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NATURAL PRODUCTIVITY OF ACRYLIC ACID AND DIMETHYL SULPHIDE DURING A SUMMER BLOOM OF *PHAEOCYSTIS POUCHETII* IN ANTARCTIC COASTAL WATER

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Abstract Water samples were collected over *P. pouchetii* bloom period beginning in November 1988, in 15m water column, from 10km offshore of Davis Station, Vestfold Hill, Antarctica. The concentrations of acrylic acid and dimethyl sulphide (DMS), which are toxic compounds in the water samples, were determined by HPLC and GC. The result shows that the concentration of acrylic acid varies in 0.001—0.510μmol • L^{-1} and the concentration of DMS in 0.003—0.588 μmol • L^{-1} during *P. pouchetii* bloom. Both the increased since late December 1988 and reached the highest concentration in early January 1989, then they decreased rapidly and returned to lower level from middle January to February in agreement with variation in cell number of the unicell alga *P. pouchetii*. The correlation coefficients between acrylic acid and *P. pouchetii* and between DMS and *P. pouchetii* are all 0.998. It is undoubted that *P. pouchetii* produced acrylic acid and DMS. The highest productivity of acrylic acid and DMS were 9.76×10^{-8} μmol • $cell^{-1}$ and 13.09×10^{-8} μmol • $cell^{-1}$, respectively, during *P. pouchetii* bloom. A cellular product, dimethylsulphonium propionate (DMSP), is decomposed into acrylic acid and DMS, and the formation of DMSP is probably from methionine which could be utilized by *P. pouchetii*.

Key words Antarctica, acrylic acid, DMS, prouductivity, *P. pouchetii*.

Introduction

The prymnesiophyte *Phaeocystis pouchetii* (Hariot) Lagerheim is a unicell alga distributed extensively in cool temperature, subpolar and polar water (Kashkin, 1963). The mucilagious alga clogs commercial fishing nets (Boney, 1970) and appearance of the bloom is associated with poor towing to the extent that the livelihood of fishermen is threatened. The appearance of *P. pouchetii* bloom became an ill omen for fishermen and colloquial disdainful names, such as "the fishermen's sign", "weedy water", and "stinking water". Savage (1930) confirmed fishermen's claims that herring can avoid *P. pouchetii*

bloom and suggested that the alga was unpalatable and its blooms were consequently avoided. The widespread observation of low fishing catches associated with *P. pouchetii blooms* would infer that why the avoidance of bloom by herrings has the same effect upon species of fish (Chang, 1983). Some results of investigation of krill show that there is a negative correlation between krill numbers and phtoplankton cells in Antarctic sea. It is possible that it responses to chemical effect such as toxic compounds. *P. pouchetii* utilized amino acids as a source of carbon and nitrogen and released toxic compounds, acrylic acid and dimethylsulfide (DMS). These compounds may be a source of chemical effect to drive fishs and krills.

It is important to understand how much acrylic acid and DMS were released to environment and to obtain productivity of them in *P. pouchetii* bloom in Antarctic coastal water. Here we will give the concentaration of acrylic acid and DMS and their natural productivity which was simply estimated during *P. pouchetii* bloom in Antarctic coastal water, in order to further study the chemical effets of those toxic compounds on biota in Antarctic ocean water.

Materials and Methods

Sea water samples were collected at 15m level at a coastal site 10km north of Davis Station on Vestfold Hill of Antarctica (Fig. 1). Acrylic acid and DMS were determined by HPLC and GC at Davis Station Biological Laboratory and *P. pouchetii* were fixed for later enumeration in Australia Antarctic Division Biological Laboratory.

High Pressure Liquid Chromatography (HPLC) system is used to determine acrylic acid and orther voletil fatty acids. The system consists of a Kortec ETP pump; a Kortec mixing chamber; a Rainin microsorb 7125 injector with valve with 100μL loop; a LDC milton roy variable UV spectrophotometer and Aminrx ion exclusion HPX－87H organic acids column. The operation conditions are as follows: Elent, 0.05N H_2SO_4 pH = 1.68; Flow rate, 0.6mL minute^{-1}; Absorbance wave length 210nm (UV) and 0.002 AUFS. Samples injection volume was 100μL. The peaks were identified by their characterstic retention times and the quantification was measured by peaks area linearly compared with standard samples in CL－10B integrator.

The method used to determine DMS is based on the publication "Determination of Trace Quantities of Dimethylsulphide in Aqueous Solution" (Andreae, 1983). The method involves removing the DMS from a fixed volume of sea water by a helium sparge and trapping it on silated glass beads at －95 C, After being released from the cold trapping, the compounds collected are separated by gas chromatographic flame ionization detection. The cryogenic enrichment gas chromatographic system comprise of Varian aerograph series 3700 TCD － FID － EPD gas chromatographic mass flowmaters LDC milton Ron CL － 10B

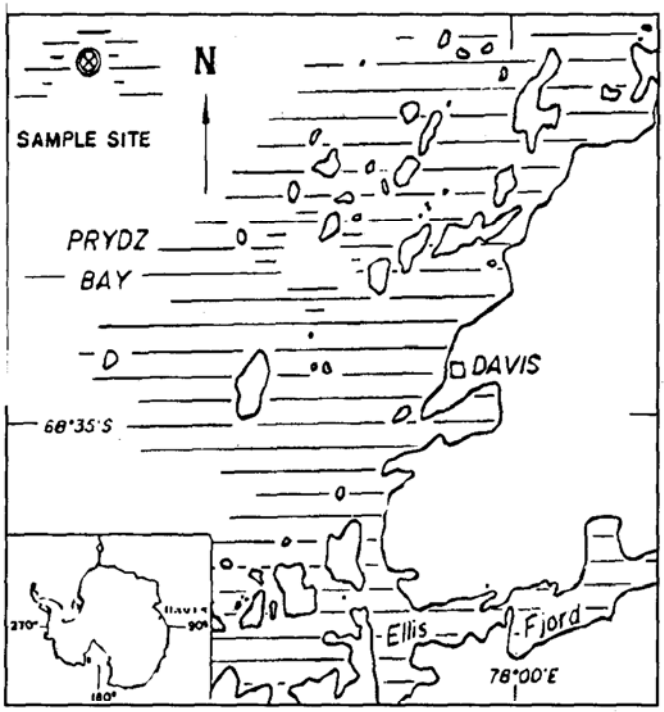

Fig. 1. Sampling site at 10km northwest of Davis Station.

integrator NESLAB cryocol immersion cooler CC100, NESLAB DR－2 digital readout thermomter, ACTIBON indicating oxygen trapper; KCW isotherm constant temperature bath and six way stainless steel switching valve (VALCO instrument company). The analytical column was a 52.0×3.0mm I.D. teflontube packed with acetone washed porapak QS4.

Results

The growth of *P. pouchetii* was observed since 23 November 1988 at a sampling site on Prydz Bay, Antarctica. From December 1988 to January 1989, it froms an important number of *P. pouchetii* bloom. The maximum cells number is approximately 6.00×10^{7} cell $\cdot L^{-1}$ that were found in third January, 1989. Total cell mumber of *P. pouchetii* increased immediately by 226.94 times in six days (from 14 to 20 December 1988) and it declined from 3 January 1989 and recovered to lower level (1390 cell $\cdot L^{-1}$) in February 1989.

The concentrations of acrylic acid and DMS, which are acellular products were detected in Winter and a period of *P. pouchetii* bloom. Both compounds varied with the variation of *P. pouchetii*. It was found that their concentrations remained at a lower level during winter and increased rapidly with bloom of cells on 14 December 1988. The concentrations of acrylic acids and DMS increased also immediately by 78.66 times and 21 times in six days from 14 to 20 December 1988, and the highest concentrations of both were approximatly 0.

510 μmol • L^{-1} and 0.588 μmol • L^{-1}, respectively, which occurred on third January 1989. The concentrations decreased and recovered to the same lower level as that of cells in bloom times. The changeable range of both concentrations is 0.001 to 0.510 μmol • L^{-1} (acrylic acid) and 0.003 to 0.588 μmol • L^{-1} (DMS), respectively during *P. pouchetii* bloom. The results and concentration variations are shown in Table 1 and Figs. 2 and 3.

Table 1. Distribution of cell numbers and concentrations of acrylic acid and DMS during a summer bloom.

sampling date	cell numbers (cells/L)	acrylic acid (μmol • /L)	DMS (μmol • /L)
23.11.88	4715	0.0010	0.0030
14.12.88	13691	0.0015	0.0033
20.12.88	3107051	0.1880	0.0630
03.01.89	60067000	0.5100	0.5880
17.01.89	6674690	0.3190	0.3070
31.01.89	792426	0.0830	0.0940
14.02.89	1390	0.0190	0.0200

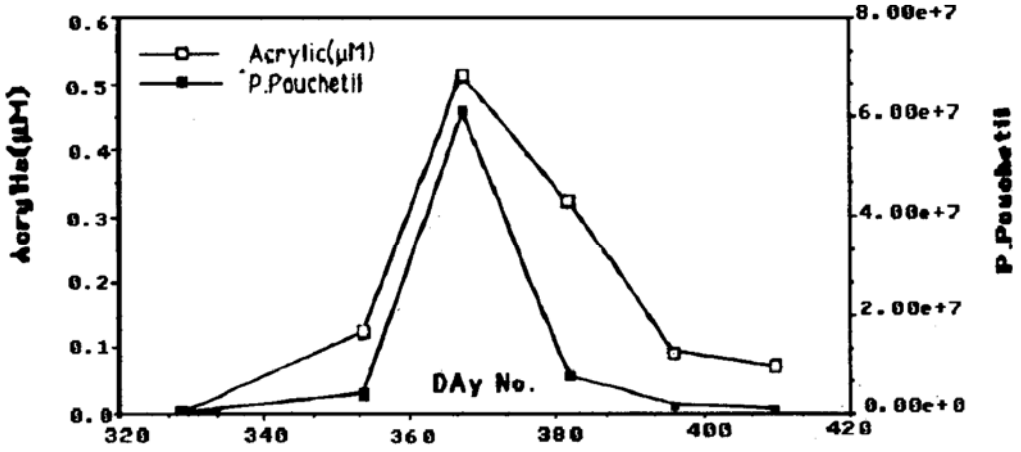

Fig. 2 Acrylic acid and *P. pouchetii* at 15m depth in coastal Antarctic sea water

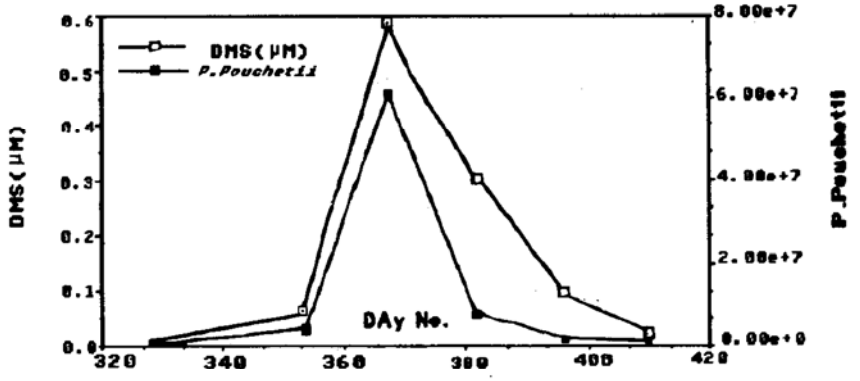

Fig. 3 DMS and *P. pouchetii* at 15m depth in coastal Antarctic sea water

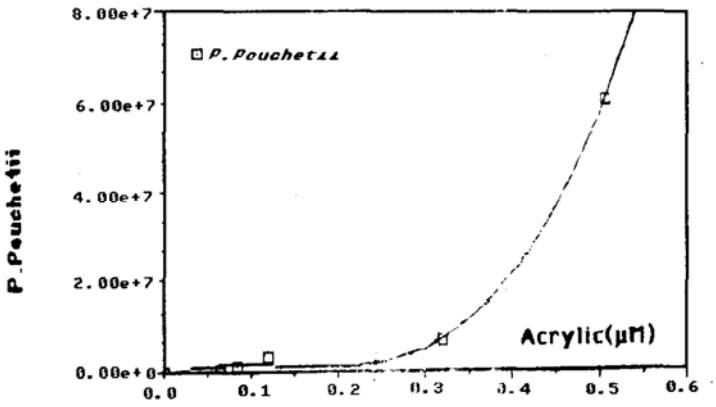

Fig. 4 Relation between *P. pouchetii* and Acrylic acid

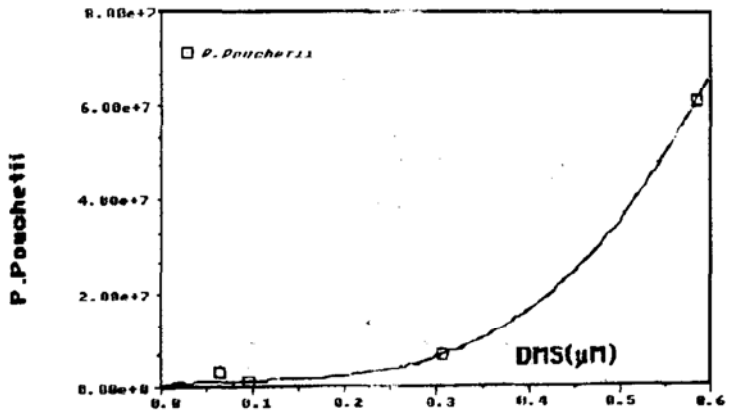

Fig. 5 Relation between *P. pouchetii* and DMS

Discussion

Table 1 and Fig. 2 and 3 show that the concentrations of arylic acid and DMS are correlated with the cells number of *P. pouchetii* during a summer bloom. During the growth phase of *P. pouchetii* bloom, the cell number increased rapidly with acrylic acids and DMS in sea water. In the senescence phase, although the concentration of acrylic acid and DMS were decreased with decreasing cell number, the amplitude of decreasing concentration were smaller than the speed of the cell declination. There is a positive correlation between acrylic acid, DMS and cell number. The calculation shows that the relative coefficient (R) was all 0.998 (see Fig. 4 and 5). It shows that the source of acrylic acid and DMS came from *P. pouchetii*. Acrylic acid produced by marine algae, which has been found in a broad spectrum of becteriocides (Siebruth, 1961). A cellular product, Dimethyl sulfonium propionate (DMSP), is cleft in acrylic acid and DMS (Sieburth, 1960), and the formation of DMSP is most probably from methionine. This compound has efficiently supplied the sulphur,

methylcarbon and hydrogen required for DMSP formation (Greene, 1962), though glycine has also been considered as the methyl donor (Vairavamurthy *et al.*, 1985). Our study indicates that dissolved free amino acids in sea water were decreased with *P. pouchetii* growth (Hefu Yang *et al.*, 1990). It suggests also that acrylic acid and DMS are produced

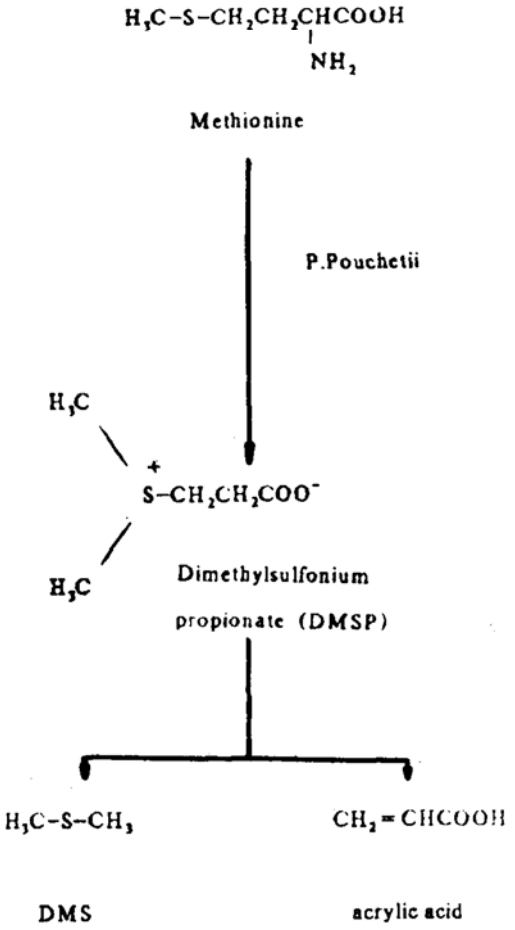

Fig. 6 Production pathway of acrylic acid and DMS.

by *P. pouchetii* through the way as show in Fig. 6. Table 2 shows the different natural productivity of acrylic acid and DMS during each growth and decline phases of *P. pouchetii*. In early growth phase (23 November to 14 December 1988), total increasing number of cells were 482. 8 cell • L^{-1} per day, the growth rate (r) is 0. 051 and the natural productivity of acrylic acid and DMS were 7.901×10^{-8}μmol • $cell^{-1}$ and 6.237×10^{-8}μmol • $cell^{-1}$ respectively. Both of the increasing rates (r) were 0. 019 and 0. 005, respectively. After that, the increasing rate of cells number was 441908. 5 cells per day from 14 to 20 December, and the growth rate (r) was 0. 904. This growth rate is 17. 725 times as that during early growth phase. However the natural productivity of acrylic acid and DMS was 9.758×10^{-8} μmol • $cell^{-1}$ and 13.090×10^{-8} μmol • $cell^{-1}$, and their increasing rate (r) was 0. 808 and 0. 491, respectively. Thus the natural productivity was just 1. 235 times and 2. 098 times respectively as those in early growth phase. The increasing rate were 42. 36

times and 83. 8 times. During 20 December 1988 and third January 1989, cells number increased to 3797329. 9 cell • L^{-1} per day, but the growth rate (r) of cells decreased to 1. 261 times, r = 0. 212, and the natural prodctivity of both compounds also decreased to 2. 034 × 10^{-8} μmol • $cell^{-1}$ and 1. 521 × 10^{-8} μmol • $cell^{-1}$, respectively, which had decreased by 1. 797 times and 8. 606 times as those in early growth phase. Their increasing rate (r) was 0. 071 and 0. 159, which had decreased by 11. 338 times and 3. 088 times as those in early phase.

Table 2. The natural productivity and increased rate of acrylic acid, DMS and cells growth rate during a summer bloom of P. pouchetii.

line days	cell growth rate (r*)	cell production cells/Ld	acrylic acid increased (r*)	DMS Increased rate (r*)	acrylic acid productivity μmol/cell	DMS productivity μmol/cell
328—349	0. 051	482. 8	0. 019	0. 005	7. 901 × 10^{-8}	6. 237 × 10^{-8}
349—355	0. 904	441908. 5	0. 805	0. 491	9. 758 × 10^{-8}	13. 090 × 10^{-8}
355—369	0. 212	3797329. 2	0. 071	0. 159	2. 034 × 10^{-8}	1. 521 × 10^{-8}
369—383	−0. 157	−3559487. 3	−0. 034	−0. 047	1. 838 × 10^{-8}	2. 930 × 10^{-8}
383—397	−0. 152	−392180. 9	−0. 096	−0. 084	3. 018 × 10^{-8}	2. 542 × 10^{-8}
397—411	−0. 454	−52735. 7	−0. 104	−0. 111	2. 399 × 10^{-8}	2. 106 × 10^{-8}

* $r = \frac{\log_e N_t - \log_e N_0}{t - t_0}$ $\quad N_t = N_0 e^{rt}$

These results show that the cell number increased with time, but the growth rate varied during the growth phase, the concentrations of acrylic acid and DMS were the same as cells number increased with time, but thier increasing rate and natural productivities were the same as growth rate of cells and have only a little difference between them in various areas.

The correlation of increasing rates of acrylic acid, DMS and cells growth rate is : the relation between acrylic acid and cells growth rate is, $Y = 0.905X - 0.035$, $R = 0.980$; and the relation between DMS and cells growth rate is, $Y = 0.558X - 1.957$, $R = 0.990$.

These excellent correlations indicate that acrylic acid and DMS were produced by cells division and metabolism during growth phase of *P. pouchetii*.

There exists some difference in variations of cell number, decreasing rate of acrylic acid and DMS between the growth phase and senescence phase of *P. pouchetii*. These variations were smoother during senescence phase of *P. pouchetii*. Total cell number decreased to 355947. 3 cell • L^{-1} during first decline phase from third January to 17 January 1989. The growth rate was −0. 157. and cells number decreased to 392180. 9 cell • L^{-1} from 17 Janurary to 31 January. The growth rate was −0. 152, close to that in first decline phase on third January. In the last decline phase the cell dead rate was higher than that in early phase, r = −0. 488.

The concentrations of acrylic acid and DMS decreased with the decreasing cells number during decline phase, but both productivities increased. From 3 to 17 January, the

concentration of acrylic acid decreased from 0.510 μmol • L^{-1} to 0.319 μmol • L^{-1}, and the increasing rate (r) was −0.034. The concentration of DMS also decreased from 0.588μmol • L^{-1} to 0.307μmol • L^{-1} and decreasing rate (r) was −0.047. Both of the lowest productivities found in this phase were 1.938×10^{-8}μmol • $cell^{-1}$ and 2.930×10^{-8} μmol • $cell^{-1}$, respectively. After that, the concentrations and increasing rate of both continuously decreased, but their natural productivities increased. The increasing rates (r) of acrylic acid and DMS were −0.096 and −0.084, respectively, The natural productivities were 3.018×10^{-8}μmol • $cell^{-1}$ and 2.542×10^{-8} μmol • $cell^{-1}$ from 17 to 31 Jannuray 1989. This increasing rate of acrylic acid reduced by 2.824 times than that in early phase, but the natural prodctivity increased by 16.42 times. The increasing rate of DMS was 1.787 times and its productivity increased by 8.675 times relative to that in early phase. During the last decline phase (from 31 Jan. to Feb.) of *P. pouchetii*, the concentration and increasing rate (r) continuously reduced. The concentrations of acrylic acid and DMS decreased by 4.368 and 4.700 times, and the increasing rate (r) reduced by 1.08 times and 1.321 times, respectively. The productivity of acrylic acid also decreased to 2.399×10^{-8} μmol • $cell^{-1}$, but productivity of DMS increased to 2.916×10^{-8} μmol • $cell^{-1}$. However, the concentrations and increasing rates of acrylic acid and DMS reduced with the cell's lessening, and the productivities of them increased with the cell's lessening during all decline phase of *P. pouchetii* (see Table 2). This may be related to the rate of diffusion loss of both compounds in water except the material released by cell division (diffusion coefficient of DMS, K=0.133d)

Conclusion

Acrylic acid and DMS were mainly produced by cells division and metabloism during growth phase of *P. pouchetii*. The growth rate of cells was restricted to the natural productivity of acrylic acid and DMS and the concentrations of acrylic acid and DMS were controlled by total cells number of *P. pouchetii* in sea water.

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