

Trehalose, digeneaside, and floridoside in the Florideophyceae (Rhodophyta) – a reevaluation of its chemotaxonomic value

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The qualitative and quantitative occurrence of low-molecular-weight carbohydrates (LMWCs) in the Florideophyceae was surveyed using ¹³C-nuclear magnetic resonance spectroscopy and high-performance liquid chromatography. Besides the joint occurrence of the heterosides floridoside and digeneaside in various Florideophycean orders (Ceramiales, Rhodymeniales, Gelidiales and Gigartinales), the disaccharide trehalose was detected only in several members of the Ceramiales. While some taxa of the latter order such as *Aglaothamnion* exhibited only trehalose, others, such as *Delesseria sanguinea*, showed trehalose together with digeneaside. The biosynthesis and physiological function of trehalose in red algae remains an open question. In addition, recent data from the literature indicate strong variation between phenotypic and genotypic formation of trehalose among red algal orders, and hence there exists some uncertainty to use this disaccharide as a chemotaxonomic marker. The occurrence of digeneaside in so many phylogenetically different Florideophycean taxa clearly negates any diagnostic value for this particular heteroside in the Ceramiales. In conclusion, while trehalose and digeneaside are unsuitable as chemotaxonomic markers for red algal phylogeny, other LMWCs, such as polyols and D-/L-isofloridoside, well support recent molecular taxonomic treatments.

KEY WORDS: Compatible solutes, Low-molecular-weight carbohydrates, Osmolytes

INTRODUCTION

The use of chemical characters, other than those based on molecular genetics, as taxonomic markers has long been recognized in algal systematics. One of the best-known examples is the wide use of specific primary and accessory photosynthetic pigments such as chlorophylls, carotenoids and phycobiliproteins as diagnostic of the different divisions of algae (Jeffrey & Veski 1997). However, for an organic compound to be useful as a chemosystematic character, it must be specific to a certain taxon or group of organisms and should be sufficiently abundant to detect and identify with confidence. Another important consideration is the stability of the respective compound within a lineage since multiple losses or gains could lead to erroneous taxonomic conclusions. However, the concentration of a chemical character is of low taxonomic value, if at all, since environmental factors strongly control the intracellular pool size.

The various low-molecular-weight carbohydrates (LMWCs) synthesised during photosynthesis by different algae have hitherto been considered as promising chemotaxonomic character (Meeuse 1962; Craigie 1974; Kremer 1980; Karsten *et al.* 1999, 2003), at least at higher taxonomic rankings. The heteroside, floridoside (α -D-galactopyranosyl-(1–2)-glycerol), for example, is considered to be the main photosynthetic and reserve product in all orders of the Rhodophyta except the Ceramiales. Members of the Ceramiales in general synthesise and accumulate the chemically related digeneaside (α -D-mannopyranosyl-(1–2)-glycerate). Although the distribution of

these different heterosides within red algal orders had been regarded as chemotaxonomically useful (Kremer & Vogl 1975; Kremer 1978b), it has been demonstrated that the genera *Laurencia* and *Osmundea* (Rhodomelaceae, Ceramiales) produce and accumulate floridoside and not digeneaside (Barrow *et al.* 1995). Other biochemical exceptions of major carbohydrates are known in the genera *Bostrychia* and *Stictosiphonia* (Rhodomelaceae, Ceramiales) and *Caloglossa* (Delesseriaceae, Ceramiales), which are characterized by the polyols sorbitol, dulcitol and mannitol, compounds that are otherwise rare in red algae (Karsten *et al.* 1992a, b, 1999, 2003). However, it should be mentioned that recently *Bostrychia* and *Stictosiphonia* were subsumed by Zuccarello & West (2006). In addition, various species of the genus *Hypoglossum* (Delesseriaceae, Ceramiales) synthesise and accumulate digalactosylglycerol as the main carbohydrate, a compound so far not known from other red algae (Karsten *et al.* 2005). Other recent studies indicate an even more complex picture on the diversity and distribution of LMWCs among the Rhodophyta. All known LMWCs found in the Ceramiales, that is, heterosides and polyols, are also found in the morphologically simple, early diverging red algae such as, for example, the Porphyridiales 2 (according to Müller *et al.* 2001; see also Karsten *et al.* 1999, 2003).

Because of the biochemical diversity in red algal LMWCs, ¹³C-nuclear magnetic resonance spectroscopy (¹³C NMR) was used to qualitatively screen for further new compounds among the Florideophyceae, followed by high-performance liquid chromatography (HPLC) for quantification (Karsten *et al.* 2005). The presence of trehalose was noted in some members of the Ceramiales, as well as the occurrence of floridoside in

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combination with digeneaside in various other orders. The observed LMWC patterns are reevaluated and discussed in terms of their chemosystematic value.

MATERIAL AND METHODS

Algal material

Collection locations and dates of the species used for the LMWC analysis are shown in Table 1. Most algae were grown as unialgal cultures in sterile seawater (30–32 psu) enriched with modified PES/2 (West & McBride 1999; West 2005). Cultures were maintained in 500-ml Pyrex dishes at 23–25°C, 20–30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ natural and fluorescent lighting of 12–18 h variable day length. The algae were grown in continuous immersion culture; the medium was changed every 2 weeks. Some species were collected in the field by SCUBA. All algal samples were air-dried prior to chemical analysis.

Carbohydrate screening using NMR

For NMR spectroscopy, 100–200 mg of algal dry weight (DW) was extracted in 5 ml of aqueous ethanol (70% v/v) for 3 h in a water bath at 70°C. After centrifugation at $6200 \times g$, the supernatant was evaporated to dryness *in vacuo* and redissolved in 0.5 ml of D_2O (99.98%) for NMR spectroscopy. The ^{13}C NMR spectra were recorded with a Bruker AVANCE 500 spectrometer. (^{13}C : 125.7 MHz). Chemical shift values δ (in ppm) are given relative to the signal for internal TMS ($\delta = 0$). The calibration of spectra was carried out using the signals of added dioxane (δ (^{13}C) = 67.6).

Carbohydrate quantification using HPLC

Trehalose, digeneaside and floridoside were separated and quantified by HPLC. For these analyses, 10–15 mg DW of the algal samples were extracted in 1 ml of 70% (v/v) ethanol (3 h in a water bath at 70°C). After centrifugation for 5 min at $6200 \times g$, 700 μl of the supernatant were evaporated to dryness under vacuum (Speed Vac Concentrator SVC 100H). Dried extracts were redissolved in 700 μl distilled water, sonicated for 5 min and vortexed for 30 s. After centrifugation at $16,000 \times g$, the supernatants were analysed with an isocratic Agilent HPLC system equipped with a differential refractometer. Carbohydrates were separated on a stainless-steel Phenomenex Rezex ROA-Organic Acid column ($300 \times 7.8\text{-mm}$ I.D.) protected with a Phenomenex Carbo- H^+ guard cartridge ($4 \times 3\text{-m}$ I.D.). The mobile phase was 5 mM H_2SO_4 run isocratically at a flow rate of 0.4 ml min^{-1} and a temperature of 75°C. LMWCs were identified by comparison of retention times with those of standard compounds and quantified by peak areas. This method proved to be extremely reliable and reproducible. While trehalose was verified using a commercial standard (Sigma-Aldrich, Taufkirchen, Germany), digeneaside was isolated from the red alga *Ceramium rubrum* (Hudson) C. Agardh (Karsten *et al.* 2005) and floridoside from the red alga *Delisea pulchra* (Greville) Montagne (Karsten *et al.* 1993).

All concentrations given are expressed as mmol kg^{-1} DW and represent the mean value $\pm s$ of three replicate measurements of samples taken from different thalli.

Cluster analysis

To visualise the LMWC pattern with respect to the taxonomic position of the samples, a hierarchical cluster analysis was performed on presence–absence data of the compounds digeneaside, floridoside and trehalose. The squared euclidean distance was used as the distance metric, and clusters were merged by the average linkage method (SPSS 13.0 for Windows).

RESULTS

The ^{13}C -NMR spectroscopic measurements of all Florideophycecean extracts investigated gave characteristic chemical shifts typical for specific LMWCs. The heterosides floridoside and digeneaside were distinguished easily by the respective NMR signal of the anomeric C-1 atom of the appropriate hexose (galactose or mannose) at 99.1 and 99.6 ppm, respectively; these signals represent a chemical fingerprint for both chemically related compounds (data not shown, but for details, see Karsten *et al.* 2003). The disaccharide trehalose showed six typical resonances at 94.4, 73.7, 73.3, 72.2, 70.9 and 61.7 ppm.

By using a new HPLC method (Karsten *et al.* 2005), it was possible to separate and quantify trehalose, digeneaside and floridoside (data not shown). Under the chromatographic conditions described, trehalose, digeneaside and floridoside showed retention times of 13.3 min, 13.8 min, and 16.1 min, respectively, well apart from each other to guarantee full separation of all compounds.

The ^{13}C -NMR spectra and HPLC analysis demonstrated that many but not all members of the Ceramiales contained digeneaside. While some taxa of this order such as *Aglaothamnion* exhibited trehalose only without any trace of digeneaside (Table 1), others such as *Delesseria sanguinea* (Hudson) J.V. Lamouroux showed digeneaside plus trehalose (Table 1). The presence of trehalose was only proven for representatives of the Ceramiales. In various members of the orders Rhodymeniales (e.g. *Champia parvula* (C. Agardh) Harvey), Gelidiales (e.g. *Gelidium* sp.) and Gigartinales (e.g. *Ahnfeltiopsis leptophylla* (J. Agardh) P.C. Silva & De Cew), floridoside together with digeneaside was determined (Table 1).

There was considerable variation in the concentrations of LMWCs ranging from very low digeneaside contents of about 3 mmol kg^{-1} DW in *Champia parvula* up to 1068 mmol kg^{-1} DW in *Claudea batanensis* Tanaka (Table 1). A similar range was measured for floridoside. In contrast, the trehalose concentration amounted only between 4 and 145 mmol kg^{-1} DW (Table 1) and was very similar among all positively tested taxa studied.

The cluster analysis showed four clusters and one unassociated sample (*Gigartina ornithorhynchos*) (Fig. 1, Table 2). Most investigated species (34) grouped in cluster III, that is, synthesised digeneaside as the only LMWC, and belong to four families (Ceramiaceae, Dasyaceae, Delesseriaceae, Rhodomelaceae) within the Ceramiales. However, the LMWC pattern of the Ceramiales is more diverse. While some species of the Ceramiales synthesised in addition to digeneaside also floridoside (cluster I) or trehalose (cluster II) (Fig. 1), others were not able to form digeneaside at all but produced instead

Table 1. Sources of specimens analysed, their taxonomic position and culture number and (low-molecular-weight carbohydrates) patterns of different members of the Florideophyceae as detected and quantified by high-performance liquid chromatography. The concentrations are expressed as mmol kg⁻¹ dry weight. Results are given as mean values $\pm s$ ($n = 3$); n.t., no trace.

| Taxa | Collection site | Culture no. | Digeneaside | Floridoside | Trehalose |
|---|---|-------------|-------------------|---------------|-----------------|
| Rhodophyta, Ceramiales, Ceramiaceae | | | | | |
| <i>Acrothamnion preissii</i> (Sonder) Wollaston | Catanduanes, Philippines, 14.5.1988 | 2885 | 115.8 \pm 15.6 | n.t. | n.t. |
| <i>Aglaothamnion byssoides</i> (Arnott ex Harvey) L'Hardy-Halos & Rueness | OR 34, sent by Jan Rueness 16.9.2003 | 4356 | n.t. | n.t. | 66.2 \pm 0.7 |
| <i>Aglaothamnion byssoides</i> | OR 34, sent by Jan Rueness 16.9.2003 | 4357 | n.t. | n.t. | 72.0 \pm 0.5 |
| <i>Aglaothamnion byssoides</i> | R 3648, sent by Jan Rueness 16.9.2003 | 4358 | n.t. | n.t. | 94.2 \pm 0.2 |
| <i>Aglaothamnion byssoides</i> | culture from G.H. Kim, 20.1.2004 (C. Maggs) | 4404 | n.t. | n.t. | 32.6 \pm 5.0 |
| <i>Aglaothamnion oosumiense</i> Itono | from G.H. Kim, Korea, 13.12.1999 | 4036 | n.t. | n.t. | 41.8 \pm 6.7 |
| <i>Aglaothamnion obstipum</i> Cowling, Kraft and J.A. West | Williamstown, VIC, Australia, 13.2.1995 | 3443 | n.t. | n.t. | 64.2 \pm 6.4 |
| <i>Aglaothamnion obstipum</i> | Williamstown, Gloucester Reserve, VIC, Australia, 28.4.1995 | 3486 | n.t. | n.t. | 38.6 \pm 2.7 |
| <i>Aglaothamnion roseum</i> (Ross) Maggs & l'Hardy-Halos | UTEX 2292, J. & M. Rueness, Norway | 3785 | n.t. | n.t. | 145.1 \pm 9.1 |
| <i>Aglaothamnion roseum</i> | UTEX 2293, M. Wynne, Woods Hole, MA, USA, 10.7.1972 | 3786 | n.t. | n.t. | 32.3 \pm 3.1 |
| <i>Aglaothamnion</i> sp. | Parra Açu Maranhão, Brasil, 20.11.1996 | 3651 | n.t. | n.t. | 42.4 \pm 0.1 |
| <i>Anotrichium</i> sp. | Dumaguete, Philippines, 4.5.1987 | 2783 | 134.0 \pm 0 | n.t. | n.t. |
| <i>Anotrichium furcellatum</i> (J. Agardh) Baldock | San Diego Marina, USA, 9.3.1982 | 2600 | 210.4 \pm 1.0 | n.t. | n.t. |
| <i>Antithamnion densum</i> (Suhr) M.A. Howe | from G.H. Kim, Korea, 13.12.1999 | 4035 | 152.0 \pm 3.6 | n.t. | n.t. |
| <i>Antithamnion pectinatum</i> (Montagne) Brauner | from G.H. Kim, 20.1.2004 | 4402 | 130.5 \pm 6.2 | n.t. | n.t. |
| <i>Antithamnion tenuissimum</i> (Hauck) Schiffner | Sundene, Norway, Oct. 1966 | 533 | 91.3 \pm 3.1 | n.t. | n.t. |
| <i>Antithamnionella glandulifera</i> (Kylin) E.M. Wollaston | Berkeley Marina, California, USA, 20.2.1988 | 2878 | 142.0 \pm 32.1 | n.t. | n.t. |
| <i>Antithamnionella glandulifera</i> | from G.H. Kim, 20.1.2004 | 4403 | 456.8 \pm 19.5 | n.t. | n.t. |
| <i>Balliella</i> sp. | Aride Island, Seychelles, 12.5.1982 | 2656 | 98.0 \pm 22.7 | n.t. | n.t. |
| <i>Balliella</i> sp. | Moorea, Tahiti, 5.3.1980 | 2422 | 55.7 \pm 3.1 | n.t. | n.t. |
| <i>Centroceras</i> sp. | Horn Island, QLD, Australia, 21.6.2002 | 4233 | 185.9 \pm 0.3 | 4.6 \pm 0.1 | n.t. |
| <i>Ceramium boydenii</i> A. Gepp & E.S. Gepp | Iwaya, Awaji Island, Okinawa, 28.5.1996 | 4234 | 159.5 \pm 2.0 | n.t. | n.t. |
| <i>Ceramium</i> sp. | Morib, Selangor, West Malaysia, 12.5.1998 | 3839 | 78.8 \pm 0.8 | n.t. | n.t. |
| <i>Crouania</i> sp. | Moorea, French Polynesia, 1.7.1995 | 3508 | 8.9 \pm 0.4 | n.t. | 29.5 \pm 0.2 |
| <i>Dasyphila plumarioides</i> Yendo | Cantanduanes, Philippines, 14.5.1988 | 2884 | 74.8 \pm 6.8 | n.t. | 4.0 \pm 0.2 |
| <i>Gymnothamnion elegans</i> (Schousboe ex C. Agardh) J. Agardh | Cantanduanes, Philippines, 14.5.1988 | 2882 | 176.3 \pm 0.5 | n.t. | n.t. |
| <i>Haloplegma duperryi</i> Montagne | Cantanduanes, Philippines, 14.5.1988 | 2883-1 | 584.5 \pm 167.6 | n.t. | n.t. |
| <i>Haloplegma duperryi</i> | Cantanduanes, Philippines, 14.5.1988 | 2883-2 | 908.2 \pm 67.0 | n.t. | n.t. |
| <i>Haloplegma duperryi</i> | Chuuk, Federated States of Micronesia, 1.10.1975 | 1521-1 | 488.8 \pm 2.1 | n.t. | n.t. |
| <i>Haloplegma duperryi</i> | Chuuk, Federated States of Micronesia, 1.10.1975 | 1521-2 | 269.9 \pm 73.8 | n.t. | n.t. |
| <i>Haloplegma duperryi</i> | Yucatan, Mexico, 27.7.1980 | 2413 | 90.7 \pm 0.4 | n.t. | n.t. |
| <i>Pterothamnion pectinatum</i> (Kylin) Athanasiadis & Kraft | Friday Harbor, Washington, USA, 1.4.1965 | 364 | 212.1 \pm 1.6 | n.t. | n.t. |
| <i>Pterothamnion villosum</i> (Kylin) Athanasiadis & Kraft | Ft. Point, San Francisco, USA, 28.2.1988 | 2877 | 206.8 \pm 10.7 | n.t. | n.t. |
| <i>Pterothamnion villosum</i> | Berkeley Marina, California, USA 30.5.1987 | 2811 | 153.8 \pm 5.2 | n.t. | n.t. |
| <i>Pterothamnion yezoense</i> (Inagaki) Athanasiadis & Kraft | from G.H. Kim, 20.1.2004 | 4401 | 221.9 \pm 25.6 | n.t. | n.t. |

Table 1. Continued

| Taxa | Collection site | Culture no. | Digeneaside | Floridoside | Trehalose |
|---|--|-------------|----------------|-------------|------------|
| <i>Seirospora seirosperma</i> (Harvey) P.S. Dixon | Woods Hole, Massachusetts, USA, 20.4.1979 | 2174 | n.t. | n.t. | 89.9 ± 8.1 |
| <i>Seirospora interrupta</i> (J.E. Smith) F. Schmitz | UTEX 1510, F.D. Ott, 4.1966 | 4587 | n.t. | n.t. | 26.5 ± 0.4 |
| Rhodophyta, Ceramiales, Dasyaceae | | | | | |
| <i>Dasya iridescens</i> (Schlech) A.J.K. Millar & Abbott | Kahala, Oahu, Hawaii | 2400-1 | 308.6 ± 63.1 | n.t. | n.t. |
| <i>Dasya iridescens</i> | Kahala, Oahu, Hawaii, 10.10.1979 | 2400-2 | 478.8 ± 2.1 | n.t. | n.t. |
| <i>Dasya sinicola</i> (Setchell & N.L. Gardner) E.Y. Dawson | Station Beach, Puerto Penasco, Mexico, 12.4.1968 | 646 | 1136 ± 117.4 | n.t. | n.t. |
| <i>Dasya</i> sp. | Williamstown, Victoria, Australia, 31.2.1995 | 3446 | 849.4 ± 131.8 | n.t. | n.t. |
| <i>Heterosiphonia gibbesii</i> (Harvey) Falkenberg | Puerto Morales, Yucatan, Mexico, 27.7.1980 | 2401 | 176.8 ± 7.5 | n.t. | n.t. |
| Rhodophyta, Ceramiales, Delesseriaceae | | | | | |
| <i>Branchioglossum woodii</i> (J. Agardh) Kylin | Station Beach, Puerto Penasco, Mexico, 12.4.1968 | 653 | 422.4 ± 134.1 | n.t. | n.t. |
| <i>Claudea batanensis</i> Tanaka | Siayan Island, Batanes, Philippines, 3.6.1986 | 2715 | 1067.7 ± 167.6 | n.t. | n.t. |
| <i>Delesseria sanguinea</i> (Hudson) J.V. Lamouroux | Artificial Reef, Nienhagen, Rostock, Germany, 1.3.2005 | Field | 330.6 ± 54.1 | n.t. | 26.7 ± 1.7 |
| <i>Martensia fragilis</i> Harvey | Oahu, Hawaii, 10.2.1978 | 2112 | 73.9 ± 10.0 | n.t. | n.t. |
| <i>Nitophyllum adhaerens</i> M.J. Wynne | Cooks Bay, Moorea, French Polyne- sia 1.7.1995 | 3504 | 107.3 ± 2.9 | n.t. | n.t. |
| <i>Phycodrys rubens</i> (Linnaeus) Batters | Artificial Reef, Nienhagen, Rostock, Germany 1.3.2005 | Field | 105.7 ± 0.6 | n.t. | n.t. |
| <i>Phycodrys setchelli</i> Skottsberg | Santa Cruz, California, USA, 20.4.1988 | 2218 | 227.3 ± 0.4 | n.t. | n.t. |
| <i>Phycodrys setchelli</i> | Bodega Bay, California, USA, 11.9.1967 | 589 | 129.4 ± 0.3 | n.t. | n.t. |
| <i>Zellera tawallina</i> G. Martens | Cantanduanes, Philippines, 14.5.1988 | 2888 | 786.5 ± 0.4 | n.t. | n.t. |
| Rhodophyta, Ceramiales, Rhodomelaceae | | | | | |
| <i>Melamansia glomerata</i> (C. Agardh) R.E. Norris | Oahu, Hawaii, 28.9.1979 | 2289 | 556.7 ± 98.7 | n.t. | n.t. |
| <i>Laurencia</i> sp. | Port Dickson, Selangor, West Malay- sia, 14.5.1998 | 3850 | 7.1 ± 0.4 | 142.9 ± 5.1 | n.t. |
| <i>Murrayella pericladus</i> (C. Agardh) F. Schmitz | Cayo Caracoles, La Parguera, Puerto Rico, 20.3.1981 | 2513 | 750.5 ± 166.9 | n.t. | n.t. |
| <i>Osmundaria fimbriata</i> (Lamouroux) R.E. Norris | Port Douglas, QLD, Australia 12.6.1987 | 2841 | 454.7 ± 174.4 | n.t. | n.t. |
| <i>Polysiphonia accuminata</i> N.L. Gard- ner | Berkeley Marina, California, USA, 2.2.1988 | 2942 | 452.0 ± 47.4 | n.t. | n.t. |
| <i>Polysiphonia howei</i> Hollenberg | Catanduanes, Philippines, 14.5.1988 | 2922 | 122.2 ± 5.1 | n.t. | n.t. |
| <i>Polysiphonia morrowi</i> Harvey | 1988 | 2943 | 448.5 ± 1.2 | n.t. | n.t. |
| <i>Polysiphonia pacifica</i> Hollenberg | 46 JZ | 46 JZ | 387.3 ± 0.6 | n.t. | n.t. |
| <i>Pterosiphonia bipinnata</i> (Postels & Ruprecht) Falkenberg | Agate Beach, Santa Cruz Co., Cali- fornia, USA, 12.1.1988 | 2938-1 | 298.1 ± 0.3 | n.t. | n.t. |
| <i>Pterosiphonia bipinnata</i> | Agate Beach, Santa Cruz Co., Cali- fornia, USA, 12.1.1988 | 2938-2 | 788.5 ± 56.8 | n.t. | n.t. |
| Rhodophyta, Gelidiales, Gelidaceae | | | | | |
| <i>Gelidium</i> sp. | Morib, Selangor, West Malaysia, 12.5.1998 | 3849 | 3.7 ± 0.8 | 179.8 ± 3.7 | n.t. |
| Rhodophyta, Gigartinales, Gigartinaceae | | | | | |
| <i>Gigartina</i> sp. | Monterey Bay, California, USA, 1978 | 1781 | 2.8 ± 0.2 | 292.8 ± 1.6 | n.t. |
| <i>Gigartina ornithorhynchus</i> J. Agardh | Monterey Bay, California, USA, 1978 | 1783 | n.t. | 260.6 ± 3.1 | n.t. |
| <i>Ahnfeltiopsis flabelliformis</i> (Harvey) Masuda | Oshoro Bay, Hokkaido, Japan, 4.11.1977 | 2456 | 52.9 ± 2.7 | 270.5 ± 2.3 | n.t. |
| <i>Ahnfeltiopsis leptophylla</i> (J. Agardh) P.C. Silva & De Cew | Duxbury Reef, Marin Co., Califor- nia, USA, 30.4.75 | 2196 | 78.4 ± 0.3 | 900.9 ± 8.2 | n.t. |
| <i>Ahnfeltiopsis leptophylla</i> | Stillwater Cove, Monterey Co., Cali- fornia, USA, 11.7.1979 | 2337 | 51.2 ± 0.1 | 335.9 ± 0.5 | n.t. |
| <i>Ahnfeltiopsis leptophylla</i> | Stillwater Cove, Monterey Co., Cali- fornia, USA, 15.3.1980 | 2377 | 32.3 ± 0.5 | 293.6 ± 1.0 | n.t. |

Table 1. Continued

| Taxa | Collection site | Culture no. | Digeneaside | Floridoside | Trehalose |
|---|--|-------------|-------------|--------------|-----------|
| Rhodophyta, Gigartinales, Hypneaceae | | | | | |
| <i>Hypnea</i> sp. | Oahu, Hawaii, 29.9.1979 | 2281 | 19.6 ± 1.0 | 117.1 ± 3.2 | n.t. |
| Rhodophyta, Gigartinales, Phylloporaceae | | | | | |
| <i>Phyllophora pseudoceranoides</i> (S.G. Gmelin) Newroth & A.R.A. Taylor | Artificial Reef, Nienhagen, Rostock, Germany, 1.3.2005 | Field | 28.0 ± 1.0 | 299.0 ± 2.4 | n.t. |
| <i>Schottera nicaeënsis</i> (J.V. Lamouroux ex Duby) Guiry & Hollenberg | Grève St Michel, Brittany, France, 10.1.1977 | 1460 | 46.0 ± 1.4 | 1037.5 ± 0.3 | n.t. |
| Rhodymeniales, Champiaceae | | | | | |
| <i>Champia parvula</i> (C. Agardh) Harvey | Sacco de San Francisco, Sao Paulo, Brasil, 2.7.1982 | 2645 | 3.2 ± 0.3 | 71.1 ± 2.3 | n.t. |

only trehalose (cluster IV). Furthermore, the combination of digeneaside and floridoside (cluster I) occurred next to the Ceramiales also in species of the Gelidiales, Gigartinales and Rhodymeniales (Fig. 1, Table 2).

Gigartina ornithorhynchos (Gigartinales) did not associate with any of the clusters, as it contained only floridoside. However, another *Gigartina* sample synthesised next to floridoside also digeneaside (cluster I), the latter in very low concentrations of 2.8 ± 0.2 mmol kg⁻¹ DW. Neither the combination of floridoside and trehalose nor the joint occurrence of all three LMWCs was found in any single sample investigated.

DISCUSSION

In algal taxonomy the occurrence or lack of specific carbohydrate components such as major storage compounds, cell-wall constituents or low-molecular-weight photosynthates has been considered useful for distinguishing groups at several levels (Percival 1979; Kremer 1980; Barrow *et al.* 1995). Although the physiological state of an algal species and the environmental conditions are recognized to strongly influence the intracellular concentrations of LMWCs (Karsten *et al.* 1992a, 1993, 2005), the principal biochemical capability to produce particular compounds could still be useful for chemotaxonomic considerations. LMWCs typically originate as photoassimilatory products in primary metabolism and hence are usually present in sufficient concentrations for analytical detection. In recent years various ¹³C-NMR investigations were undertaken on carbohydrate patterns in red algae and proved to be an excellent qualitative (i.e. chemical fingerprinting) tool (Karsten *et al.* 1999, 2003, 2005; Simon-Colin *et al.* 2004 and references therein). On the other hand, accurate quantitative information is very difficult to obtain with this technique because the NMR is rather insensitive, is time consuming, and needs a relatively high amount of biomass compared to chromatographic methods. Therefore, the HPLC method described here for the analysis of trehalose, digeneaside and floridoside provides a simple and reliable technique for quantitative measurements of these compounds.

Different LMWC patterns could be detected within the various Florideophyceae (Table 1, Fig. 1). Based on the presence or absence of the three LMWCs digeneaside, floridoside and

trehalose, the cluster analysis revealed only four main clusters among the red algal taxa studied. This low number of clusters already implies an insensitive character of these particular carbohydrates with respect to the chemotaxonomy of the Florideophyceae.

Most interesting is the observation that trehalose represents a major compound in various families of the Ceramiales (e.g. Ceramiaceae, Delesseriaceae). While the Ceramialean taxa *Aglaothamnion* and *Seirospora* exclusively contained only this disaccharide, others such as *Delesseria sanguinea* exhibited trehalose together with digeneaside (Table 1). Trehalose has been reported as one of the most abundant end products of photosynthetic carbon reduction in three species of freshwater Rhodophyta (*Batrachospermum boryanum* Sirodot, Batrachospermales; *Compsopogon coeruleus* (Balbis ex C. Agardh) Montagne as *Compsopogon hookeri*, Compsopogonales; *Paralemanea annulata* (Kützing) M.L. Vis & R.G. Sheath as *Lemanea annulata*, Batrachospermales) (Kremer 1978a). However, since this author used a rather nonspecific thin-layer chromatographic method, the identification of trehalose is doubtful. This is supported by a more recent study on LMWCs in *Compsopogon coeruleus*, which clearly indicates the sole presence of floridoside using HPLC and NMR techniques (Karsten *et al.* 2003). Although in various other red algal taxa trehalose has been reported (Craigie 1974), the occurrence has to be verified with modern analytical methods. However, so far, quantitative data on this disaccharide, as well as information on its physiological function in red algae, are missing. Trehalose concentrations up to 145 mmol kg⁻¹ DW (Table 1) as measured in the present study, which is compared to other LMWCs relatively high, indicate an important role in metabolism.

In freshwater cyanobacteria, trehalose contributes to the osmotic acclimation (Reed *et al.* 1984) but plays only, if at all, a minor role as a compatible solute (Warr *et al.* 1988) because the three-dimensional structure of this disaccharide is generally known to interfere with enzyme function (Hinton *et al.* 1969). More recent publications indicate that trehalose is strongly involved in anhydrobiosis (Yancey 2005 and references therein). During desiccation this disaccharide may bind to macromolecules and membranes by replacing water and maintaining their basic structure. Trehalose forms a glass-like state under dry conditions, contributing to the preservation of

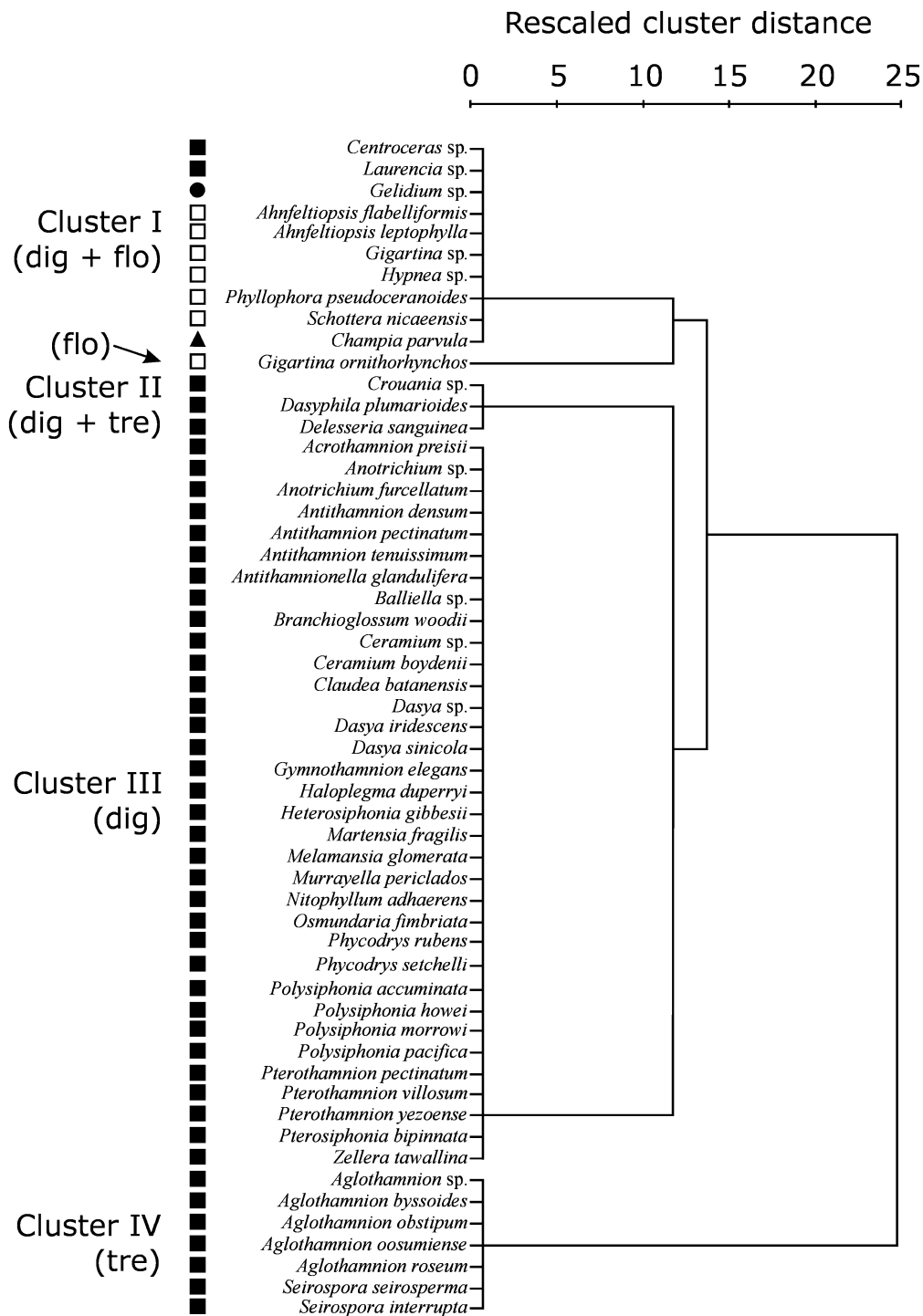


Fig. 1. Cluster analysis of all screened red algal samples based on the presence and absence of the low-molecular-weight carbohydrates (LMWCs) digeneaside (dig), floridoside (flo) and trehalose (tre). The taxonomic position of the species studied is shown by various symbols: black square – Ceramiales, white square – Gigartinales, black circle – Gelidiales, black triangle – Rhodymeniales. The analysis reveals four clusters (I to IV) and one unassociated sample (*Gigartina ornithorhynchos*). The corresponding LMWC are given in parentheses.

cellular structures (Yancey 2005). In addition, freezing and heat tolerance in many organisms such as yeast, higher plants, insects, and so on is related to the presence of trehalose (Yancey 2005). Similar physiological functions, however, have still to be experimentally proven for red algae.

Although the present data indicate that only a few Cera-

mialean taxa are capable of synthesizing trehalose, the summarized results in the review of Craigie (1974) point to other Florideophyceean species also forming this disaccharide. Therefore, this compound may be more widely distributed among the Rhodophyta than thought. Although we did not detect trehalose in various *Porphyra* species using NMR and

Table 2. Characterisation of the four clusters as obtained by the cluster analysis (Fig. 1).

| Cluster | Low-Molecular-weight carbohydrates | No. of samples | Families | Orders |
|---------|------------------------------------|----------------|--|---|
| I | digeneaside and floridoside | 10 | Ceramiaceae, Rhodomelaceae Geldiaceae Gigartinaeaceae, Hypneaceae, Phylloporaceae Champiaceae | Ceramiales Gelidiales Gigartinales Rhodymeniales |
| II | digeneaside and trehalose | 3 | Ceramiaceae, Dasyaceae | Ceramiales |
| III | digeneaside | 34 | Ceramiaceae, Dasyaceae, Delesseriaceae, Rhodomelaceae | Ceramiales |
| IV | trehalose | 7 | Ceramiaceae | Ceramiales |

GC techniques (data not shown), a trehalose-6-phosphate synthase gene was recently screened out from a large-DNA-fragment library constructed from *Porphyra yezoensis* (Dai *et al.* 2004). This result strongly supports the genotypic presence of trehalose biosynthesis in a representative taxa of the order Bangiales (Bangiophyceae), which is considered as sister group of the Florideophyceae (Saunders & Hommersand 2004). Therefore, the monophyly of the Bangiophyceae and Florideophyceae, which is based on molecular data and ultrastructural features such as Golgi-ER associations (Saunders & Hommersand 2004), seems to be further biochemically supported by the joint ability to form this rather uncommon disaccharide. The reason why trehalose can not be phenotypically measured in *Porphyra* may be explained by silenced genes, missing transcription factors or a high metabolic turnover that leaves no detectable traces of this compound. Because of this variation between phenotypic and genotypic formation of trehalose among red algae, there exists some uncertainty to use this disaccharide as chemotaxonomic marker, and hence more studies on other red algal taxa are needed to address this question.

Among the Rhodophyta studied Kremer (1978b, 1980) and Kirst (1980) considered digeneaside to be an important chemosystematic character for representatives of the Ceramiales only. However, the present study clearly documents that in various species of the orders Rhodymeniales, Gelidiales and Gigartinales digeneaside occurs together with floridoside (Table 1, Fig. 1). In addition, while the Ceramialean genera *Laurencia* and *Osmundea* synthesize and accumulate floridoside instead of digeneaside (Barrow *et al.* 1995), various ancestral taxa such as *Rhodosorus*, *Stylonema* and *Rhodochaete* produce digeneaside (Karsten *et al.* 1999, 2003). Consequently, the occurrence of digeneaside in so many Florideophycean taxa and other ancestral orders clearly negates any diagnostic value for this particular heteroside in the Ceramiales.

Although high digeneaside concentrations up to 1068 mmol kg⁻¹ DW were measured in *Claudea batanensis* Tanaka (Table 1), it is questionable whether this compound is actively involved in the process of osmotic acclimation. Kirst & Bisson (1979) reported an almost twofold accumulation of digeneaside in *Centroceras clavulatum* (C. Agardh) Montagne after increasing the salinity from 26 to 51 psu. However, these authors considered the contribution of this heteroside to the internal osmotic potential of the cells as low and therefore physiologically unimportant. Similarly in *Caloglossa leprieurii* (Montagne) Martens and *Hypoglossum barbatum* Okamura, the digeneaside concentration was unaffected after exposure to a range of salinities (Karsten *et al.* 1994, 2005). Therefore,

all the published work indicates that digeneaside plays only a minor role as an organic osmolyte.

In conclusion, members of the Florideophyceae exhibit a much more diverse range of LMWC patterns than thought. The data also indicate that some red algal LMWCs such as trehalose and digeneaside are unsuitable as chemotaxonomic markers, while others such as D- and L-isofloridoside, as well as polyols, represent reliable characters to support recent molecular phylogeny of various red algal lineages (Karsten *et al.* 2003; Yoon *et al.* 2006). Some peaks of the HPLC chromatograms could not be chemically identified thus far (data not shown), suggesting that in the near future more biochemical surprises can be expected. Consequently, Rhodophyta seem to exhibit a broad range of metabolic pathways to fix inorganic carbon into photoassimilates. However, except for mannitol (Karsten *et al.* 1997), the underlying anabolic and catabolic processes are not well understood.

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