ELSEVIER

Thermochimica Acta 397 (2003) 37–47

thermochimica acta

www.elsevier.com/locate/tca

The energetic stress response of the microalgal *Chlorella vulgaris* to the mycoplasma, *Acholeplasma laidlawii* as a model system for plant–pathogen interaction☆

N.L. Loseva[a,*], A.Ju. Alyabyev[a], L.Kh. Gordon[a], I.N. Andreyeva[a], O.P. Kolesnikov[a], A.A. Ponomareva[a], V.M. Chernov[a], R.B. Kemp[b]

[a] *Institute of Biochemistry and Biophysics, Russian Academy of Sciences, 420503 Kazan, Russia*
[b] *Institute of Biological Sciences, University of Wales, Aberystwyth, SY23 3DA Wales, UK*

Received 11 September 2001; received in revised form 25 April 2002; accepted 29 April 2002

Abstract

This paper reports the early energetic response of plant cells to infection by pathogenic mycoplasma. The microalgal cells of *Chlorella vulgaris* were used as a plant model system to investigate the stress responses against the Mollicute (mycoplasma) *Acholeplasma laidlawii*. The chlorella cells were cultured under various physiological conditions and the dependence of the defence responses to the action of the different concentrations of pathogen were studied in detail. The mycoplasma triggered a substantial increase in the rate of heat production and caused an enhanced oxygen uptake rate, which were regarded to be manifestations mostly of the production of the superoxide anion radical as one of the active oxygen species (AOS).

Differences were observed in the intensity and duration of the "heat burst" and the "oxygen burst" under the different conditions of the experiments and were interpreted in terms of the oxygen metabolism to form AOS, particularly, the superoxide anion radical, that are important in the host defence. Control chlorella cells gave a calorimetric–respirometric (CR) ratio of -509 ± 73 (S.E.) kJ/mol O_2, within the calculated range for the oxycaloric equivalent (-509 ($\pm 15\%$) kJ/mol O_2) and meaning that the catabolic process was fully oxidative. At least part of the reason for the differences between the two "bursts" to give a CR ratio of -575 kJ/mol O_2 at 2.5×10^6 mycoplasma cells/ml chlorella culture (5.3×10^8 cells/ml) is the fact that the mycoplasma are facultative anaerobes. There is evidence that a humoral factor produced by the mycoplasma elicits an early response by the host when added as a cell-free supernatant. Besides the immediate, primary response to the interaction seen in energetic terms, electron microscopic evidence is presented to show that the mycoplasma attached to the host cells and probably served to prolong the host defence response.

Keywords: *Chlorella vulgaris*; *Acholeplasma laidlawii*; Heat production rate; Superoxide anion radical; Plant–pathogen ultrastructure

1. Introduction

It is known that the infection of plant cells by a variety of different pathogens results in the appearance of a transient rise in the oxygen uptake rate, the so-called "oxidative burst" [1]. It is supposed that an oxidase in the plasma membrane catalyses the formation of

☆ Presented at the 12th Conference of the International Society for Biological Calorimetry, 7–11 September 2001, Santiago de Compostela, Spain.

* Corresponding author. Tel.: +7-8432-38-3203; fax: +7-8432-38-7577.
E-mail address: loseva@sci.kcn.ru (N.L. Loseva).

0040-6031/02/$ – see front matter
PII: S0040-6031(02)00314-3

active oxygen species (AOS) that include the superoxide anion radical, hydrogen peroxide from NADPH and molecular oxygen [2–4]. According to our previous data, microalgal cells of *Chlorella vulgaris* in the dark react to the attack by the Mollicute (mycoplasma), *Acholeplasma laidlawii* by generating considerably enhanced amounts of the superoxide anion radical [5]. A heat "burst" measured by direct calorimetry accompanied this increase. As a necessary requirement for the claimed oxygen metabolism [5], the rate of oxygen uptake by the chlorella cells determined by indirect calorimetric polarography in the dark also increased to a marked extent [6]. It seems probable that the formation of the superoxide is only the initial step in a cascade of defence reactions by the chlorella cells to the mycoplasma infection.

In some cases, plants under the stress of pathogen attack are also in environmental conditions that exacerbate the stress, for instance, drought and/or extreme temperature. The effects of these combined insults require the type of investigation that can be achieved by using the present model system with its potential for rigorous biothermochemical analysis. Earlier work on this model has concentrated on the consequences of the infection for the catabolic processes operating in the dark and recorded by direct calorimetry. Clearly, there is a need to examine the effect on photosynthesising plant cells that evolve oxygen, while recognising that the formation of AOS should cause an apparent decrease in the rate of oxygen evolution. It is also important to investigate the nature of the host–pathogen interaction in physical terms. For this aspect, it was decided to employ electron microscopy.

The aims of this research are to study: (i) by direct and indirect methods the early energetic responses of chlorella cultures to infection by mycoplasma at different cell number concentrations, under temperature-induced stress and in the dark as well as light; and (ii) by transmission electron microscopy (TEM), the short-term interaction of the host with the pathogen.

2. Experimental

The unicellular microalga *C. vulgaris* and the Mollicute *A. laidlawii* were the subjects of this investigation. Chlorella cells were grown in the Tamiya medium, pH 6.8–7.2 [7] at 30 °C, and illuminated at 1×10^4 lx with a photoperiod of light/dark of 12/12 h. Cell suspensions were bubbled with 0.3% CO_2 in air. The optical density was maintained at $(1–1.5) \times 10^8$ cells/ml.

A. laidlawii was grown at 37 °C in test tubes using the Edward medium [8]. Before each experiment, the mycoplasma suspension was centrifuged at 20,000 × *g* in phosphate buffer for 10 min. Mycoplasma were re-suspended in the buffer with a titre of 10^7/ml viable cells and added to the cultures of chlorella in Tamiya medium at the ratio of 1:4. When appropriate, the suspension was further diluted in phosphate buffer prior to adding it to the host cells. The supernatant acted as the cell-free test material and was added to the chlorella cells in the same ratio. The numbers of living mycoplasma were determined both spectrophotometrically by measuring the culture density and observationally by isolating the mycoplasma on agar-medium, counting the colonies and expressing the culture titre as colony forming units (CFUs).

As an essential preliminary to the series of experiments, separate controls were made to discover if there was any influence on the physiological activity of both organisms of the mixed solution of Tamiya medium with the phosphate buffer in the ratio 4:1. Analysis of the data showed that the rates of heat production and oxygen uptake by the chlorella cells in the mixed solution were identical to that of the control in the Tamiya medium alone. The metabolic rates of *A. laidlawii* in the mixed medium were slightly greater than those in the phosphate buffer.

Chlorella cells were infected with the mycoplasma suspension immediately before the experimental observations (zero time). The samples were then divided into four aliquots in order to: (i) measure the rate of heat production; (ii) measure the rate of oxygen uptake/evolution; (iii) estimate the amount of superoxide formation with time; and (iv) fix the cells at specific time intervals for ultrastructural examination. With the exception of the direct calorimetry, the suspensions were cultured with continuous stirring and illuminated at 3×10^3 lx. The rate of heat production was measured in the dark, without stirring.

The oxygen uptake and evolution rates were measured by the polarographic method using a Clark-type electrode [9]. Each 3.2 ml sample was placed in a measuring tube located in a tight-fitting water bath and equilibrated to 30 °C in 3 min. Then a black box was fitted over the bath to allow measurement of the

oxygen uptake rate in the dark for 1 min. The box was removed and the cells were illuminated to record the rate of oxygen evolution for a further 1 min period.

The instantaneous rate of heat production was measured in the heat conduction LKB batch bioactivity monitor (BAM; the direct calorimetric successor is the thermal activity monitor (TAM) manufactured by Thermometric AB, Järfälla, Sweden) [10]. Suspensions of 1.5 cm^3 were placed in unstirred 3 cm^3 glass vessels that were hermetically sealed before thermal equilibration for 15–20 min. This means that zero time for measuring the heat production corresponded to 20 min after adding the mycoplasma to the chlorella culture.

The quantity of superoxide in the infected chlorella cultures was estimated using 1 mM epinephrine as described in [4]. The suspension was incubated with epinephrine for 10 min. Independent samples were taken at predetermined time intervals.

Growth of the chlorella culture was estimated from the optical density of the cell suspensions using a colorimeter [11].

Ultrastructural changes in the chlorella cells on interaction with the mycoplasma compared with the controls were viewed on a JEM 100 CX electron microscope. Samples were fixed in 2.5% (w/v) glutaraldehyde in phosphate buffer (pH 7.2) with post-fixation in 1% (w/v) OsO_4 solution. They were embedded in Epon-812 resin after dehydration. Thin sections were obtained with an Ultratome III microtome (LKB AB, Stockholm, Sweden) [12]. The data are the average of three experiments.

3. Results

The first set of experiments with different concentrations of mycoplasma was to determine the relationship of the chlorella stress response to the host/pathogen ratio. As seen in Fig. 1, the rate of oxygen uptake was twice that of the control after addition of *A. laidlawii* at high concentration (titre, 10^7/ml). Diluting the pathogen four-fold decreased the oxygen uptake rate to a value close to that of the control. The mycoplasma supernatant markedly enhanced the rate of oxygen uptake (Fig. 1).

As can be seen calorimetrically in Fig. 2, there was an initial steep rise in the rate of heat production approximately 15–20 min after inoculating the chlorella culture with the pathogen. This was due to the equilibration of the samples in the calorimeter. Afterwards, the rate of heat production by the treated cells was higher than by the control dependent on the cell number concentration of the pathogen. The effect

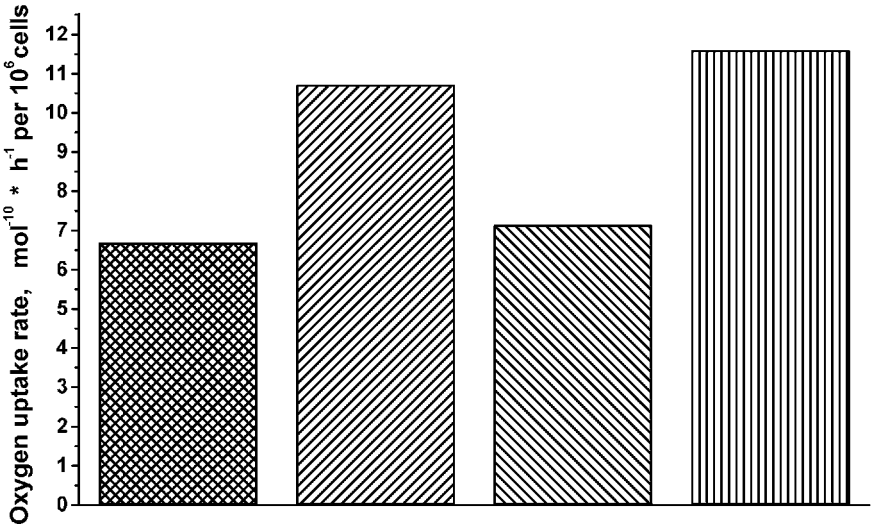

Fig. 1. The rates of oxygen uptake by *C. vulgaris* cells at 30 °C, 60 min after adding the different concentrations of *A. laidlawii*. (⊠) Chlorella alone, control (5.3×10^8 cells/ml). (▨) Chlorella + *A. laidlawii* (10^7 cells/ml). (▧) Chlorella + *A. laidlawii* (2.5×10^6 cells/ml). (▥) Cell-free *A. laidlawii* supernatant.

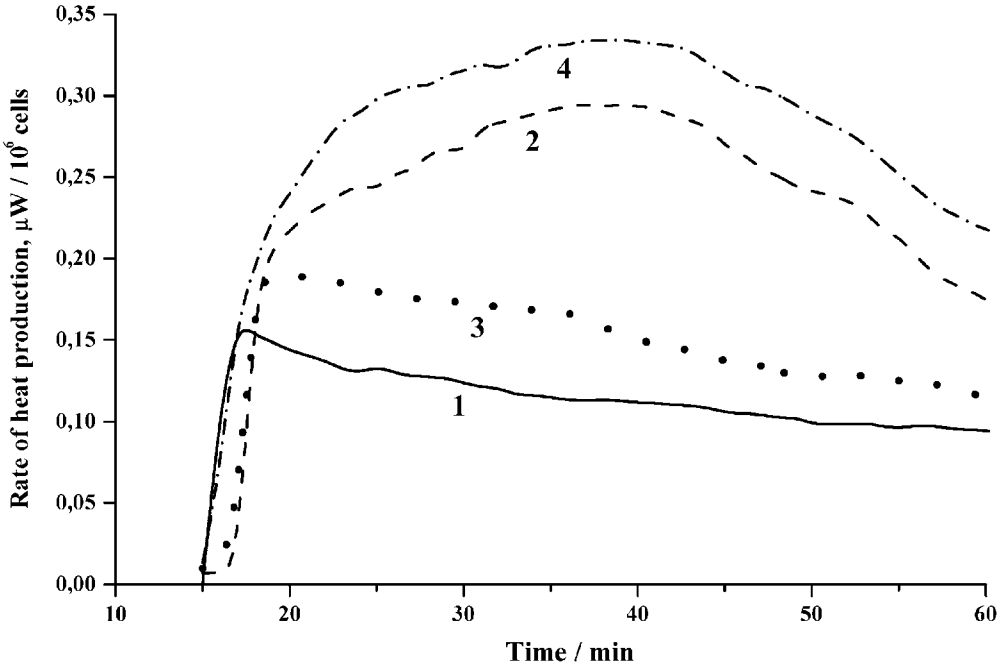

Fig. 2. The instantaneous rates of heat production by chlorella after exposure to different concentrations of mycoplasma at 30 °C. (1, —) Chlorella alone, control (5.3×10^8 cells/ml). (2, – – –) Mycoplasma (10^7 cells/ml) added to chlorella at zero time. (3, · · ·) Mycoplasma (2.5×10^6 cells/ml) added to chlorella at zero time. (4, – · – · –) Mycoplasma-free supernatant added to chlorella at zero time.

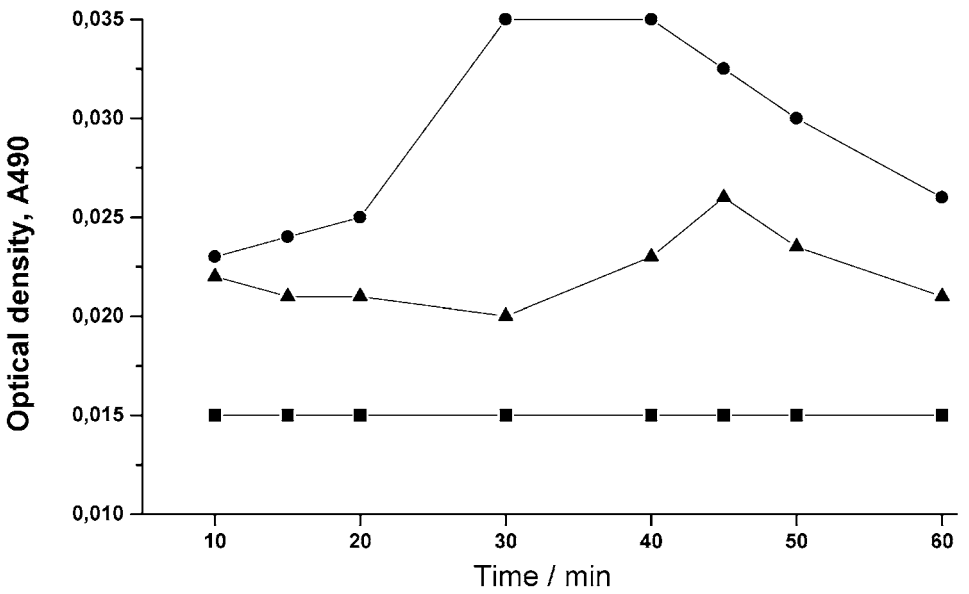

Fig. 3. Effect of mycoplasma and its cell-free supernatant on the production of the superoxide anion radical by chlorella at 30 °C. (■) Chlorella alone, control (5.5×10^8 cells/ml). (▲) Chlorella + mycoplasma (2.5×10^6 cells/ml). (●) Chlorella + mycoplasma-free supernatant.

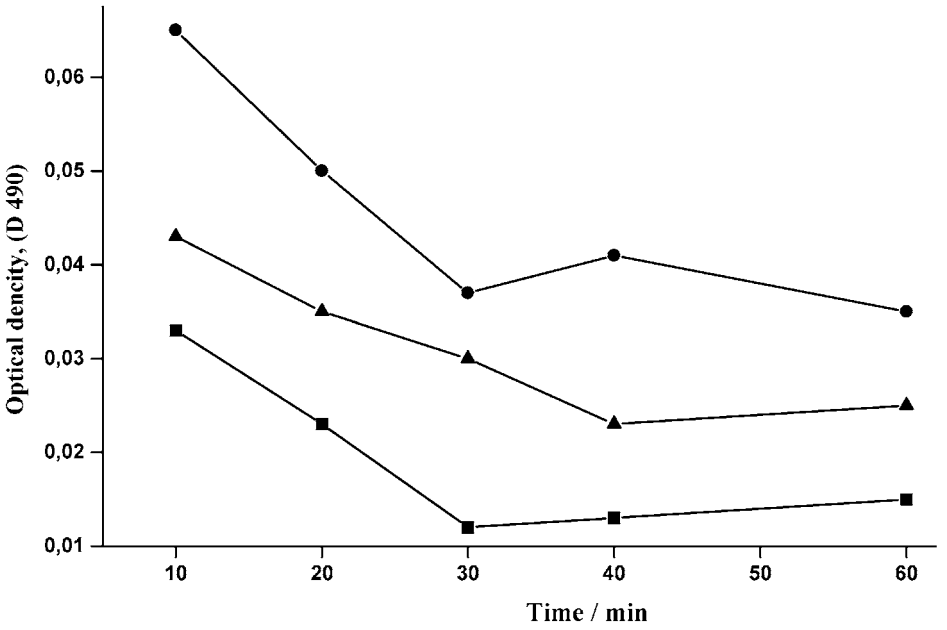

Fig. 4. Effect of heat shock on the production of the superoxide by chlorella exposed to the mycoplasma. (■) Control, chlorella at 30 °C (5.3×10^8 cells/ml). (▲) Chlorella exposure to high temperature (43.5 °C) for 1 h. (●) Chlorella exposure to high temperature (43.5 °C) and then mycoplasma (2.5×10^6 cells).

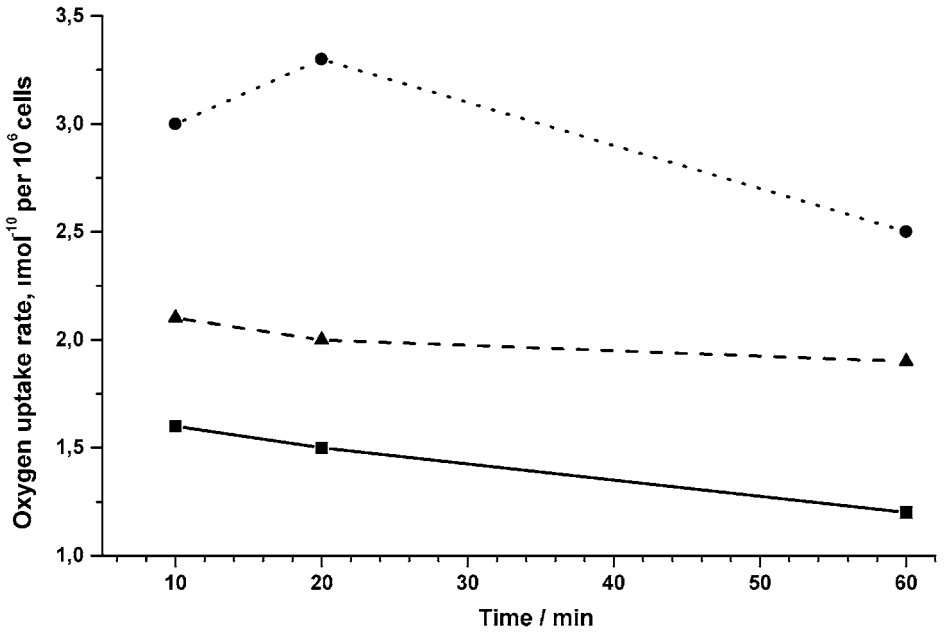

Fig. 5. The rate of oxygen uptake of chlorella cells after exposure to high temperature followed by the mycoplasma. (■) Control, chlorella at 30 °C (5.3×10^8 cells/ml). (▲) Chlorella after exposure to high temperature (43.5 °C). (●) Heat-shocked chlorella exposed to mycoplasma (2.5×10^6 cells/ml).

diminished with decreasing pathogen concentrations of four-fold (Fig. 2). The unexpected stimulatory effects of the mycoplasma supernatant on the rates of heat production and oxygen uptake by the chlorella cells were greater than the increases caused by the highest number of mycoplasma.

There was a concentration-dependent rise in the amount of superoxide produced by the chlorella cells immediately they were exposed to the mycoplasma and its supernatant at zero time (see Fig. 3). This relationship continued throughout the 1 h experimental period and the degree of it was even enhanced at certain times when there seemed to be secondary phases of rapid production of the superoxide.

In order to simulate the influence of ecological stress that can occur to plants under attack by pathogens, chlorella suspensions were exposed to high temperature (43.5 °C) for 1 h before cooling to 30 °C and adding *A. laidlawii*. The experiments showed that within 10 min, the amount of the superoxide anion radical present in the culture was much greater after heat shock than in the control (Fig. 4). There was an even greater amount of superoxide generated by the plant cells after exposure to heat and pathogen (Fig. 4). In contrast to cells without temperature stress (see Fig. 3), there was a decrease in the amount of the oxygen metabolite in the stress cultures over the remaining 50 min experimental period. As depicted in Fig. 5, similar results to those for superoxide production were obtained when the oxygen uptake rate of heat-stressed chlorella cells was measured for 60 min. The stress caused by the pathogen was additive to that of the heat shock.

There were some differences between the above heat stress results and those for the heat flow rate of similarly treated cells (Fig. 6). Although the experiments also showed that there was a heat "burst" by chlorella in response to the combined action of the heat and the pathogen (Fig. 6), it should be noted that the heat-stressed, infected cells produced a very rapid heat "burst" that was large in magnitude but transient with a maximum duration of 30 min after the addition

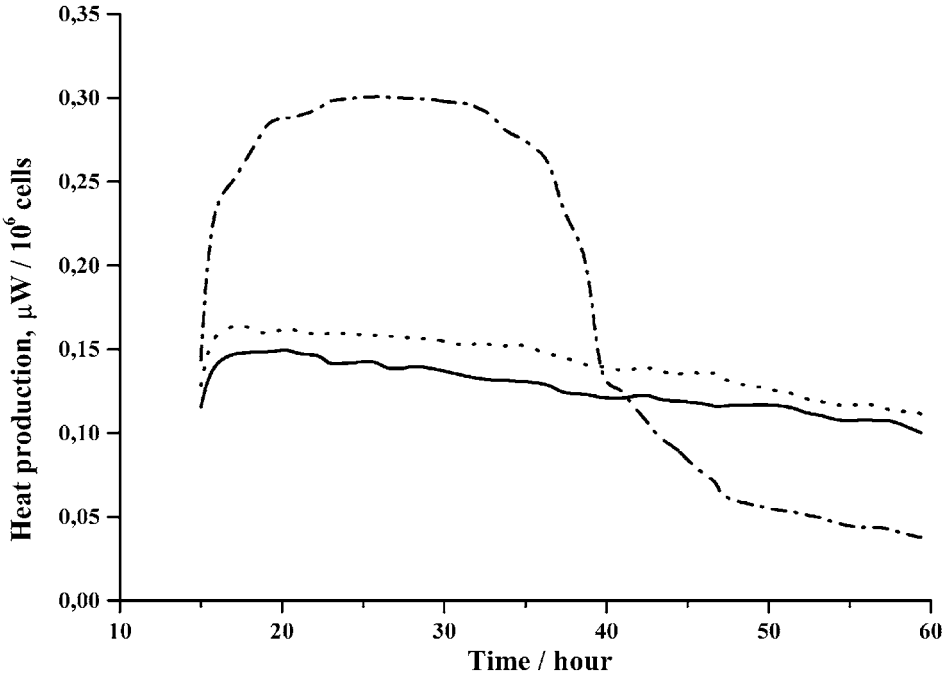

Fig. 6. The rate of heat production by chlorella cells after exposure to high temperature followed by mycoplasma. (—) Control, chlorella at 30 °C (5.5×10^8 cells/ml). (···) Chlorella treated by high temperature (43.5 °C, 1 h). (–·–·–) Heat-shocked chlorella exposed to mycoplasma (2.5×10^6 cells/ml).

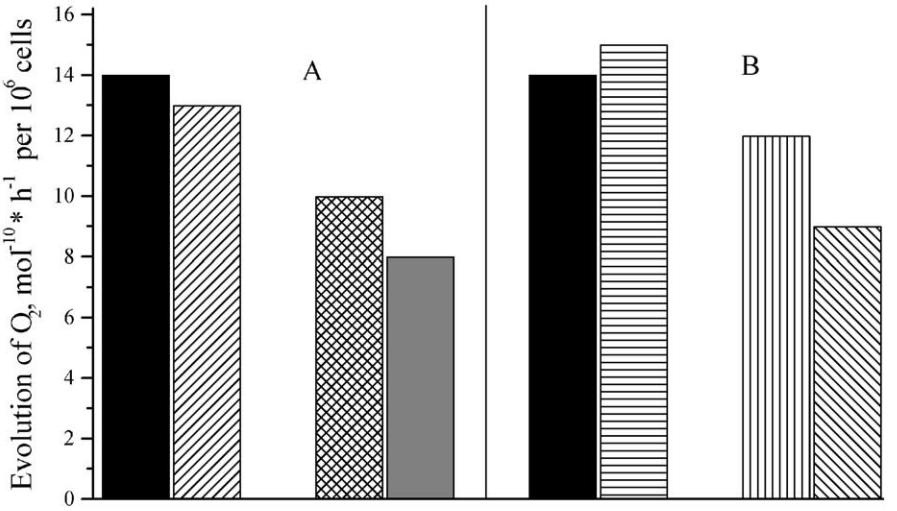

Fig. 7. The rate of oxygen evolution by heat-treated chlorella cells in the light after addition of the pathogen: (A) 10 min exposure to mycoplasma. (■) Chlorella control (5.5×10^8 cells/ml). (▨) Chlorella with 2.5×10^6 mycoplasma cells/ml (30 °C). (▩) Chlorella exposed to high temperature (43.5 °C). (▩) Chlorella exposed to high temperature (43.5 °C) followed by 2.5×10^6 mycoplasma cells; (B) 60 min exposure to mycoplasma. (■) Chlorella control (5.5×10^8 cells/ml). (▤) Chlorella with 2.5×10^6 mycoplasma cells/ml (30 °C). (▥) Chlorella exposed to high temperature (43.5 °C) (▨) Chlorella exposed to high temperature (43.5 °C) followed by 2.5×10^6 mycoplasma cells.

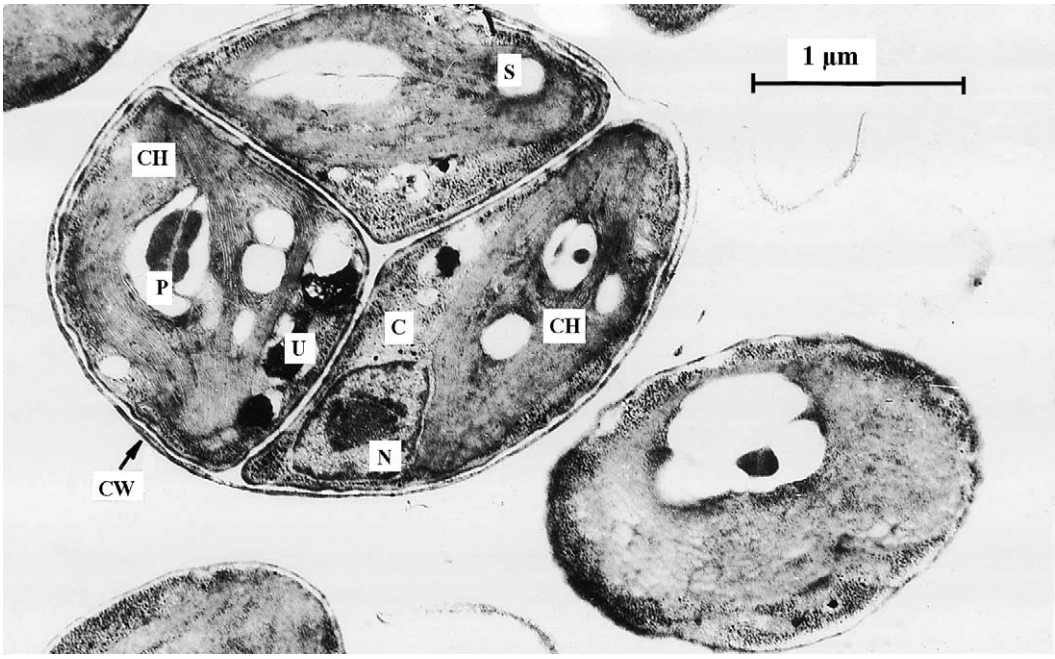

Fig. 8. An electronmicrograph of *Chlorella vulgaris* cells from a control culture at 30 °C. CH: chloroplast; CW: cell wall; C: cytoplasma; N: nucleus; S: starch; U: unrecognized body.

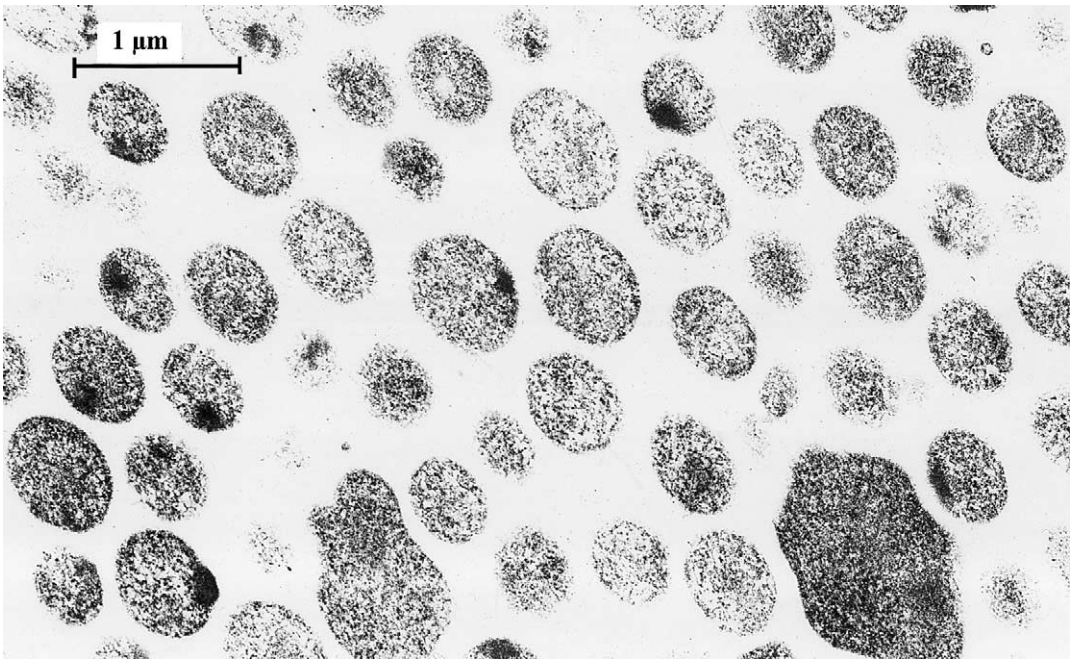

Fig. 9. The ultrastructure of *Acholeplasma laidlawii* cells in a standard culture.

of the mycoplasma. It was followed by sharp decrease after 40–45 min.

In terms of photosynthesis, the data in Fig. 7 shows that the rate of oxygen evolution decreased in heat-stressed cells. The initial rate at 10 min for infected cells not exposed to heat was close to the control. It was slightly higher than the control, however, 50–60 min after infection. The rate of oxygen evolution rapidly decreased in the heat-stressed culture and this effect was exacerbated by the subsequent addition of the pathogen.

The relationship of the physiological events to structural changes in the two organisms on their interaction was seen under the electron microscope. The basic structure of the chlorella cell is shown in Fig. 8 with its typical cell wall, nucleus, large chloroplasts, relatively small mitochondria and highly characteristic pyrenoids with starch grain. The structure of the mycoplasma is shown in Fig. 9. Within 20 min of mixing the microalgal cells with the mycoplasma, the host cell wall seemed to be in close proximity to the pathogen plasma membrane (see arrows in Fig. 10). At higher magnification (see Fig. 11), it can be seen that there is a change to the overall structure of the chlorella cell within 50 min of interaction with the pathogen. For instance, there was convincing evidence of the attachment of components of the mycoplasmal plasma membrane to elements of the microalgal cell wall. In the same section (Fig. 11), it was seen that the cellulose wall of some plant cells had broken down in regions of close approximation to the attached mycoplasma. This may be the prelude to the postulated uptake of the pathogen.

4. Discussion

As stated earlier, various pathogens as well as another stress factors have been shown to cause the so-called “oxidative burst” and “heat burst” in plants, as well as in animals [1,13,14]. The former arises directly because large amounts of oxygen are rapidly metabolised into active oxygen species, increasing the apparent rate of oxygen consumption [15]. This mainly occurs as a result of reactions at the plasma membrane that, in plant cells, is immediately subjacent to the cellulose cell wall [11–14]. In the present experiments, the evidence that the superoxide anion

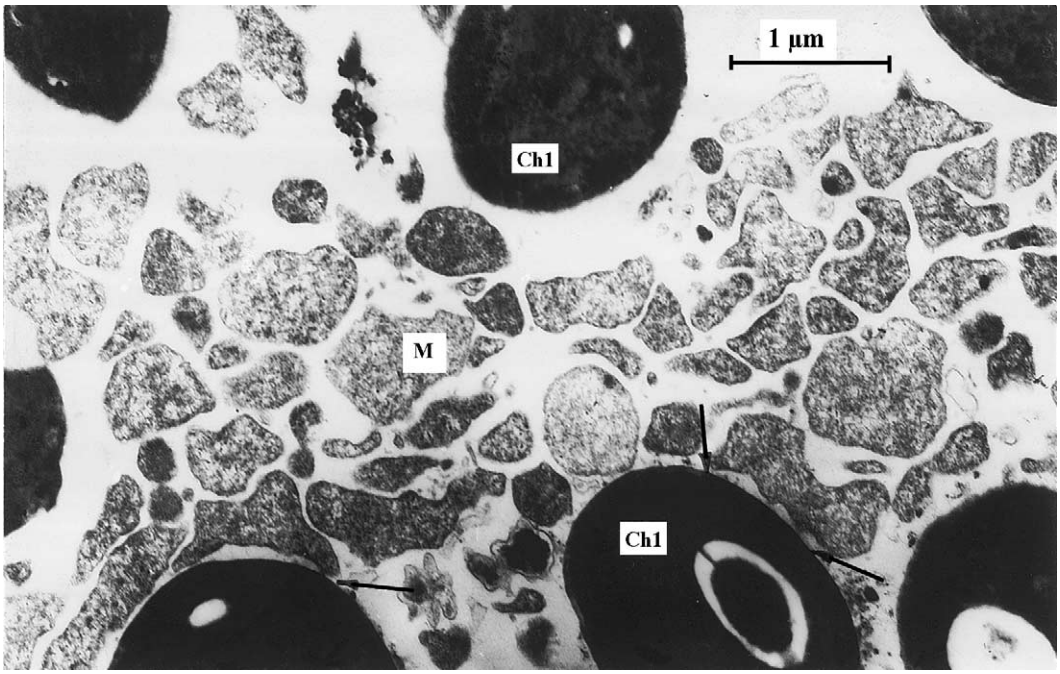

Fig. 10. Ultrastructural details of a culture of chlorella and mycoplasma, 20 min after mixing. Chl: chlorella; M: mycoplasma; →: points of close contact between the mycoplasma and chlorella.

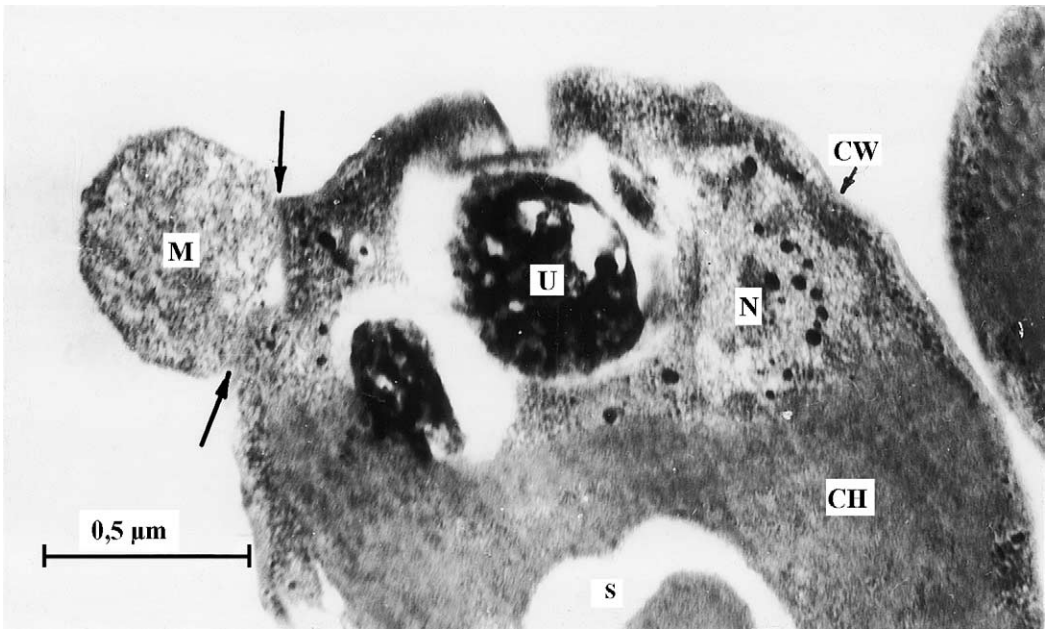

Fig. 11. An electronmicrograph of a mixed culture of chlorella and mycoplasma, 50 min after initial contact. Ch: chloroplast; CW: cell wall; M: mycoplasma; S: starch; U: unknown body; →: points of close contact between the mycoplasma and chlorella.

was formed permits the suggestion that an oxidase in the chlorella plasma membrane was stimulated by the presence of the *A. laidlawii* cells. If this is correct, then it is this oxidase that stimulated the production of the superoxide anion radical from NADPH and molecular oxygen. It is probable that other AOS were produced but these were no identified in the current investigation.

The enhanced generation of AOS, as exemplified by the superoxide, is likely to have been the most important part of the primary defensive response of the host to the mycoplasma infection. This is similar to the response of mammalian phagocytes to bacterial attack [15], but it cannot exclude the formation of the superoxide anion radical by the mycoplasma itself. While defending against attack by producing superoxide, the chlorella cells still have to response to the normal demands in energy metabolism and in fact these stressed microalgae may need to increase the rate of oxygen uptake to answer the demand for more energy from respiration. For instance, there may be increased energy expenditure for the maintenance of ion homeostasis and the active ion transport as well as in biosynthetic processes [4].

In previous studies, it was found that the respiratory burst induced by phorbol-12-myristate-13-acetate (PMA) in mouse macrophages was accompanied by a significant increase in heat production [15]. Similar data were obtained in the present experiments when chlorella was challenged with either the mycoplasma (Fig. 2) or the heat stress (Fig. 6). The principal reason in both cases was the greatly increased demands for NADPH in oxygen metabolism, which probably were serviced by greater flux through the hexose monophosphate shunt (HMPS) [15,16]. Thus, the event is essentially oxidative in type, meaning that the experimental calorimetric–respirometric (CR) ratio should be close to the theoretical oxycaloric equivalent of -455 ($\pm 15\%$) kJ/mol O_2 calculated by Gnaiger and Kemp [17] for a range of carbohydrates, amino acids and fatty acids catabolised by cells. The CR ratio for chlorella cells in the controls without exposure to the pathogen was calculated from the data shown in Figs. 1 and 2 and found to be -509 ± 73 kJ/mol O_2, within the known variation of the above-mentioned types of carbon substrate [17]. In research on the thermobiochemical origins of the "heat burst", the event was triggered by chemical mimics, such as PMA [15,18], but living cells are used in the current study and their metabolism is additive to that of chlorella. *A. laidlawii* is a facultative anaerobe [19] and this fact may explain the finding (see Fig. 2) that the increased heat produced during the plant–pathogen interaction contained an anaerobic element, i.e. -575 kJ/mol O_2 at 2.5×10^6 cells/ml and -583 kJ/mol O_2 at 10^7 cells/ml.

It was shown that the cell-free mycoplasma supernatant caused very large increases in the rates of heat production and oxygen uptake, together with a considerable rise in the amount of the superoxide produced by the cells (see Figs. 1–3). One possible explanation is that the mycoplasma exocytosed a water-soluble, humoral factor (elicitor) that acted as a signal to the chlorella cells by penetrating the cell wall, possibly through pores, and attaching to transmembrane receptors on the plasma membrane. In this hypothesis, it is reasoned that the binding of the factor to the receptor triggered a cascade to induce the operation of the plant cell defence systems, and in particular, activated the mechanism for the production of AOS to destroy the supposed pathogen.

It was somewhat surprising that the apparent rate of photosynthesis as measured in the light by the rate of oxygen evolution was similar in the pathogen-infected culture to that in the control (Fig. 7). It should be remembered, though, that the quantity of oxygen metabolised to AOS detracts from the true rate of photosynthesis. Oxygen consumed by the mitochondria also depresses the apparent rate of photosynthesis, but there is some evidence in chlorella that light inhibits the dark respiration [20]. It is only when the microalgal culture was subjected to the temperature stress that there was a marked inhibition of the rate of oxygen evolution, presumably because this activated the defensive production of AOS, particularly superoxide. As also found for the rates of heat production and oxygen uptake (Figs. 5 and 6), the temperature stress potentiated the effect of the supernatant on oxygen evolution (Fig. 7). Interpretation of this result may be complicated by the possibility that the mycoplasma infection partly destroyed the algal chlorophyll. Chernov et al. [19] have reported that *A. laidlawii* destroyed the green pigments of the pea leaf.

It is interesting to note from Fig. 6 that the heat-potentiated effect of the mycoplasma is quantitatively and qualitatively different to that of simply adding the pathogen to the chlorella culture. This change was

found in the profiles for the heat shock effect on the oxygen uptake rate, so it is due to an unknown phenomenon that appears not to be connected with the oxygen metabolism to form AOS. The causative agent for this phenomenon is under investigation.

The contribution of this investigation to our knowledge of the plant cell defence against pathogens is best judged against the information from electron microscopy. Examination of electronmicrographs showed the pathogen attached to the host cell only 20 min after mixing the two organisms (see Fig. 10). This means that the primary response to pathogen attack must have been elicited by a humoral factor exocytosed by the mycoplasma (see Figs. 1–3). Evidently, the elicitor was received at the chlorella plasma membrane and it seems likely that the resulting receptor complex initiated a cascade leading to the production of the superoxide and probably other AOS.

The research produced evidence of continued high rates of catabolic processes and production of the superoxide over the 60 min experimental period (see Figs. 1–3). It seems likely that the necessary augmentation of the elicitor signal after initial insult of the host by the mycoplasma was caused by the direct cell-mediated attachment of the pathogen to the host cell. This suggestion is supported by the finding of increases in the production of the superoxide anion later in the 60 min experimental period.

5. Conclusions

The algal cells reacted to the presence of pathogenic mycoplasma by the enhanced production of active oxygen species, which was accompanied by increases in the rate of heat production, oxygen consumption in the dark and oxygen evolution as an indicator of the apparent photosynthetic rate in the light.

There were differences in the intensity and duration of “oxidative burst” and “heat burst” under different conditions of the experiments that require explanation.

The data allow us to suppose that the mycoplasma exocytosed a water-soluble elicitor that acted as a signal to chlorella plasma membrane receptors. It is possible that the binding of elicitor to its receptor triggered a cascade that induced the action of the cellular defence system.

Acknowledgements

The authors are grateful to INTAS for the award of Grant no. 99-1390 that enabled completion of much of the work to be completed and to The Royal Society, UK, and the Russian Academy of Sciences, which provided funds to allow the necessary personal contact in each of our institutions.

References

[1] N. Doke, Y. Miura, L.M. Sahchez, H.J. Park, T. Noritake, H. Yoshioka, K. Kawakita, Gene 179 (1996) 45.
[2] I.A. Tarchevsky, N.N. Maksjutova, V.G. Jacovleva, A.N. Grechkin, Fisiol. Rast. (Russian) 46 (1999) 23.
[3] G.P. Bowell, D.R. Davies, C. Gerrish, C.-K. Auh, T.M. Murphy, Plant Physiol. 116 (1998) 1379.
[4] F.V. Minibayeva, O.P. Kolesnikov, L.K. Gordon, Protoplasma 205 (1998) 101.
[5] V.P. Skulachev, Energetics of Biological Membranes, Nauka, Moscow, 1989, p. 564.
[6] F.V. Minibayeva, N.L. Loseva, L.Kh. Gordon, A.Ju. Alyabyev, V.M. Chernov, R.B. Kemp, in: Proceedings of the 3rd INTAS Interdisciplinary Symposium, Moscow SU Press, Moscow, 2000.
[7] T.H. Tamiya, T. Yamura, K. Shibata, E. Hase, T. Nihei, Biochim. Biophys. Acta 12 (1953) 23.
[8] D.G. Edward, J. Gen. Microbiol. 1 (1974) 238.
[9] G.S. Grishina, L.N. Bell, Fiziol. Rast. (Russian) 13 (1966) 737.
[10] I. Wadso, in: K.N. Marsh, P.A.G. O’Hare (Eds.), Experimental Thermodynamics, Vol. IV, Solution Calorimetry, Blackwell Scientific, London, 1994, p. 267.
[11] V.E. Semenenko, L.A. Krasimikov, Fiziol. Biochem. Kult. Rast. (Russian) 1 (1969) 235.
[12] O.O. Polygalova, A.B. Ivanova, L.Kh. Gordon, Cytology (Russian) 41 (1999) 230.
[13] A. Hernandez, F.J. Corpas, M. Gomez, L.A. del Rio, F. Sevilla, Plant Physiol. 89 (1993) 103.
[14] A.C. Cazale, M.A. Rouet-Mayer, H. Barbier-Brygoo, Y. Mathieu, C. Lauriere, Plant Physiol. 16 (1998) 659.
[15] R.B. Kemp, A. Belicic-Kolsek, S. Hoare, M. Schmalfeldt, C. Townsend, P.M. Evans, Thermochim. Acta 250 (1995) 259.
[16] A.S. Romanenko, R.K. Saljaev, Endicitos in Plants, Nosibirsk (in Russian), Nauka, Russia, 1991, 101 pp.
[17] E. Gnaiger, R.B. Kemp, Biochim. Biophys. Acta 1016 (1990) 328.
[18] C. Eftimiadi, G. Rialdi, Cell Biophys. 4 (1982) 231.
[19] V.M. Chernov, O.A. Chernova, A.B. Ivanova, S.N. Borchsenius, Recent Advances in Mycoplasmology, Gustav Fischer Verlag, Stuttgart, 1990, p. 937.
[20] O.V. Zalensky, N.S. Mamuchina, Bot. J. (Russian) 9 (1980) 1141.