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Do vesicle cells of the red alga *Asparagopsis* (*Falkenbergia* stage) play a role in bromocarbon production?

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Abstract

The Rhodophyceae (red algae) are an established source of volatile halocarbons in the marine environment. Some species in the Bonnemaisoniaceae have been reported to contain large amounts of halogens in structures referred to as vesicle cells, suggesting involvement of these specialised cells in the production of halocarbons. We have investigated the role of vesicle cells in the accumulation and metabolism of bromide in an isolate of the red macroalga *Asparagopsis* (*Falkenbergia* stage), a species known to release bromocarbons. Studies of laboratory-cultivated alga, using light microscopy, revealed a requirement of bromide for both the maintenance and formation of vesicle cells. Incubation of the alga in culture media with bromide concentrations below 64 mg l^{-1} (the concentration of Br^- in seawater) resulted in a decrease in the proportion of vesicle cells to pericentral cells. The abundance of vesicle cells was correlated with bromide concentration below this level. Induction of vesicle cell formation in cultures of *Falkenbergia* occurred at concentrations as low as 8 mg l^{-1}, with the abundance of vesicle cells increasing with bromide concentration up to around 100 mg l^{-1}. Further studies revealed a positive correlation between the abundance of vesicle cells and dibromomethane and bromoform production. Interestingly, however, whilst dibromomethane production was stimulated by the presence of bromide in the culture media, bromoform release remained unaffected suggesting that the two compounds are formed by different mechanisms.

Keywords: Bromide; Biosynthesis; Bromoform; Dibromomethane; Bonnemaisoniaceae

1. Introduction

Refractive vesiculate cells occur throughout the Rhodophyceae and have been termed 'gland', 'vesicle' or secretory cells. For more than a century, phycologists have attempted to define their structure and chemical nature (Pelligrini et al., 1989). It has become increasingly apparent that these specialised cells are of several types and have divergent functions (Fritsch, 1945). Vesicle cells of the Rhodomeniales have been interpreted as mucilage producing cells whilst those of *Peysonnelia* appear to have a similar function to cystoliths found in higher plants (Dixon and Irvine, 1977). However, in some algae of the Bonnemaisoniaceae (*Trailliella*, *Asparagopsis*) vesicle cells have been implicated in halide accumulation (Wolk, 1968). Using electron microprobe spectroscopy, a tool suited for elemental studies of cell composition, Wolk (1968) confirmed that the vesicle cells of *Trailliella* concentrated both bromine and iodine, albeit in an unknown form. Bromine and iodine concentrations within the vesicle cells were estimated to be respectively 30- and 3-fold higher than their concentrations

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in neighbouring cells. A later study by Young et al. (1980) used energy dispersive fluorescence to localise the brominated compound β-snyderol within a cytoplasmic vesicle in *Laurencia snyderae*.

An isolate of the *Falkenbergia* sporophyte stage of a tropical red macroalga *Asparagopsis* species, characterised by vesicle cells and known to produce several bromocarbons (Marshall et al., 1999), has been used in an investigation of a possible role of these specialised cells in bromide accumulation and bromocarbon production.

2. Materials and methods

2.1. Algal material

The isolate of the *Falkenbergia* stage of *Asparagopsis* sp. used in this study was obtained from the collection of Professor M. Guiry of the Department of Botany, National University of Ireland, Galway, Ireland. This specimen (culture collection no. 0985) was originally collected from Key Largo, Florida. The taxonomic status of this isolate is discussed by Ní Chualáin (1997) who, on the basis of DNA restriction fragment length polymorphism, has tentatively assigned it to a species distinct from both *A. armata* and *A. taxiformis*, provisionally described as the Caribbean strain.

2.2. Culture conditions

Cultures of the alga were initially established in seawater supplemented with von Stosch (1964) stock solution (20 ml l^{-1}). The alga was placed in petri dishes (50 mm × 20 mm) containing culture medium (15 ml) and incubated at 20 °C, illuminated from above with Thorn Gro-lux and Sylvana Lifeline fluorescent lights at an irradiance of 30 μmol photons m^{-2} s^{-1} (16 h light:8 h dark cycle). The alga was re-suspended in fresh medium every 7 d. The alga was later subcultured into fresh medium (100 ml) contained in 250 ml conical flasks with cotton wool bungs, shaken at 100 rpm at 20 °C under a similar light regimen. For the purpose of this study the alga was transferred to 100 ml artificial seawater (ASP 6F) (Fries, 1975; modified by Wolk, 1968), without the addition of bromide, and fragmented using a homogeniser. Aliquots (5 ml) of the fragmented culture were then pipetted into 250 ml conical flasks containing 20 ml of ASP 6F culture medium with bromide concentrations ranging from 0 to 256 mg l^{-1}. Flasks were then incubated under the above conditions.

For the monitoring of halocarbon release the alga was removed from its culture flask and washed thoroughly with ASP 6F containing no bromide. The alga was then suspended in 100 ml of ASP 6F without bromide, and fragmented. Aliquots (10 ml) of the fragmented cultures were pipetted into a series of screw- capped vials (25 ml) fitted with PTFE septa and incubated in ASP 6F medium or ASP 6F medium without bromide under an irradiance of 30 μmol photons m^{-2} s^{-1}, for 6 h. Following the incubation period medium was withdrawn from each vial for analysis.

2.3. Quantification of halocarbon release

Dibromomethane and bromoform were quantified using a DANI 3750 purge and trap sampler linked to a Hewlett Packard 5890 gas chromatograph/5970 mass selective detector (MSD) system. Incubation medium (5 ml) was placed in a vial (20 ml) and 1,2 dibromoethane (1 ml of a 10 μg l^{-1} solution) was added as an internal standard. Previous work in the laboratory had established that the release of this halocarbon did not occur under the conditions employed in these experiments. Vials were then sealed with aluminium crimp caps fitted with PTFE-coated butyl rubber seals, placed in the purge-and-trap sampler and allowed to equilibrate at 50 °C for 1 h prior to purging. Helium was employed as the purge gas at a flow rate of 30 ml min^{-1} for a purge time of 30 s. The volatiles were trapped on Tenax TA and thermally desorbed at 250 °C for 1 min and transferred via a heated line (150 °C) onto a Chrompack Poroplot Q capillary column (10 m × 0.32 mm i.d. with 10 μm of divinylbenzene/styrene polymer as bonded phase). The injector port temperature and MSD transfer line were maintained at a temperature of 250 °C and the gas chromatograph oven was held initially at 40 °C for 1 min, then programmed to 230 °C at 10 °C min^{-1} and maintained at this temperature for 5 min. The MSD was operated in the selective ion mode with ions monitored as follows: bromoform m/z 250, 252, 254 and 256; dibromomethane m/z 172, 174 and 176; and 1,2 dibromoethane m/z 107 and 109. The limits of detection for bromoform and dibromomethane were 2.5 and 1 μg l^{-1} respectively.

2.4. Enumeration of vesicle cells in algal cultures

To quantify the proportion of vesicle cells in algal cultures three samples (0.1 ml) were removed from each culture flask using a Pasteur pipette. The vesicle cell content of each sample was assessed under the light microscope (Unilux-11) at 100× magnification. The total numbers of vesicle cells and pericentral cells were counted for three random fields of view, for each individual sample. For each field of view, the proportion of vesicle cells to pericentral cells was calculated. The proportion of vesicle cells to pericentral cells for each culture flask was taken as the mean of the nine proportions measured. The cultures were set up in triplicate.

2.5. Monitoring of algal growth

The measurement of algal growth was adapted from the procedure employed by Dring (1967) using compacted cell volume to estimate the growth of *Conchocelis*—filaments of *Porphyra*. Aliquots (0.7 ml) were removed from culture flasks, placed in blood sedimentation tubes and centrifuged at 8000 rpm for 30 min. The volume of plant material was read from the graduations on the tubes and taken as a representative sample for the flask from which it had been removed. This volume was used to calculate the total volume present in the flask. Cultures were set up in triplicate.

3. Results and discussion

Initial experiments to investigate a possible role for the vesicle cells of *Falkenbergia* in the production of bromocarbons and bromide accumulation involved assessing the effects of exogenous bromide on the formation and abundance of these structures. Preliminary observations were conducted on cultures transferred from von Stosch enriched seawater to ASP 6F culture medium with or without bromide (64 mg l^{-1}). Observations of the cultures under the light microscope showed that, over an incubation period of four weeks, the frequency of the vesicle cells decreased in those cultures incubated in the absence of bromide. Indeed after four weeks there appeared to be no vesicles present (Fig. 1).

Having established the importance of bromide for maintaining vesicle cells, subsequent experiments attempted to quantify the effects of different concentrations of bromide on cultures which had been maintained in von Stosch enriched seawater (64 mg l^{-1} bromide). The alga was incubated at eight concentrations of bromide ranging from 0 to 256 mg l^{-1} and samples were taken at intervals over 10 d after transfer (Fig. 2). All algae incubated at bromide concentrations below 64 mg l^{-1} displayed a decrease in vesicle cell abundance. This decrease did not occur immediately, only becoming apparent after 2 d at 0 mg l^{-1} and 4 d at 32 mg l^{-1}. Furthermore, the concentration of the ion determined the magnitude of the decrease in vesicle cells. The saturation concentration, above which there was no decrease in the proportion of vesicle cells was similar to levels of bromide observed in natural seawater (64 mg l^{-1}).

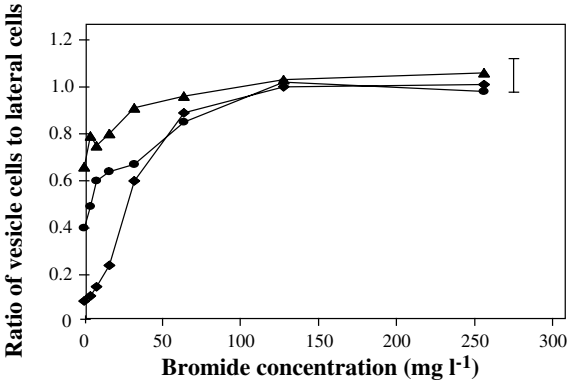

Fig. 2. The ratio of vesicle cells to pericentral cells of *Falkenbergia*, previously incubated in the presence of 64 mg l^{-1} bromide, after 2 (▲), 4 (●) and 10 d (◆) at different bromide concentrations. Bar in top right hand corner represents the LSD value ($p = 0.05$).

Such findings would support the report of Wolk (1968) that the vesicle cells of *Asparagopsis armata*, *Bonnemaisonia hamifera* and *Trailliella intricata* disappeared when the algae were transferred to bromide-free media. Wolk (1968) stated that the subsequent transfer of these algae without vesicles to media containing bromide resulted in the formation of vesicle cells.

Further experiments were therefore conducted to assess whether the formation of vesicle cells in *Falkenbergia* stage cultures previously incubated in bromide-free media occurred on the re-introduction of different

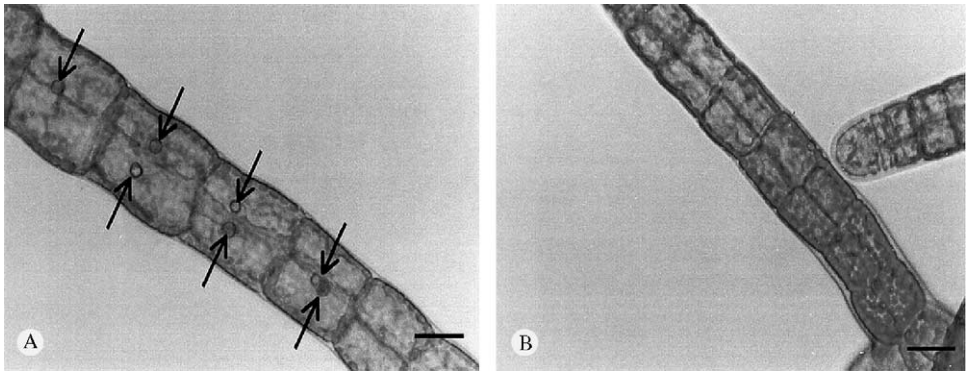

Fig. 1. Filaments of *Falkenbergia* 0985 incubated in the presence (64 mg l^{-1}) (A) and absence (B) of bromide for four weeks. Arrows indicate vesicle cells. Scale bar represents 10 μm.

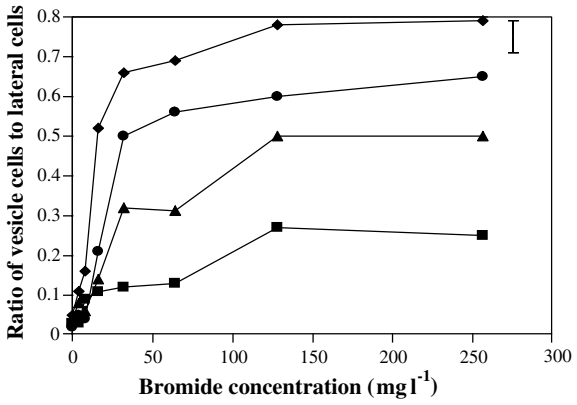

Fig. 3. The ratio of vesicle cells to pericentral cells of *Falkenbergia*, previously incubated in the absence bromide, after 3 (■), 5 (▲), 7 (●) and 10 d (◆) at different bromide concentrations. Bar in top right hand corner represents the LSD value ($p = 0.05$).

levels of bromide to the cultures. Algae with no vesicle cells (having been grown in the absence of bromide for four weeks) were incubated at eight different concentrations of bromide (0–256 mg l^{-1}). Samples were taken at 1–3 d intervals over a 10 d.

Induction of vesicle cells appeared to occur at bromide concentrations as low as 8 mg l^{-1} (Fig. 3). Interestingly this concentration falls between the concentrations of 6.4 and 19.2 mg l^{-1} previously stated to be critical for the formation of vesicle cells in cultures of *Asparagopsis armata* and *Trailliella intricata* (Wolk, 1968). In the present study a lag period of a day was observed before a significant number of vesicle cells were formed, after which the formation of the cells continued steadily for the next 8 d. There appeared to be a saturation concentration of about 100 mg l^{-1}, at which the daily rate of increase in the proportion of vesicle cells reached a maximum.

Growth experiments conducted with *Falkenbergia* stage cultures over the same time period and under the same environmental conditions as the vesicle cell experiments (Figs. 2 and 3) showed that bromide had no significant effect on the growth of the alga. Fries (1966) demonstrated that the growth of various species of red alga was not adversely affected by the absence of bromide in the culture medium. In contrast, studies on *Polysiphonia urceolata* showed the alga to require bromide for growth. In the present study with *Falkenbergia* stage cultures, increased vesicle cell abundance was not linked to a requirement of the ion for growth. However, it would appear that bromide is required by the alga to maintain vesicle cell numbers.

Having established that bromide was essential for the formation and maintenance of vesicle cells, the relationship between vesicle cells and the production of organobromine compounds was investigated. *Falkenbergia* stage cultures, characterised by a range of vesicle cell frequencies, after growth at different concentrations of bromide (0–64 mg l^{-1}), were selected for experiments. The algae were incubated in either the presence or absence of bromide (64 mg l^{-1}) for 6 h, having previously been washed thoroughly with ASP 6F medium containing no bromide, to prevent carry-over of bromide. Following the incubation, the concentrations of both dibromomethane and bromoform present in the culture medium were determined (Fig. 4). Regression analysis showed a significant relationship between the production of dibromomethane and bromoform and the proportion of vesicle cells in the presence and in the absence of exogenous bromide ($p < 0.05$ for each compound in both the presence and absence of bromide). A two-way ANOVA also showed that the presence of exogenous bromide significantly stimulated dibromomethane production ($p < 0.001$) but had no effect on bromoform production ($p > 0.05$).

These findings would support the hypotheses made from earlier studies that the vesicle cells of the Bonnemaisoniaceae are involved in halide metabolism (Wolk, 1968; Fenical, 1975). However, no insight is given as to the exact mechanism involved. The observation that dibromomethane production increased when the alga was incubated in the presence of bromide whilst bromo-

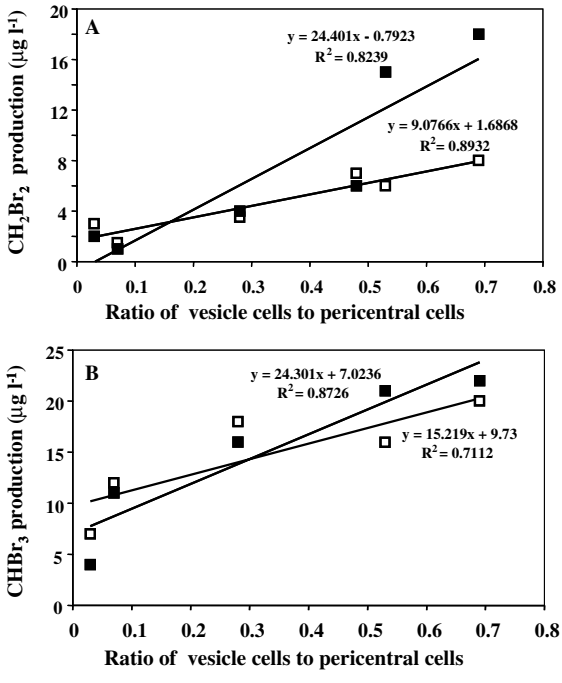

Fig. 4. The relationship between the proportion of vesicle cells to pericentral cells and the production of dibromomethane (A) and bromoform (B) by cultures of *Falkenbergia* incubated for 6 h in the presence (64 mg l^{-1}) (■) and absence (□) of bromide.

form production was not affected suggests that the two compounds are formed by different mechanisms. Furthermore, the production of the bromocarbons in both the presence and absence of exogenous bromide would imply an endogenous source of the ion. Further studies are clearly required to establish whether the vesicle cells are the site of the ion within the algal filament of this species. Electron probe X-ray microanalysis (Marshall, 2000) has shown that, although bromine is distributed throughout the algal thallus of *Asparagopsis armata* and *Trailliella intricata*, the vesicle cells accumulate higher concentrations of the ion. In *Trailliella intricata* the bromine detected in the vesicle cells was as much as 30-fold higher than that found in neighbouring cells. However, attempts to localise bromine within the thallus of *Falkenbergia* stage cultures using similar methods have so far proved to be unsuccessful (Marshall, 2000). The development of a procedure allowing the localisation of this element within the filaments of *Falkenbergia* would be useful in providing a deeper insight into the role of these vesicle cells in bromine metabolism.

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