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Mechanism of action of bacterial phytotoxin, syringomycin. Simultaneous measurement of early responses in yeast and maize

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Metabolic events following the addition of the phytotoxin syringomycin were characterized in cell suspensions of the yeast *Rhodotorula pilimanae* and maize callus cells. The toxin induced K^+ efflux, a cessation in the acidification of the suspension medium, and a change in oxygen consumption. In yeast cells, an increased electric field over the plasma membrane was found as measured by enhanced tetraphenylphosphonium (TPP^+) uptake. At high concentrations of the toxin, the electric field increase was transient, the medium acidification was reversed, and the respiration rate was lowered. Restriction of the oxygen supply of the cells allowed the separation of two syringomycin-induced TPP^+ uptake phases. The first component, observed with a minimal supply of oxygen, was accompanied by a net influx of protons and K^+ efflux. The second component was observed with subsequent aeration of the cell suspension and coincided with the reestablishment of medium acidification. We postulate that a transport protein (probably a H^+/K^+ antiporter) in the plasma membrane is activated allowing an electrogenic K^+ efflux down its concentration gradient and a simultaneous H^+ influx. In addition, an energy dependent proton flux from the cytoplasm to another cell compartment is postulated to account for the second TPP^+ uptake phase.

Introduction

Syringomycin is a small, peptide-containing molecule produced by the phytopathogen *Pseudomonas syringae* pv. *syringae* [1]. Syringomycin inhibits the growth of several fungi [1] and produces necroses and cankers in stone fruit trees, bean and maize [1,2].

In previous work it was shown, that syringomycin enhances the membrane potential ($\Delta\psi$) and the pH gradient (ΔpH) across the plasma membrane in two different yeast species [3,4]. These were measured by the cellular uptake of TPP^+ and DMO. It was shown also that the toxin activates the plasma membrane ATPase in isolated plasma membrane vesicles of *R. pilimanae* [3] and of red beet storage tissue [5]. A limitation in these studies was the inability to discern how these various responses to syringomycin are related. For example, it is not known if all of these events occur simultaneously and independently or if they are interrelated such that primary responses result in one or more sequential secondary effects.

A variety of ion and proton flux changes across the plasma membrane of plant cells are known to

Abbreviations: TPP^+, tetraphenylphosphonium cation; Mes, 4-morpholineethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; PVC; polyvinylchloride; DMO, dimethyloxazolidine.

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occur following different stimuli such as physical injury [6,7], hormones [8], reducing agents [9], polypeptides of microbiological origin [10], bacteria [11,12], environmental changes [6], protein synthesis inhibitors [13], and toxins [14]. Fungal cells respond with K^+ efflux to plasma membrane ATPase inhibitors [15,16,17], energy depletion [18], and calmodulin antagonists [19,20,21]. In general, ion flux changes of fungal and plant cells seem to indicate a disturbed metabolic state of the attacked cell. But again, how the variety of ion fluxes are related is largely unknown.

In this work, we describe the sequence of early responses in the yeast *R. pilimanae* and maize callus cells upon exposure to syringomycin. A multielectrode device enabled us to monitor simultaneously TPP^+ uptake, K^+ efflux, pH and respiratory changes in a cell suspension. The data indicate that changes in these parameters occur simultaneously suggesting multiple primary effects of syringomycin.

Material and Methods

Cells

Embryonic maize callus cells (BMS No. 32, Calgene, Davis, CA) were grown at 26°C in N6 medium [22] supplemented with 100 mg casamino acids, 1 mg thiamin hydrochloride, 2 mg glycine, 0.5 mg pyridoxine hydrochloride, 0.6 mg niacin and 2 mg 2,4-dichlorophenoxyacetic acid per liter [23]. The medium (100 ml) was placed in a 500 ml Erlenmeyer flask and shaken at 100 rpm at 26°C (New Brunswick Scientific Psychrotherm G-27, Edison, NY). After approximately one week the culture was transferred by 1 : 2 dilution with fresh medium. Cells were harvested and washed twice with distilled water by filtration through Whatman 1 filterpaper. Immediately after filtration equal aliquots of plant cells (600 mg fresh weight) were dispensed in glass tubes sealed with Parafilm and used within 2 h.

The yeast *Rhodotorula pilimanae* (ATCC 26423) was grown overnight at room temperature in 200 ml of potato dextrose broth (24 g/l, Difco Laboratories, Detroit, MI) in 1-l Fernbach flasks on a reciprocal shaker. Cells were centrifuged and washed once or twice by centrifugation (200 × *g*, 5 min) with distilled water or with 20 mM Tris-Mes (pH 6.5) and 2 mM $CaCl_2$ (TMC buffer) at 4°C and resuspended in distilled water or the TMC buffer to an absorbance between 20 and 60 (600 nm, Bausch and Lomb Spectronic 20; measured in a 100–300-fold diluted cell suspension). Cells were stored on ice and used within 4 h. An absorbance of 1 corresponded to $(6.5 \pm 0.9) \cdot 10^7$ cells/ml.

Electrode holder

The electrode holder (Fig. 1) was built from a plexiglass block to support pH, oxygen, potassium or TPP^+, and reference electrodes, All electrodes were sealed with O-rings in drillings on the four sides of the block. The electrodes were supported and shielded against electromagnetic fields by aluminum housings. A vertical center hole could be sealed from the top with a removable plexiglass stopper and from the bottom with an O-ring sealed disk. The stopper had a closeable hole for making sample additions and also supported a stirring rod. The position of the stopper could be changed to allow sample volume adjustments (4.25–5.0 ml). The stirring rod was driven at 1 rpm by a synchronous clock motor (60 Hz, 120 V).

Ion and oxygen selective electrodes

The ion selective barrier between the sample compartment and measuring electrode was a PVC film glued to the end of a PVC housing [24]. For the TPP^+ electrode, 250 mg PVC (Sigma, St.

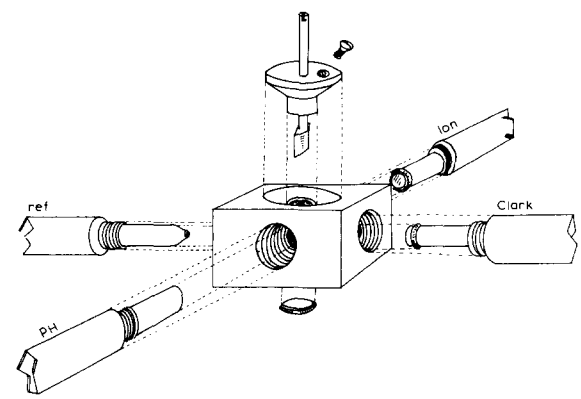

Fig. 1. Electrode compartment (symbols: pH, pH electrode; Clark, Clark electrode; Ion, K^+ or TPP^+ selective electrode; ref, reference electrode for the ion-selective electrode). Further explanations are in the text.

Louis, MO) were dissolved in 4 ml tetrahydrofuran and 400 μl di(2-ethylhexyl)phthalate (Polysciences, Warrington, PA). Then 8 mg tetraphenylboron (Sigma), dissolved in 1 ml tetrahydrofuran were added to the PVC solution which was poured into a horizontal glass petri dish (diameter 89 mm) to allow solvent evaporation. For preparation of the potassium sensitive electrode, tetraphenylboron was replaced by 5–8 mg valinomycin (Sigma, St. Louis, MO). For the pH electrode, a flat-end combination electrode was used (Type 91-36, Orion, Cambridge, MA). A Clark electrode (YSI 5331 oxygen probe, Yellow Springs Instruments, Yellow Springs, OH) was used for oxygen level recording. Ion concentrations (K^+, TPP^+) were recorded as voltage between ion selective and a calomel reference electrode with ceramic junction (476109, Corning, Medfield, MA) filled with 3 M NaCl.

Electronic recording

The Clark electrode was connected to a Model 55 Yellow Springs oxygen monitor. The potential between ion and reference electrode was measured with a Corning 12 pH meter and the pH was monitored by a Corning 150 pH/ion meter. The output of the first two devices was amplified by a differential DC amplifier (Ectron, San Diego, CA) and digitized (DT2801, Data Translation, Marlborough, MA). The serial output of the Corning 150 pH/ion meter was directly connected to the RS232 port of the IBM PC-XT computer. All three signals were simultaneously monitored and the data were stored in 10-s intervals by aid of a data acquisition software program (Labtech Notebook, Wilmington, MA).

It was essential that all electrode holders and the stirring motor enclosure were grounded and that a high resistance (100 kOhm to 10 MOhm) existed between sample compartment, all grounded parts, and both connections of the Clark electrode. Only with these precautions was interference-free function of the electrodes possible (Fig. 2). Interference of pH changes with the TPP^+ electrode were negligible in the presence of more than 5 μM TPP^+. The K^+ electrode was found to respond to TPP^+, but pH interference was absent with pH changes smaller than 1 pH unit.

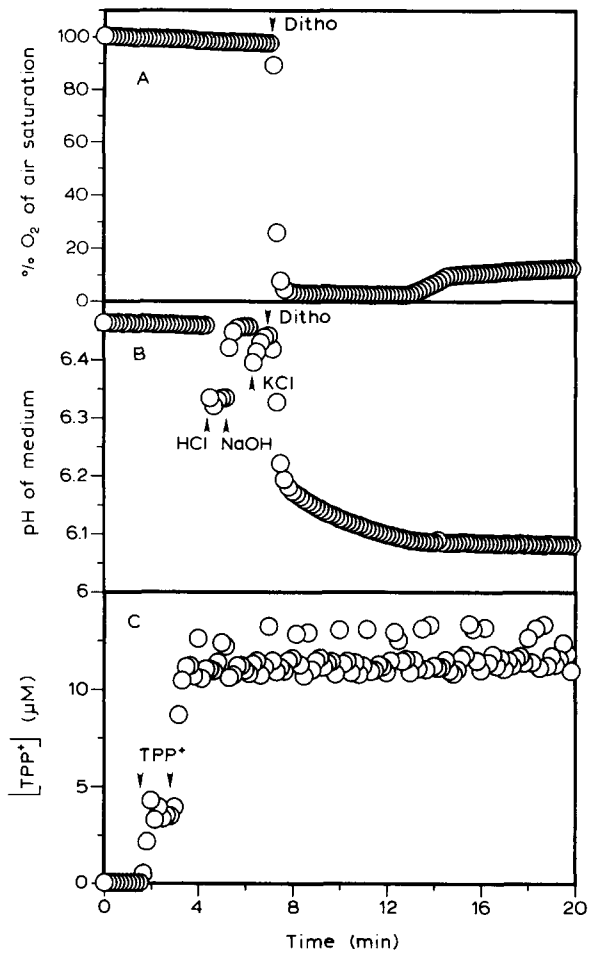

Fig. 2. Simultaneous measurement of the O_2 level (A), pH (B), and TPP^+ concentration (C). 2 mM Tris-Mes (pH 6.5), 20 mM NaCl and 100 μM $CaCl_2$. Additions: TPP^+, 3.7 and 7.3 μM TPP^+; HCl and NaOH, 11 μM each; KCl, 1.1 mM; Dithio, a few grains of solid $Na_2S_2O_4$. KCl was added to verify that K^+ level changes in the range observed have no effect on the TPP^+ electrode response. The slight rise in the oxygen level at 13 min was due to the open hole for sample additions which was closed at 15 min. The drop of pH at 7 min originates from sulfurous acid liberated following dithionite addition.

Experiments. All experiments were started with aerated buffer in the compartment. We used Tris-Mes buffer (0.5 or 2 mM), rather than unbuffered solutions, to limit the pH changes to a few tenths of a pH unit, resulting in constant H^+ efflux rates from the cells. Cells were added, followed by 5–10 μl TPP^+ (3.3 mM stock solution), when this ion was monitored. In aerobic experiments, the suspension was aerated with either pure oxygen or

with an O_2/CO_2 mixture (97.5 : 2.5, v/v) when the oxygen level fell below 10%. In anaerobic experiments, the cells were allowed to deplete the oxygen within the covered compartment, before the effects of syringomycin were measured. The voltage of the K^+ electrode was converted to a K^+ concentration assuming a linear relationship between log $[K^+]$ and voltage U:

$$U = m(\log[K] + c) \quad (1)$$

where m is the slope and c is a constant. The parameters m and c were determined by calibration. The electrode film was renewed when m became smaller than 43 mV. Eqn. 1 was found to apply for $[K^+]$ higher than about 30 μM. Data from the TPP^+ electrode were treated analogously.

Syringomycin

Syringomycin was prepared as described earlier [3]. It was found that different syringomycin preparations and storage conditions gave different toxicities. Therefore, the toxin was quantified in units/ml. One unit (u) inhibits the growth of *R. pilimanae* totally on a petri dish when applied in a 10 μl droplet [3]. Preparations showed 20–50 u/ml.

Results

Effect of syringomycin on R. pilimanae

The oxygen level, pH and K^+ concentration in a suspension of *R. pilimanae* cells were monitored simultaneously (Fig. 3). After addition of cells ($t = -8$ min), the dissolved oxygen decreased gradually (Fig. 3A), and protons were excreted from the cells thereby acidifying the medium (Fig. 3B). The K^+ level of the medium increased to about 100 μM after cell addition (Fig. 3C). We observed that immediately after cell addition the acidification rate was highest. Therefore, in most experiments the oxygen levels was brought up again to 100% and the respiration and acidification rates were followed for a few minutes. Addition of syringomycin (40 u/ml) did not affect the respiration rate largely (Figs. 3A, 4A), but stopped the acidification of the medium (Figs. 3B, 4B) and induced a potassium efflux (Fig. 3C). K^+ efflux ended after 5 min, but medium alkalinization continued for 7 min after addition of syringomycin. The very rapid transient pH drop observed within the first 10 or 20 s after syringomycin addition was due to the acidity of the syringomycin solution. The addition of syringomycin caused the extracellular TPP^+ level to decrease immediately, and it continued to decrease for 8 min (Fig. 4C). With TPP^+ (15 μM) in the medium, the pH increased sharply at about 2 min (Fig. 4B).

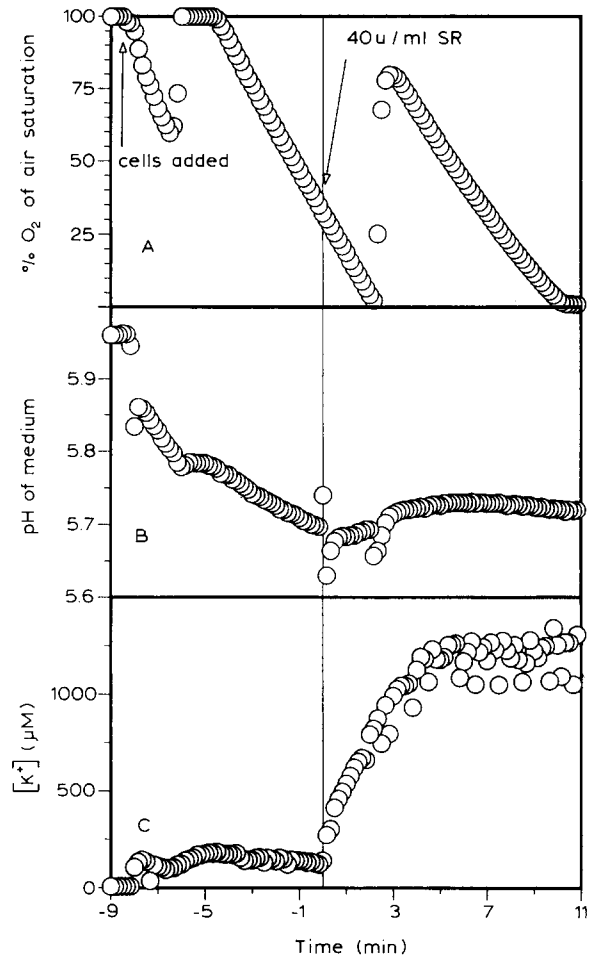

Fig. 3. The effect of syringomycin on O_2 level (A), pH (B), and K^+ concentration (C) in a cell suspension of *R. pilimanae*. The buffer contained 2 mM Tris-Mes, 100 μM $CaCl_2$. Cells were added corresponding to an absorbance of 2. At -6 min and 2 min the suspension was aerated. Oxygen levels slightly higher than 100% air saturation could be reached, but were electronically not registrated (as in the interval -6 to -4 min). At time zero syringomycin (40 u/ml) was added.

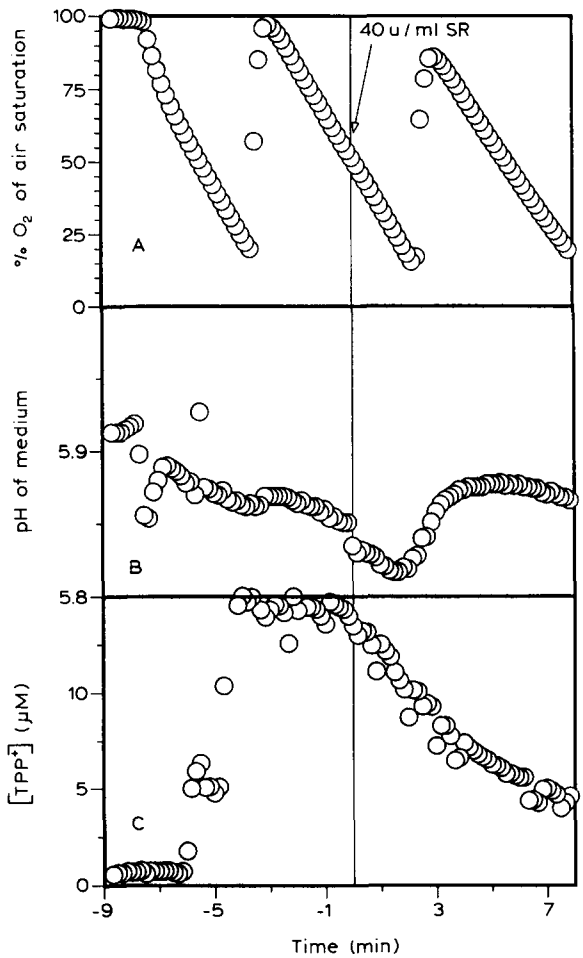

Fig. 4. The effect of syringomycin on O_2 level (A), pH (B), and TPP^+ concentration (C) in cell suspension of *R. pilimanae*. Buffer and cell concentrations were as in Fig. 3. Cells were added at −8 min, TPP^+ at −6 and −5 min and syringomycin (40 u/ml) at time zero. The cell suspension was aerated at −3 and +2.5 min. The small pH shift at time zero originates from addition of syringomycin.

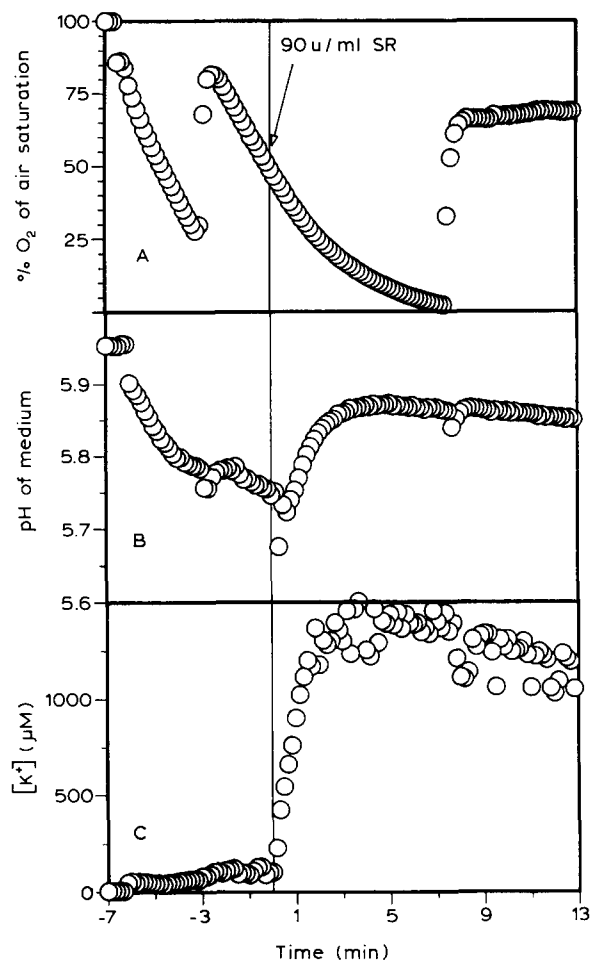

Fig. 5. The effect of syringomycin on O_2 level (A), pH (B) and K^+ concentration in cell suspension of *R. pilimanae*. Buffer and cell concentrations were as in Fig. 3. At time zero syringomycin (90 u/ml) was added. The cell suspension was aerated at −3 and 8 min.

Higher concentrations of the toxin (90 u/ml) induced a rapid and pronounced K^+ efflux (Fig. 5C). In parallel or slightly later the medium pH rose sharply (Fig. 5B). Approximately 2 min following syringomycin addition, the respiration rate started to decline gradually (Figs. 5A, 6A). The kinetics of TPP^+ uptake were different than with the lower syringomycin concentrations. High syringomycin induced a rapid uptake of TPP^+ which was followed by a gradual release of the lipophilic ion (Fig. 6C). Even higher toxin concentrations (> 150 u/ml) gave a greater pH increase but no detectable TPP^+ uptake (data not shown).

The influence of calcium

In the presence of calcium (100 μM), the addition of cells had little effect on the K^+ level of the suspension medium (Fig. 7A). The addition of syringomycin (30 u/ml) caused a rapid K^+ extrusion. In the absence of calcium, the cells added to the buffer immediately lost K^+ which could be seen as a gradual rise in the K^+ level of

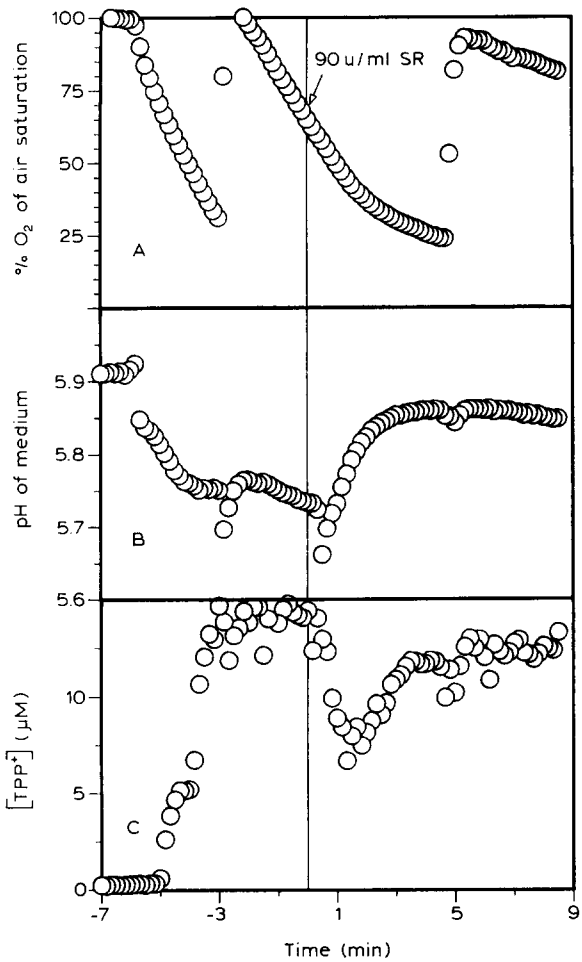

Fig. 6. The effect of syringomycin on O_2 level (A), pH (B), and TPP^+ concentration (C) in cell suspension of *R. pilimanae*. Buffer and cell concentrations were as in Fig. 3. At time zero syringomycin (90 u/ml) was added. The cell suspension was aerated at −2.5 and +5 min.

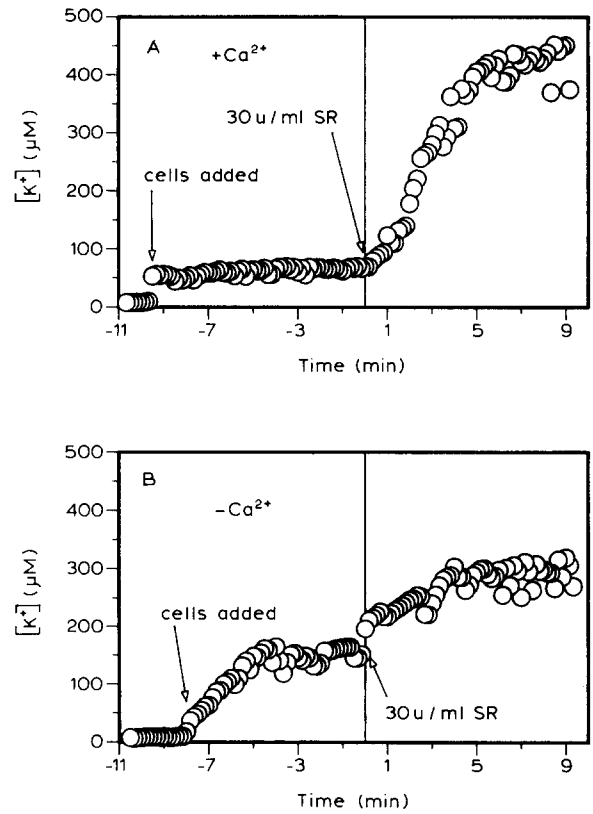

Fig. 7. The effect of syringomycin on the K^+ level in an aerobic cell suspension of *R. pilimanae*. Cell concentration corresponded to an absorbance of 1. (A) 0.5 mM Tris-Mes, 20 mM NaCl, 100 μM $CaCl_2$ (pH 6.5). (B) as A but no $CaCl_2$.

the medium. The addition of syringomycin only enhanced slightly the further K^+ efflux (Fig. 7B).

Influence of oxygen supply

We examined the responses of *R. pilimanae* to syringomycin under conditions of minimal oxygen supply. In the aerated buffer, oxygen was consumed within 3 min after cell addition (Figs. 8A, 9A). Thereafter, the pH of the medium reached a constant value (Figs. 8B, 9B). The addition of 30 u/ml of syringomycin induced a small but significant TPP^+ uptake, which stopped after 3 to 4 min (Fig. 8C). This TPP^+ uptake was not accompanied by an acidification of the suspension medium (Fig. 8B). Subsequent aeration of the cell suspension reestablished medium acidification and initiated a further pronounced TPP^+ uptake (Fig. 8C).

It is possible that the decreased acidification observed with syringomycin (Figs. 3B, 4B, 5B, 6B) was due to a change in buffer capacity (e.g., release of macromolecules from the cells). To examine this, equal amounts of acid and base were sequentially added before the addition of syringomycin followed later by an equal amount of acid (not shown). The pH changed equally with acid addition either before or after addition of the toxin. Thus, syringomycin does not change significantly the buffer capacity.

Under limited oxygen conditions, syringomycin (200 u/ml) induced medium alkalinization and

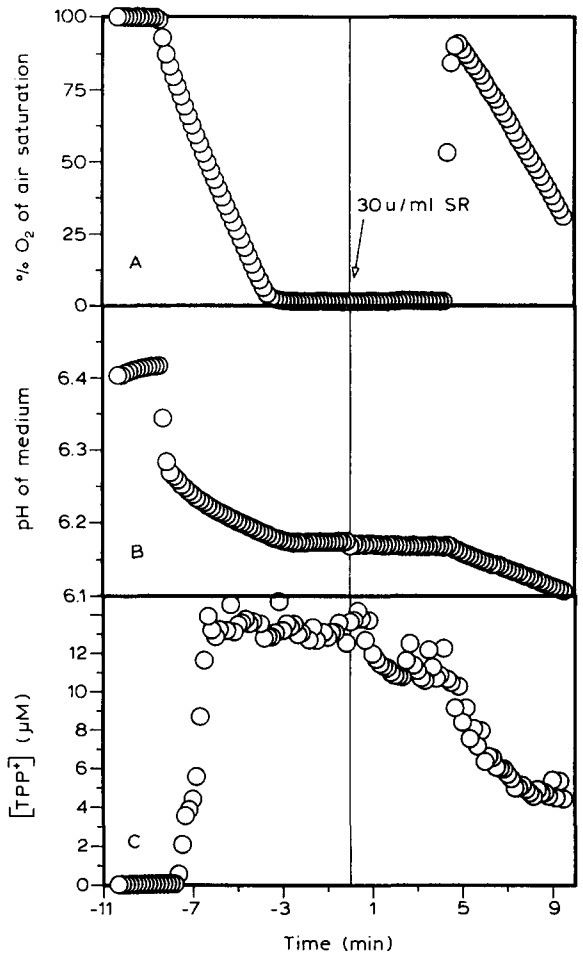

Fig. 8. The effect of syringomycin on O_2 level (A), pH (B), and TPP^+ concentration (C) in *R. pilimanae.* Cell concentration corresponded to an absorbance of 2. The buffer consisted of 2 mM Tris-Mes (pH 6.4), 100 μM $caCl_2$ and 20 mM NaCl. At -7.5 and -6.5 min 3.7 and 7.4 μM TPP^+ and at time zero syringomycin (30 u/ml) was added. The cell suspension was aerated at $+4.5$ min.

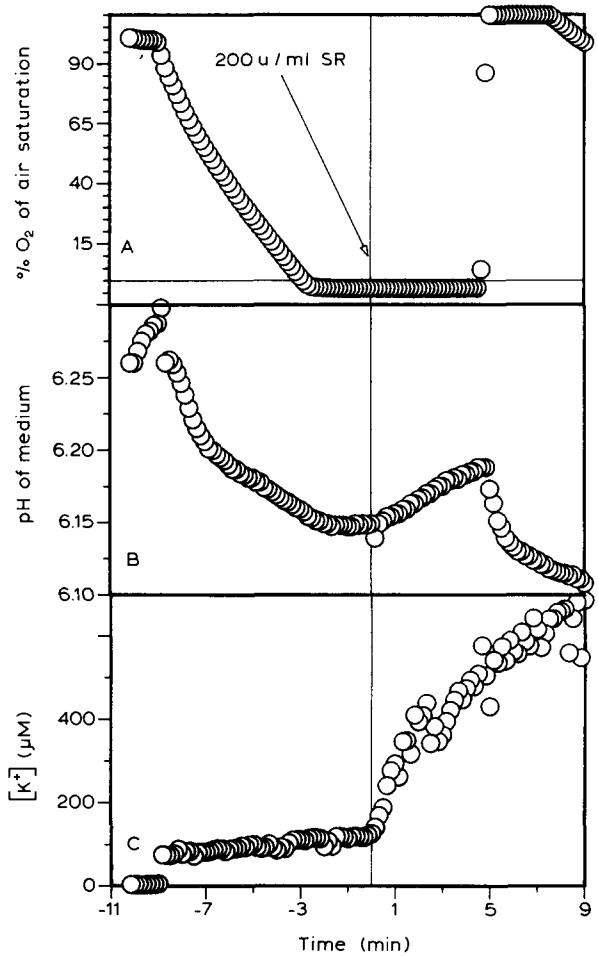

Fig. 9. The effect of syringomycin on O_2 level (A), pH (B) and K^+ concentration in cell suspension of *R. pilimanae.* Cells were added at -9 min, buffer and cell density as in Fig. 8. At time zero syringomycin (200 u/ml) was added. The suspension was aerated at 5 min.

K^+ efflux (Figs. 9B, 9C). The K^+ efflux even under these high toxin concentrations showed a lower rate than under aerobic conditions (Fig. 5C). With the following aeration a medium acidification occurred at a high rate. About 1 min after this aeration, the acidification decreased and showed about the same rate as before anaerobiosis.

Effect of high TPP^+ concentrations

R. pilimanae cells responded differently to syringomycin when the TPP^+ concentration was raised from 15 to 25 μM. At this higher TPP^+ level, even low amounts of syringomycin (20 u/ml) caused an increase in respiration rate and simultaneously an increased acidification rate (Figs. 10A, 10B). TPP^+ uptake occurred immediately after syringomycin addition, followed by an TPP^+ efflux beginning 2.5 min later (Fig. 10C). Note that TPP^+ itself does not change the respiration or acidification rate.

The effect of syringomycin on maize embryonic cells

In contrast to *R. pilimanae,* syringomycin in-

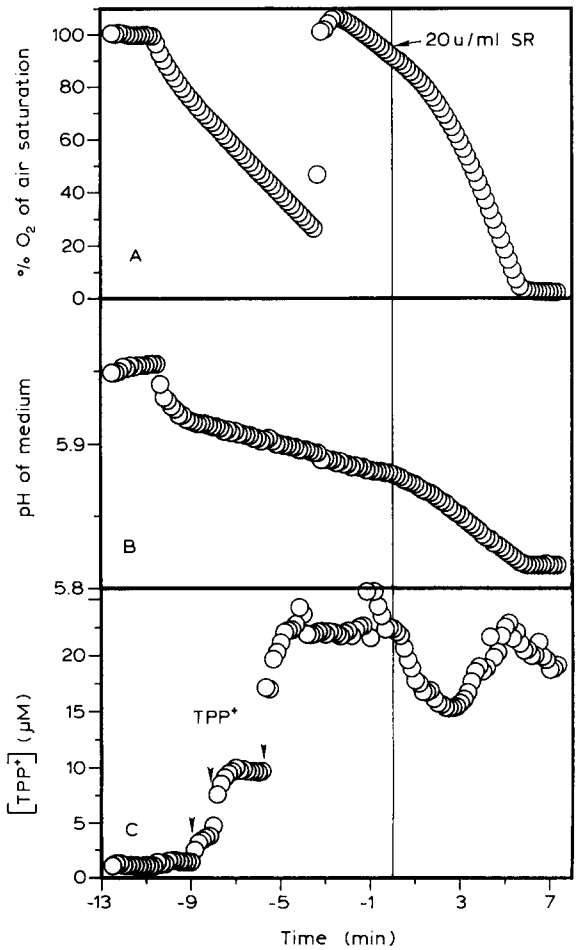

Fig. 10. The effect of syringomycin on O_2 level (A), pH (B), and TPP^+ concentration (C) in cell suspension of *R. pilimanae*. Buffer and cell density was as in Fig. 8. At −9, −8 and −6 min, 3.7, 7.4 and 14.8 μM TPP^+, and at time zero 20 u/ml syringomycin were added. The cell suspension was aerated at −3.5 min.

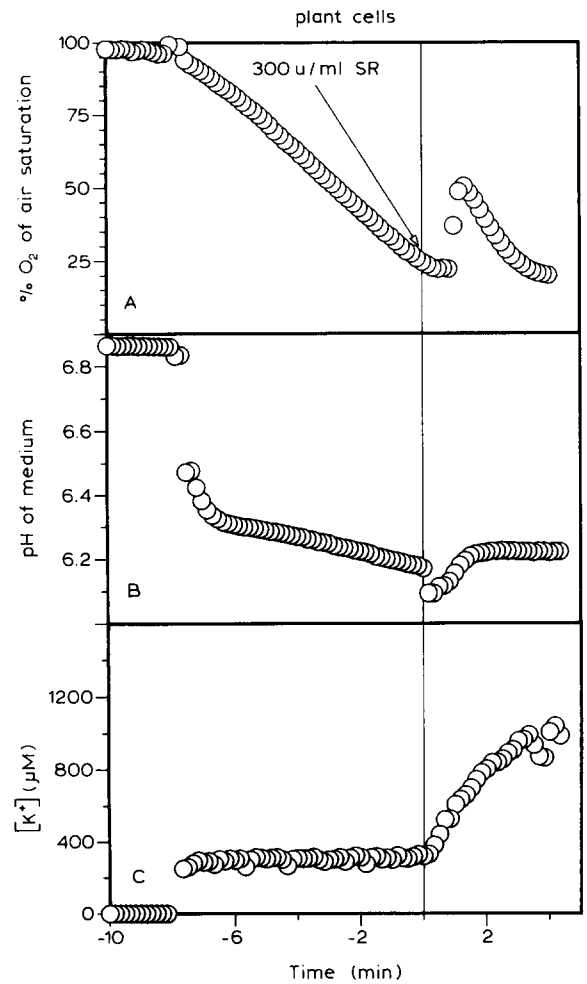

Fig. 11. The effect of syringomycin on O_2 level (A), pH (b) and K^+ concentration in suspension of BMS maize cells (133 mg fresh weight/ml). The buffer consisted of 0.5 mM Tris-Mes (pH 6.5). At time zero syringomycin (300 u/ml) was added. The suspension was aerated at 1 min.

duced an increase of the respiration rate in maize cell suspensions (Fig. 11A). The same effect was observed over a range of toxin concentrations (80–300 u/ml). Higher syringomycin levels, however, lowered the respiration rate (not shown). As with *R. pilimanae,* the acidification stopped and a transient alkalinization could be detected (Fig. 11B). K^+ efflux was also found after syringomycin addition (Fig. 11C). Maize cells differed from yeast cells with respect to TPP^+ uptake. Addition of maize cells to a medium containing TPP^+ led to an immediate uptake of the lipophilic cation. Addition of syringomycin in different concentrations did not significantly change the TPP^+ level observed (not shown).

Discussion

Low concentrations of syringomycin (20–50 u/ml) induced an increase of $\Delta\psi$ over the *R. pilimanae* plasma membrane. An efflux of K^+ was found to occur in parallel to the $\Delta\psi$ increase. K^+ is known to play a fundamental role in poising ΔpH against $\Delta\psi$ in plants and fungi [25]. Under

normal conditions plant and fungal cells maintain a high K^+ level in the cytoplasm and in the vacuole [26–28]. It is postulated that the energy contained in the K^+ gradient supplements the energy reserve of the proton gradient in yeast [29]. In all observed cases of the present study, the syringomycin-induced K^+ efflux was at least four times larger than the net H^+ influx, as calculated from the buffer capacities and K^+ efflux. Taking all these facts, an electrogenic K^+ efflux could be responsible for the increased $\Delta\psi$ with syringomycin.

The kinetics of K^+ efflux corresponded with the kinetics of TPP^+ uptake. At low syringomycin concentrations (40 u/ml), a slow K^+ efflux and a slow TPP^+ uptake was observed (Figs. 3C and 4C); at higher syringomycin concentrations (90 u/ml) a faster K^+ efflux and a faster TPP^+ uptake occurred. TPP^+ release from cells coincided with the end of the K^+ efflux (Figs. 5C, 6C). This release which followed the rapid TPP^+ uptake indicates a decrease of $\Delta\psi$ which can be explained by either an anion efflux or a cation influx following the stop of K^+ extrusion. In both cases the ion flux probably is driven by the high electric field over the plasma membrane which is then gradually dissipated. It will be discussed below that a bursting of cells [3] cannot explain the collapse of $\Delta\psi$ and the medium alkalinization.

Finally, K^+ efflux and TPP^+ uptake occurred under oxygen limited conditions. Without oxygen, proton extrusion via the ATPase ceased (Fig. 8B), but K^+ efflux was observed (Fig. 9C). Therefore, the TPP^+ uptake induced by syringomycin reflects most probably the contribution of K^+ to the buildup of $\Delta\psi$ (Fig. 8C). The K^+ efflux shown here was induced by a much higher syringomycin concentration than the TPP^+ uptake, but K^+ efflux was found to take place following the addition of various syringomycin concentrations independent of the oxygen level of the cell suspension (not shown). The significance of the second TPP^+ uptake phase (Fig. 8C) which followed the aeration of the cell suspension will be discussed below.

The effects of syringomycin on medium pH are more difficult to interpret. It was postulated earlier, that syringomycin stimulates the *R. pilimanae* plasma membrane ATPase to explain the electric field increase and the stimulated cellular uptake of DMO [3]. Despite this, in the present work, we did not observe an increased acidification rate of the medium with addition of the toxin. Low concentrations of syringomycin resulted in a slow TPP^+ uptake with no change in acidification rate (not shown). Increasing syringomycin concentrations first caused a stop in the medium acidification and then a transient medium alkalinization.

One could postulate that the plasma membrane ATPase is stimulated by syringomycin and that a simultaneous backflux of protons into the cell takes place. In this case protons would not be a source of the increased electric field over the plasma membrane. Comparing the kinetics of K^+ efflux with changes in the medium pH allows speculations on the sequence of events which might occur with syringomycin addition: Initially, K^+ efflux, the ATPase, and proton backflux are stimulated simultaneously. As the $\Delta\psi$ increases, especially at high syringomycin concentrations, the proton backflux dominates over proton pumping. When K^+ efflux was ceased a new H^+ flux steady state is reached. The backflux of protons may take place via proton leaks or via a K^+/H^+ antiporter which is known to exist in yeast [30,31]. This antiporter cannot be functional under normal metabolic conditions since it would dissipate both the H^+ and K^+ gradient. Similarly, in a marine bacterium the H^+/K^+ antiporter is only active when the cytoplasm becomes too alkaline [32]. Since more K^+ ions appear than protons disappear from the cell suspension medium, either this antiporter does not function with a 1 : 1 ion ratio or an additional K^+ transport is activated. We observed that the syringomycin-induced K^+ efflux is slower under conditions of minimal oxygen supply. This is consistent with the model of an activated H^+/K^+ antiporter because the driving force of the higher ΔpH under aerobic conditions then would drive faster the K^+ efflux.

The above model does not explain the DMO uptake of *R. pilimanae* with syringomycin added [3]. DMO is a weak acid which can penetrate membranes in the uncharged form but not in the charged form. DMO uptake of cells indicates an increased ΔpH over the plasma membrane with a more alkaline cytoplasm. For this we postulate

that besides the effect on a component in the plasma membrane, syringomycin induces a cell energy dependent proton pumping out of the cytoplasm into one or more intracellular compartments, thereby increasing the pH of the cytoplasm. When this proton flux is not simultaneously compensated by the movement of other ions, the positive charges disappearing from the cytoplasm would increase also the $\Delta\psi$ over the plasma membrane. This may cause the second TPP^+ uptake phase seen with aeration of oxygen limited cells (Fig. 8C). The vacuole is the most likely candidate for such a compartment since it is large in fungi and plant cells and possesses a proton pump.

By using TPP^+ to monitor $\Delta\psi$, it is normally assumed that the TPP^+ concentration in the cell suspension is low enough not to decrease $\Delta\psi$ itself. High K^+ levels inside plant and yeast cells make it impracticable to use valinomycin for dissipating $\Delta\psi$. In plant cells, TPP^+ in high concentrations has been used to lower this electric potential [33]. We found that in the presence of high TPP^+ levels (25 μM), a low syringomycin concentration (20 u/ml) induced a higher acidification rate (Fig. 10B) together with a higher respiration rate (Fig. 10A) in *R. pilimanae.* The high level of TPP^+ alone did not stimulate the acidification rate. Although the TPP^+ uptake following syringomycin addition indicates an increased $\Delta\psi$, the plasma membrane ATPase seems to be stimulated as indicated by the higher acidification rate. We attribute this stimulation to the fact that the high TPP^+ level did not allow the $\Delta\psi$ to increase to the extent seen in the experiments with lower TPP^+ levels. This shows further that the plasma membrane ATPase is activated by syringomycin and that the toxin does not prevent the regulation of its activity by $\Delta\psi$. A stimulated plasma membrane ATPase also could explain the high acidification rate following immediately the aeration of the anaerobic cell suspension (Fig. 9B, at 5 min). Finally, the TPP^+ efflux following the syringomycin-induced uptake cannot be due to cell rupture because under the conditions of high TPP^+ levels, the efflux of the lipophilic ion does not correlate with medium alkalinization.

The response of maize cells to syringomycin was similar to yeast. A transient alkalinization and K^+ efflux was also detected (Fig. 11). However, a different behavior was found with respect to the respiration rate. With maize cells the respiration increased with syringomycin (Fig. 11A). In yeast, under most conditions a reduction of respiration rate was found. We have no explanation for this opposite behavior of maize and yeast cells. The reduction of respiration is not compatible with an earlier reported uncoupling effect of syringomycin on plant mitochondria [34].

Analyses of membrane potential with these cells were restricted because high TPP^+ uptake rates occurred immediately after the addition of cells (not shown). This made it difficult to resolve the effect of syringomycin on this parameter.

In most of the experiments described, calcium (100 μM) was included in the buffer medium (Fig. 7A). It is known for plant cells that the presence of calcium prevents the loss of electrolytes, especially K^+ [35]. This may be true for *R. pilimanae* as well. The syringomycin-induced K^+ efflux was present also in the absence of calcium (Fig. 7B) but it had to be monitored against the high background concentration of K^+.

Conclusion

Syringomycin appears to cause multiple effects in a simultaneous fashion. Early observable events affecting the plasma membrane as well as the cytoplasm could be separated by a restricted oxygen supply of the cells.

In yeast and plant cells a H^+/K^+ antiporter in the plasma membrane seems to be activated. This activation can take place also under low oxygen conditions. At high cellular energy levels, a syringomycin-induced H^+ flux inside the cell from the cytoplasm to one or more cellular compartments (e.g. vacuole) was postulated for *R. pilimanae*, to account for DMO uptake and the TPP^+ uptake which is not accompanied by an increased rate of proton or K^+ extrusion. In addition, the respiratory activity was found to be altered by syringomycin in yeast and plants. Syringomycin also seems to stimulate the plasma membrane ATPase, but the stimulation cannot be seen normally when an increased $\Delta\psi$ over the plasma membrane reduces the turnover rate of this enzyme.

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