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EFFECT OF HYDROSTATIC PRESSURE ON THE CELL WALL OF *CALLITHAMNION CORYMBOSUM* (SMITH) LYNGB.*

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Abstract

Callithamnion corymbosum exposed to 400 atm for 5,10 & 20 hr caused no visible after effect, while subjection to 500 atm resulted in the swelling of longitudinal and transverse walls. The intensity of swelling increased with the rise of pressure level as well as the duration of its action. The thickening produced after short exposures gradually disappeared, but a long exposure for 20 hr caused an irreversible swelling resulting in ultimate death of the organisms. A treatment to 800 atm was lethal for the survival of the algae. No vacuolization of the protoplast and no black spots and circles were observed on the cell walls of pressure treated fronds.

Introduction

Nearly 56% of the biosphere is subjected to pressures ranging from 100 to 1150 atm, hydrostatic pressure is therefore an environmental master factor affecting biological systems. Information regarding the effects of high hydrostatic pressure on marine algae is very scanty (Shameel, 1973c), beacuse unfortunately until now the pressure is not considered as an important variable in the case of marine algae (Sleigh & MacDonald, 1972; Chapman & Chapman, 1973; Stewart, 1974). We know very little about the casuses for the observation that some of the coast inhabiting benthic algae are extreme barophobes, while others are extreme barodures (Shameel & Ohno, 1972). Studies regarding pressure effects on distribution (Vidaver, 1972), morphology (Saifullah & Steven, 1973), reproduction (Shameel, 1973b), growth (Shameel, 1974a) and the rate of O_2 gas exchange (Vidaver, 1969; Shameel, 1973a) of marine algae at the organismal level are however few, but the pressure effects at the cellular level are very poorly known (Shameel, 1974b). Observation of abnormalities in the cell walls of *Polysiphonia* spp. due to high hydrostatic pressure (Shameel, 1973d) gave a stimulus for the present work.

Materials and Methods

Callithamnion corymbosum (Smith) Lyngbye was dredged from 9-12 m depth near "Boknis Eck" (Kiel Bight, Baltic Sea) from the board of R.C. "Hermann-Wattenberg" and was determined after Pankow (1971). The algae were brought together with the sea water of their occurence (adjusted to 15 ‰ S) to the constant temperature rooms and cultured as described previously (Shameel, 1973b). After keeping the algae for 2 weeks' gradual adaptation to 15°C (experimental temperature) healthy specimens were selected and subjected to hydrostatic pressure as mentioned earlier (Shameel, 1973d). Three experimental series of 5, 10 and 20 hr durations were conducted, the pressure levels used were 400, 500, 800 and 1000 atm. After pressure release the algae were examined in culture medium as described by Shameel (1973b). The cell walls of pressure treated and control specimens were regularly observed under microscope upto 3 weeks.

*Dedicated to the memory of my teacher, Prof. Fritz Gessner on his 2nd death anniversary, who provided full facilities and desired this research to be accomplished.

TABLE 1. Effect of hydrostatic pressure applied for different durations at 15°C on the cell wall of *Callithamnion corymbosum* as observed immediately after pressure release.

Hydrostatic pressure (atm)	Duration of pressure action		
	5 hr	10 hr	20 hr
400	no effect	no effect	no effect
500	minimum thickening	increased thickening	thickening still increased
800	maximum thickening	dead cells	dead cells
1000	dead cells	dead cells	dead cells

Results and Discussion

C. corymbosum is a delicate alga and possesses filamentous, radially branched fronds, which are usually monosiphonous (Fott, 1971; Chapman & Chapman, 1973), and thus it provides suitable material for the study of cell morphology. Being a member of Ceramiaceae it offers a good opportunity for comparison with the observations made on *Polysiphonia* spp. (Shameel, 1973d) belonging to Rhodomelaceae, both are the families of Ceramiales (*Florideophyceae*, Wartenberg, 1972). Due to these reasons it was selected for study. After treatment to hydrostatic pressure of 400 atm for all the three durations of pressure action no effect was observed on the cell walls of *C. corymbosum* (Table 1) and these algae showed no after effect even upto 21 days (Table 3). But the application of a pressure of 500 atm for 5 hr caused a minor thickening of longitudinal as well as transverse walls as compared with control algae (Table 2). This thickening gradually disappeared and after 2 weeks there was practically no difference between the pressure treated and control specimens (Table 3). This appears to mark the lower limit of tolerance of the alga, as the pressure induced change was reversible. In this physiological characteristic it resembled with its taxonomic akin *Polysiphonia* quite closely. The peculiar type of black spots and circles produced on the cell walls of *Polysiphonia* spp. after a pressure treatment of 500 atm, however, could not be observed in *C. corymbosum*. Probably it is due to the reason that taxonomically related algal genera have evolved such enzyme systems under the similar environmental conditions, which resemble in certain physiological characteristics and differ in the others.

TABLE 2. Thickening of the cell wall of *Callithamnion corymbosum* as measured immediately after the application of hydrostatic pressure at 15°C.

Hydrostatic pressure (atm)	Duration of pressure (hr)	Part of the thallus	Thickness of the cell wall (μ)	
			Longitudinal wall	Transverse wall
500	5	upper	0.3— 0.7	0.3— 0.6
		middle	0.7— 2.2	0.6— 2.0
		lower	4.1— 4.7	3.9 — 4.6
500	20	upper	0.5— 1.3	0.5— 0.8
		middle	2.5— 5.0	1.5— 3.0
		lower	10.4—18.7	6.9—17.6
800	5	upper	0.8— 1.8	0.6— 0.9
		middle	2.7— 5.4	1.8— 3.6
		lower	14.4—21.6	7.2 - 20.6
Control algae		upper	0.2 — 0.5	0.2— 0.5
		middle	0.5— 1.8	0.5— 1.0
		lower	3.0— 3.6	3.0— 3.6

After a pressure treatment of 500 atm for 10 hr the peculiar swelling of cell walls in the entire thallus of *C. corymbosum* was intensified. This change was also reversible, though it required a very long time to normalise (Table 3). When the pressure duration was increased to 20 hr, the thickening of the cell wall was still intensified (Table 2), but this time it was an irreversible change. The algae died in 3 weeks. A pressure of 800 atm for 5 hr resulted in maximum thickening of cell walls (Fig. 1&2), and the algae died in 5 days. A further increase of pressure duration appeared to be lethal for the survival of algae (Table 1). This suggests that the intensity and the duration of action of pressure must be considered together, both collectively consitute the quantity of stimulus. The increasing intensity of the swelling of cell wall and a change of reversible condition to irreversible one leading to the death of treated algae indicate that hydrostatic pressure does not only bring about the structural changes in the cell but also affects its normal metabolic pathways.

The application of a hydrostatic pressure of 800 atm caused either an immediate death or killing within a few days (Table 3). This probably sets up the upper limit of tolerance of the alga. In this physiological characteristic *C. corymbosum* differed from *Polysiphonia* spp. (Shameel, 1973d). The pressure range of the general responses in algae mentioned by Vidaver (1969) are not narrowly delineated and there is an appreciable overlapping among them. In the overall behaviour under pressure *C. corymbosum* appears

TABLE 3. Percentage of changes left in the cell wall of *Callithamnion corymbosum* after subjection to hydrostatic pressure at 15°C.

Hydrostatic pressure (atm)	Duration of pressure (hr)	Days after subjection to pressure						
		0	1	3	5	7	14	21
400	20	0	0	0	0	0	0	0
500	5	100	90	70	50	30	0	0
	10	100	100	90	80	70	50	30
	20	100	100	100	100	100	100	dead
800	5	100	100	100	dead	—	—	—
	10	dead	—	—	—	—	—	—

to be pressure sensitive than *Polysiphonia* spp., though the former is constitutionally simpler than the later. This does not agree with the previous findings made on marine animals that the pressure sensitivity increases with the increasing complexity of the body organisation (Menzies & George, 1972; Ponat & Theede, 1973). Whether marine algae behave differently, in this regard, than marine animals can not be concluded with certainty at this stage. The unusual type of vacuolization observed within the protoplast of pressure treated filaments of *Cladophora glomerata* (Shameel, 1973b) was not noticed in the case of *C. corymbosum* after all the pressure levels. It suggests that different algae of similar constitution differ in their behaviour against hydrostatic pressure due to the differences in their genetically fixed characteristics.

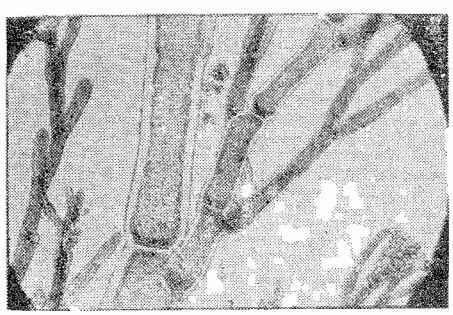

Fig. 1

Maximum thickening in the cell walls of *Callithamnion corymbosum* as observed immediately after a pressure treatment to 800 atm for 5 hr at 15°C.

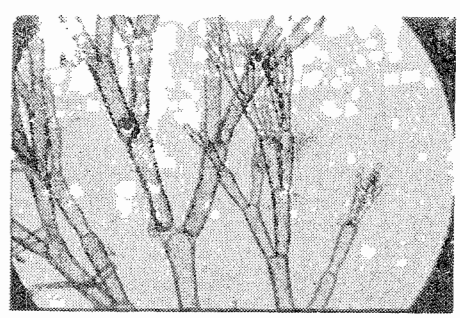

Fig. 2

Control thallus of *C. corymbosum* under the same experimental conditions, but kept at normal atmospheric pressure.

Our observation confirms that *Polysiphonia* is not an exceptional genus, but there are other algae as well, in which the cell wall undergoes a swelling under hydrostatic pressure. The causes and mechanism of this thickening are difficult to be explained. Most probably it involves the processes of deploymeration of the constituent polysaccharide microfibrills in the cell wall, whereby elongated macromolecular complexes are converted to a number of monomeres. But for a real picture we have to widen our knowledge regarding pressure effects on biological polymers and enzyme systems of marine organisms (ZoBell & Kim, 1972; Shameel, 1974c). Cellulose, chitin, starches and agar-agar are the most common biological polymers produced in the sea by various groups of marine algae (Chapman, 1970). But no attempt has so far been made to elucidate the effect of hydrostatic pressure on these polysaccharides or their degradation processes, which are equally important, as the algal polysaccharides do not accumulate in the marine environment (Kim & ZoBell, 1972). The cell wall of Rhodophyta usually contains cellulose, polygalactose sulphate esters, chitin and gulose (Chapman & Chapman, 1973), but the information regarding the arrangement of polysaccharide microfibrills in different layers and the angles in which they cross one another is highly insufficient.

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