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Growth and Cytology of *Chlamydomonas acidophila* Under Acidic Stress

I. Visviki, J. Palladino

Department of Biology, College of Mount Saint Vincent/Manhattan College, Riverdale, NY 10471, USA

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Acid deposition has attracted considerable attention in both the scientific literature and the popular press over the last thirty years. Despite reductions in SO_2, NO_x and volatile organic carbon emissions following the 1990 Clean Air Act Amendments, there have been no changes in US rain acidity (Howells 1995). Furthermore, many lakes and streams particularly concentrated in the Adirondacks, the mid-Appalachia, the mid-Atlantic Coastal Plain and the High Elevation West remain acidified or are undergoing episodic acidification.

Acidification of aquatic environments is accompanied by profound changes in phytoplanktonic communities (Anderson et al. 1997), including reduction of species richness due to the disappearance of sensitive species and the proliferation of tolerant ones (Turner et al. 1991). At the biochemical level, increasing concentrations of hydrogen ions interfere with nutrient uptake and influence the availability, speciation and toxicity of aquatic pollutants (Lustigman et al. 1995). While the effects of hydrogen or hydroxyl ions on algal physiology have been extensively documented (Gimmler et al. 1988; Tatsuzawa et al. 1996), their effects on the fine structure of algal cells have been neglected altogether. Although ultrastructure is an insensitive indicator of stress, when examined in association with more sensitive parameters, it can provide valuable insight on mechanisms of toxicity and tolerance (Visviki and Rachlin 1992; 1994). The present study attempts to remedy this paucity of data, by examining the growth response of *Chlamydomonas acidophila* Negoro to varying pH, and correlating it with alterations in the cytology of the chlorophyte. This species has been selected because some of its strains have been isolated from acidic bogs and can grow at pH 2.0 (Cassin 1974). Thus, it would be interesting to see whether it has special adaptations for survival in acidic environments.

MATERIALS AND METHODS

Algal specimens of *C. acidophila* were obtained from the University of Toronto Culture Collection (UTCC # 121). Axenic cultures were grown on liquid Bristol's medium (Bold 1949) to which proteose has been added (1g proteose/liter Bristol's). Growth conditions and growth experiments were identical to those

Correspondence to: I. Visviki

described by Visviki and Santikul (2000). Growth data were subjected to analysis of variance to establish statistically significant differences.

To evaluate the effects of hydrogen ions on the cytology of *C. acidophila*, cells were exposed to pH 3.4, 4.4 and 6.4 for five days, which is the duration of the logarithmic phase under optimum growth conditions. Subsequently, cells were prepared for transmission electron microscopy following standard procedures (Visviki and Santikul 2000).

The cells were examined with a Hitachi HS-9 transmission electron microscope at a magnification of 7000x. Over forty photomicrographs per treatment, each representing a random section through a different cell, were obtained. Sections containing only cell wall or cell wall with no identifiable features were excluded. Volume estimates of various cellular components were obtained by grid point counting. Using the procedures of morphometric analysis (Sicko-Goad and Stoermer 1979) the cells were analyzed for cell volume, relative volume of the chloroplast, pyrenoid, starch, nucleus, nucleolus, Golgi apparatus, vacuoles, cell wall and mitochondria. The mean and standard error were calculated for each measurement. In addition, morphometric data were subjected to analysis of variance to determine statistically significant differences between control and experimental cells.

RESULTS AND DISCUSSION

The majority of algae grow optimally at a circumneutral pH range. Some micro-algae can thrive in more acidic environments, such as acid streams, *Sphagnum* bogs and volcanic lakes (Cassin 1974; Hargreaves and Whitton 1976; Tatsuzawa et al. 1996). Those that grow best at pH 5 - pH 3 are considered acidotolerant, while those that grow optimally at lower pH values are considered acidophilic (Langworthy 1978). In the literature, however, the term acidophile is often applied *sensu lato* to describe any organism that can exhibit good growth below pH 6 (Hoham and Mohn 1985). Our strain of *Chlamydomonas acidophila* is not acidophilic, *sensu stricto,* growing best in media ranging from 6.4 to 8.4 (Fig. 1). Analysis of variance showed that growth at pH 5.4 is significantly reduced compared to pH 7.4. At pH 3.4 and 4.4 growth is greatly compromised, it is significantly different from that obtained at 5.4 and a logarithmic phase of growth is lacking. There is no growth at pH 1.4 and 2.4. The disparity between our data and those previously reported in the literature (Cassin 1974) is due to the fact that species are aggregations of many local populations (strains) which do not have identical ecological, physiological or biochemical characteristics. While some strains of *C. acidophila* isolated from extreme environments such as bogs, might have the ability to thrive there, other strains such as ours, which have not been subjected to such strong selective pressures lack the appropriate adaptations for survival in strongly acidic environments.

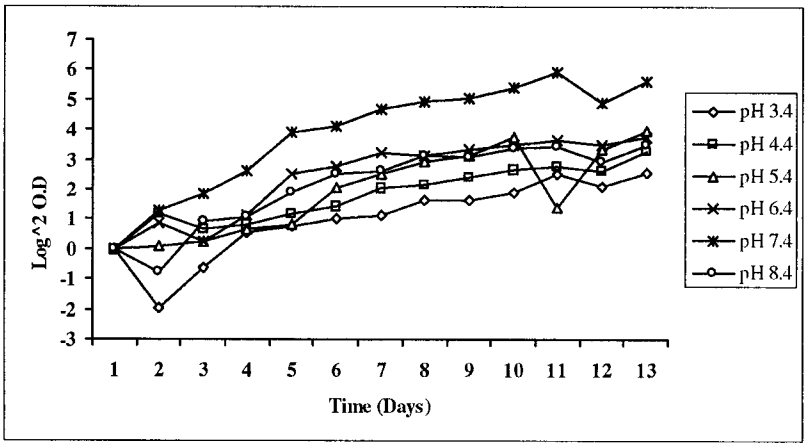


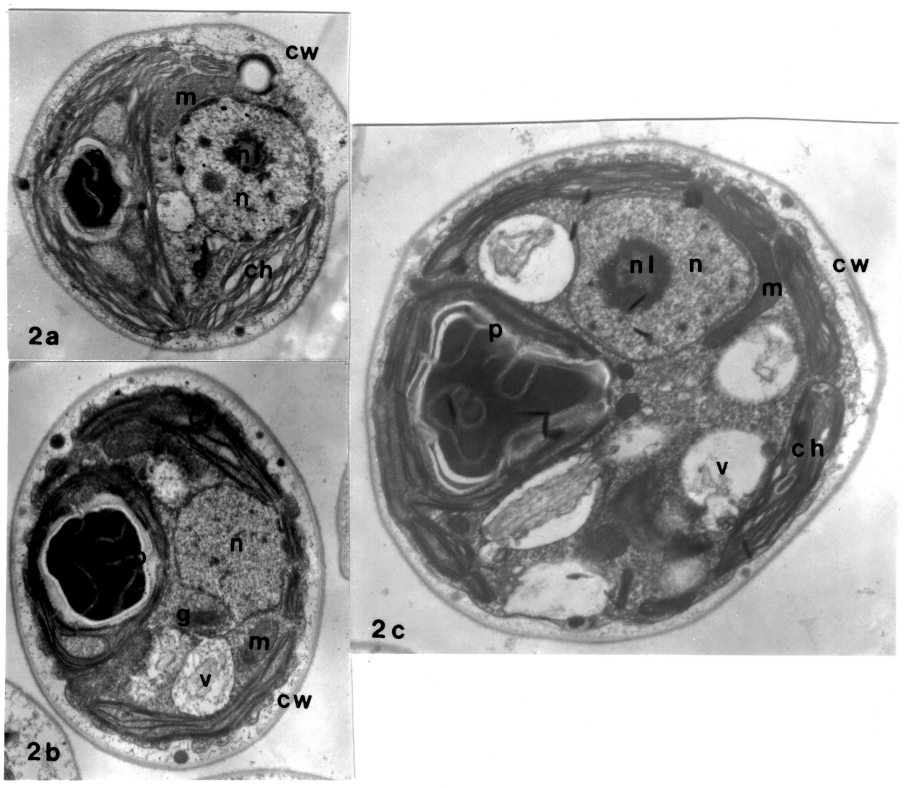
Figure 1. Growth response of *Chlamydomonas acidophila* to pH values varying from 3.4 to 8.4.

The cytological effects induced by five-day exposure to pH 4.4 and 3.4 respectively are shown in Table 1. Preliminary examination showed no cytological differences between cells exposed from pH 5.4 to 8.4, so pH 6.4 was randomly selected to serve as control. Exposure to pH 4.4 results in a heterogeneous culture consisting of autospore colonies (Fig.2), as well as single cells.

Table 1. Cytological profiles (mean ± standard error) of *C. acidophila* exposed to pH 6.4, 4.4, and 3.4.

	pH 6.4	pH 4.4	pH 3.4
Cell volume	64.78 ± 3.94	81.09 ± 4.84*	126.26 ± 8.11*
Chloroplast vol.	0.45 ± 0.03	0.39 ± 0.02	0.35 ± 0.02*
Pyrenoid vol.	0.05 ± 0.06	0.07 ± 0.01	0.06 ± 0.01
Starch vol.	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.04
Nuclear vol.	0.16 ± 0.03	0.15 ± 0.01	0.11 ± 0.01
Nucleolar vol.	0.02 ± 0.01	0.02 ± 0.004	0.02 ± 0.003
Cell wall vol.	0.08 ± 0.01	0.06 ± 0.05	0.06 ± 0.01
Golgi vol.	0.02 ± 0.003	0.01 ± 0.003	0.01 ± 0.002
Mitochondrial vol.	0.04 + 0.01	0.04 ± 0.01	0.03 ± 0.004
Vacuolar vol.	0.09 ± 0.01	0.09 ± 0.01	0.22 ± 0.02*
Chlor. lipid vol.	0.01 ± 0.003	0.02 ± 0.004	0.01 ± 0.003
Chlor. lipid number	10.6 ± 1.35	8.33 ± 0.83	6.18 ± 1.19

*Denotes statistically significant difference at $P < 0.05$.



Figures 2-5. cf = cleavage furrow, ch = chloroplast, cw = cell wall, f = flagellum, g = golgi, m = mitochondrion, n = nucleus, nl = nucleolus, p = pyrenoid, pw = parental wall, v = vacuole. Magnification =18,000x.

Figure 2. Cross section through a cell exposed to a) pH 6.4, b) pH 4.4 and c) pH 3.4.

In synchronized *Chlamydomonas* cultures, cells divide during the dark cycle to form four or more autospores contained within the parental wall. Subsequently, the parental wall breaks open releasing the daughter cells. This process is facilitated by the proteolytic enzyme V-lysin (Waffenschmidt and Jaenicke 1990), which digests the glycoproteinaceous mother cell wall. Autospores are not normally present during the light cycle in young *C. acidophila* cultures. In cultures, however, that have been exposed to pH 4.4 20% of the cells are autospores, indicating that hydrogen ions denature V-lysin. Single vegetative cells are present, but they are 25% larger than controls (Fig. 3).

Both trends are more pronounced in cultures exposed to pH 3.4. Here, approximately 95% of the cells are autospores, while the remaining vegetative cells

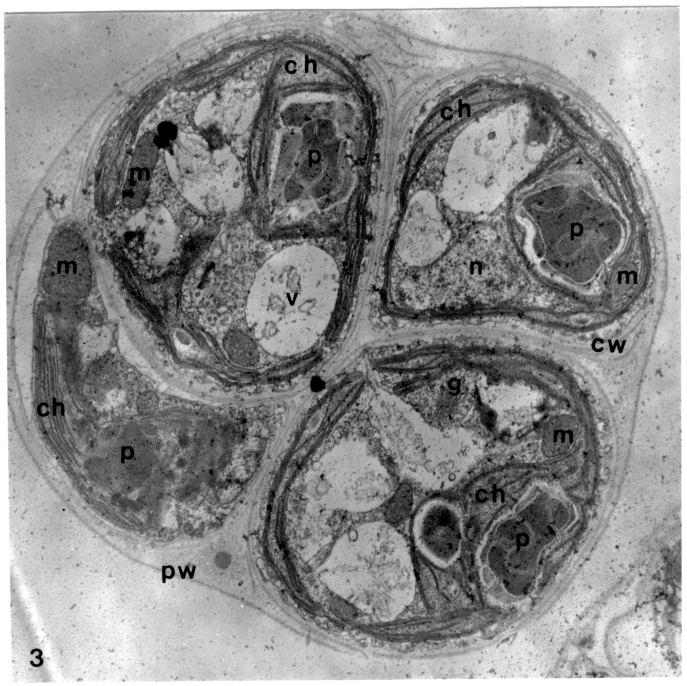


Figure 3. Four autospores contained within their parental cell wall.

are giant in size (Fig. 3) being double the volume of control cells. Similar morphological alterations were observed by Hargreaves and Whitton (1976) in *Euglena mutabilis* exposed to pH 1.3-1.5. This increase in cell volume is not due to the proliferation of the cell wall as seen in *Chlamydomonas applanata* under similar experimental conditions (Visviki and Santikul in 2000). It could be the result of decoupling of cell division and growth and/or an osmotic effect. In addition, these cells have 22% smaller chloroplasts. These results are congruent with those reported with *Hormidium rivulare* (Hargreaves and Whitton 1976) and *C. applanata* (Visviki and Santikul 2000) and indicate that the hotosyntheticability of the chlorophytes is compromised by increasing concentrations of H^+. Furthermore, the volume of the vacuome increases 144 % and is accompanied by pronounced autophagic activity (Fig. 4). Increased vacuolation is commonly seen in old or stressed cells (Visviki and Rachlin 1994). Despite such adverse effects cell divisions seem to proceed normally (Fig. 5). Indeed, *C. acidophila* exposed to pH 3.4 seems to fare better than its congener *C. applanata*. The latter exhibits a variety of cytological effects, including death, proliferation of palmelloid colonies (large aggregations of vegetative cells), appearance of akinetes (dormant cells), inhibition of motility and abnormal cell division (Visviki and Santikul 2000) not seen in *C. acidophila*.

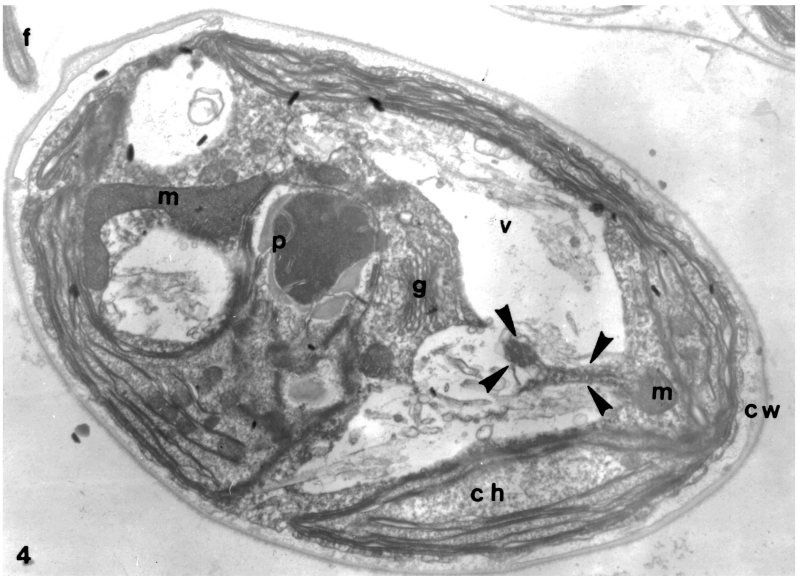


Figure 4. Longitudinal section through a cell exposed to pH 3.4. Note the segment of the mitochondrion (arrows) undergoing autolysis within the large vacuole.

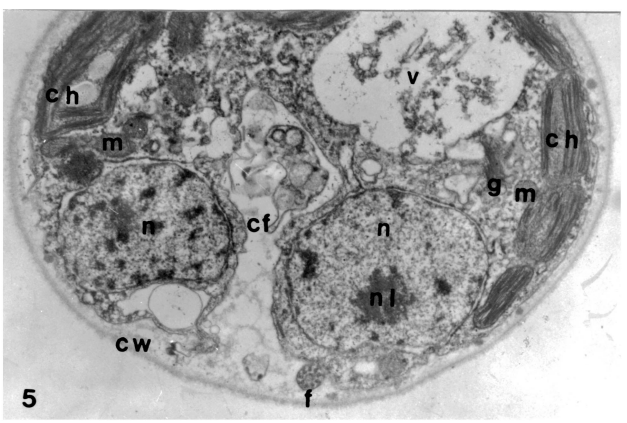


Figure 5. Cross section of a cell exposed to pH 3.4 undergoing cell division.

Several mechanisms of tolerance have been proposed to account for the ability of some microalgae to survive and thrive in acidic environments. These include:

1) The presence of special enzymes which are not denatured by hydrogen ions. 2) Increased fatty acid saturation of membrane lipids, which prevents the influx of hydrogen ions (Tatsuzawa et al. 1996). 3) The presence of a more efficient algal pump, which removes excess protons externally (Umbach 1985) or sequesters them internally in vacuoles (Raven 1976). In the absence of biochemical data it is impossible to determine whether the first two mechanisms play any role in *C. acidophila*. The third mechanism requires continuous expenditure of energy. *Chlamydomonas acidophila* possesses large mitochondria, which occupy 4% of the cell (Table 1). While this value might appear small, it is higher than those reported for other *Chlamydomonas* species (Harris 1989), as well as other chlorophytes whose mitochondrial volumes range from 1-3% (Visviki and Rachlin 1992; 94). Larger mitochondria, theoretically, could provide the energy needed to mobilize protein pumps and to counteract the passive influx of hydrogen ions. The inability of *C. acidophila* strain # 121 to thrive in acidic media, despite its presumed advantage, probably indicates that success in such extreme environments is the result of several adaptations/mechanisms rather than one.

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