their multicellular green-algal form. Furthermore, the major occurrence of these penta-amines has been reported in multicellular brown-algal seaweeds (Ochrophyta) (Hamana *et al.*, 2017). On the other hand, we found these penta-amines as a major polyamine in unicellular photosynthetic diatoms (Bacillariophyta) and unicellular non-photosynthetic Labyrinthulea belonging to the subdomain Stramenopiles (Hamana *et al.*, 2016b). The biological and phylogenetic significance of the occurrence of penta-amines in unicellular diatoms and Labyrinthulea is unclear.

CONCLUSION

We have reported the preferential distribution of thermospermine (334) and the penta-amines caldopentamine (3333), homocaldopentamine (3334) and thermopentamine (3343) in addition to common polyamines in multicellular green macroalgae and the detection of novel or unusual polyamines such as 1,6-diaminopropane (6), aminopropylcadaverine (35), aminobutylcadaverine (45), N^1 -aminopentylspermidine (534) and N^8 -aminopentylspermidine (345), homospermine (444), N^1 -aminopropylhomospermidine (3(3)3) and N^1 -aminopropylspermidine (3(3)4) as a minor polyamine in some unicellular green microalgae. Further polyamine analysis of additional endosymbiotic, photobiontic and achlorophyllous green-algal species and a comparison of polyamine distribution patterns in marine and freshwater green algal species in their environmental adaptation are in progress.

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単細胞性, 群体性, 多細胞性緑藻類のポリアミン分析
—アミノブチルカダベリン, N^1 -アミノペンチルスベルミジン, N^8 -アミノペンチルスベルミジン,
およびペンタアミン類の検出—

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緑藻類の多細胞化と細胞内ポリアミン構成との関連を知るため, 新規 59 試料 (52 種) の単細胞性, 群体性, 多細胞性の緑藻から酸抽出したポリアミン画分を HPLC と HPGC とで追加分析し, 既分析の 124 緑藻種のポリアミン構成と比較検討した. テトラアミン類のノルスベルミンとスベルミンは多細胞大型緑藻に主ポリアミン成分の一つとして顕著に分布していたが, おおの単細胞性の地衣類共生 *Trebouxia* と織毛虫共生 *Chlorella variabilis* (ともに Trebouxiophyceae) にも存在していた. Chlorophyceae では, 群体性の淡水産 *Volvox*, *Pleodorina*, *Eudorina*, *Yamagishiella*, *Pandorina*, *Tetrabaena*, *Gonium* (いずれも Volvocales) は単細胞性の *Chlamydomonas* や *Haematococcus* と同様に, プトレシン, ノルスベルミジン, スベルミジンが主ポリアミンであり, ポリアミン構成はこれらの群体形成に関係しないと思われた. ホモスベルミジンとホモスベルミンに加えて, 新規トリアミンであるアミノブチルカダベリンが別の群体性の淡水産 *Westella* (Sphaeropleales) に微量成分として検出された. さらに, カダベリン存在下に培養した場合には, アミノブチルカダベリン含量が増大し, 新規テトラアミンである N^1 -アミノペンチルスベルミジンと N^8 -アミノペンチルスベルミジンを生成した. 高含量のサーモスベルミンの存在は多細胞性で葉状の淡水産 *Prasiola* (Trebouxiophyceae) と海産 *Ulva* (Ulvophyceae) に限定していた. カルドペンタミン, ホモカルドペンタミンあるいはサーモペンタミンなどは, *Prasiola* と *Ulva* に加えて, 単細胞で多核嚢状性の *Codium*, 多細胞で分岐糸状性の *Aegagropila*, 葉状性の *Monostroma* (いずれも Ulvophyceae) および糸状性の *Spirogyra* (Zygnematophyceae) で微量ではあるが検出されたので, ペンタアミン類の存在は緑藻の多細胞化や大型化と関係すると思われた.