Effect of cesium ions on the mycelium growth and zoospores motility in the oomycete *Phytophthora infestans*

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Summary

Oomycetes are a group of parasitic eukaryotic microorganisms infecting plant and animal hosts worldwide. Many of those, for example, the crop parasite *Phytophthora infestans*, have a considerable economic impact. Previously we revealed the unexpected diversity of ion channels that could participate in the potassium transport in oomycetes. In the present study, we investigated the effects of cesium ions, which are known to nonspecifically block the activity of membrane proteins involved in the potassium transport, on the growth of *Ph. infestans* mycelium and motility of its zoospores. We showed that the addition of 3–24 mM cesium chloride to the culturing medium inhibited the oomycete growth on agar plates. Moreover, the application of 6 and 12 mM CsCl to zoospores substantially reduced the velocity of their movement in a liquid medium. The results indicate an important role of potassium transport at the different stages of *Ph. infestans* life cycle.

Key words: cesium, oomycetes, *Phytophthora infestans*, potassium channels, zoospores

Introduction

Oomycetes are a large group of osmotrophic organisms comprising marine, freshwater, and soil species. Although there are saprotrophic oomycetes, many others are obligate or facultative parasites of protists, animals, and plants (Beakes et al., 2014). A lot of them pose a serious threat to agriculture; the most economically significant are downy mildews (e.g., *Basidiophora, Peronospora, Plasmopara*), white blister rusts (*Albugo*), as well as species of the genera *Phytophthora* and *Pythium* (van Wyk et al., 1995; Sutton et al., 2006; Nowicki et al., 2012; Thines and Choi, 2016). Other oomycetes are waterborne pathogens (e.g., *Saprolegnia, Aphanomyces*) that cause various diseases of aquatic animals and are capable of infecting some commercially important fish and crustacean species (Edgerton et al., 2004; van West, 2006).

Phytophthora infestans is one of the most well-known and thoroughly studied oomycetes. It is notorious for causing a 19th-century late blight epidemic of potato in Europe, which led to the Great Famine in Ireland (1845–1849), resulting in over

one million human deaths (Yoshida et al., 2013). Today, despite the profound progress in agricultural chemistry and plant bioengineering, *Ph. infestans* remains one of the most economically significant oomycetes: it was estimated that annual losses caused by late blight reach $6,7 billion (Haverkort et al., 2008).

Cesium ions have an opposite effect on the passive and active potassium transport components. On the one hand, Cs^+ is generally considered as an unspecific inhibitor of the potassium ion channels (Cecchi et al., 1987). On the other hand, these cations are known for their stimulating effect on Na^+/K^+ or H^+/K^+-ATPases (Post et al., 1972; Sternlichi and Vassale, 1995; Ratheal et al., 2010).

Several types of potassium channels are known to be present in oomycetes. Analysis of transcriptomic data showed the presence of a significant diversity of voltage-gated potassium channel homologs (K_v-like channels) and cyclic nucleotide-gated binding domain-containing channel homologs (CNBD-channels) in these organisms, including unusual tandem K_v-like channels and CNBD-channels (Pozd-nyakov et al., 2020). In addition, there are electrophysiological evidences of potassium ion channels in the plasma membrane of the oomycete *Saprolegnia ferax* (Garrill et al., 1992; Lew et al., 1992). In *S. ferax*, Ca^{2+}-activated potassium channels contribute to the turgor pressure maintenance. Inhibition of these channels caused a rapid but transient decrease in the oomycete growth (Garrill et al., 1993). In *Pythium aphanidermatum*, K^+-transporting P-type ATPase was identified and characterized as a probable $H^+(Na^+)/K^+$-ATPase (Barrero-Gil et al., 2005).

The importance of potassium transport for zoospore motility and behavior was demonstrated for different species of *Phytophthora* (*Ph. palmivora, Ph. megakarya, Ph. infestans*) and *Pythium* (*P. apha-nidermatum, P. dyssotocum*) (Appiah et al., 2005). Zoospores of *Ph. parasitica* tend to avoid concentrations of K^+ higher than 1–4 mM (Galiana et al., 2019). Potassium ions in concentration 5 mM and higher led to encystment of the entire zoospore population of *Ph. cinnamoni*. Interestingly, CsCl at concentrations up to 50 mM caused only partial encystment in the zoospore population (Byrt et al., 1982). At the same time, potassium is essential for germination of zoospores of the animal pathogenic oomycete *Aphanomyces astaci*, and Cs^+ has a negative effect on this process (Svensson and Unestam, 1975).

Thus, the investigation of the role of potassium transport in physiology of such an economically impacting oomycete as *Ph. infestans* is of great interest and importance. In this work, we examined the effect of CsCl at concentrations of 3, 6, 12, and 24 mM on the growth of mycelium and zoospore motility in *Ph. infestans*.

Material and methods

CULTURING OF *PHYTOPHTHORA INFESTANS* AND EXPERIMENTS WITH CsCl

Phytophthora infestans mycelia (VZR18 strain, culture collection of microorganisms, pathogenic for plants and plant pests, All-Russian Institute of Plant Protection) was cultured on Rye A Agar medium (Caten and Jinks, 1968) in 90 mm Petri-dishes at 18 °C in the dark.

In order to investigate the effect of cesium ions on the phytophthora growth, parts of one-month mycelia were placed into new 90 mm Petri-dishes filled with Rye A Agar medium containing 0, 3, 6, 12, and 24 mM CsCl (Sigma-Aldrich). The growth of oomycete was observed during 62 days. The rate of the mycelium growth was determined as a slope of the curve reflecting daily increase in the diameter of mycelium (the mycelium diameter plotted against time in days).

ZOOSPORE PRODUCTION AND TREATMENT WITH CsCl

Ph. infestans mycelia, which were used to produce zoospore suspension, were grown on Rye A Agar medium without CsCl for 2–4 weeks. To trigger the release of zoospores, the following procedure was performed. Culture plates were flooded with 12–15 ml of cold (10 °C) modified Petri solution (0,25 mM $CaCl_2$, 1 mM $MgSO_4$, 1 mM KH_2PO_4, 0,8 mM KCl). To facilitate detachment of the zoosporangia, flooded thalli were gently rubbed with a sterile glass rod. Then the flooded plates were cooled on ice for 10 minutes in a cold chamber (10 °C). After that, ice was removed and thalli were incubated at 10 °C for 1,5 h.

At the next step, a modified Petri solution was used to prepare CsCl solutions of the following concentrations: 48, 24, 12, and 6 mM. Then equal volumes (40 µl) of zoospore suspension and cold (10 °C) CsCl solution of one of the concentrations listed above were mixed in a 33 mm cell imaging
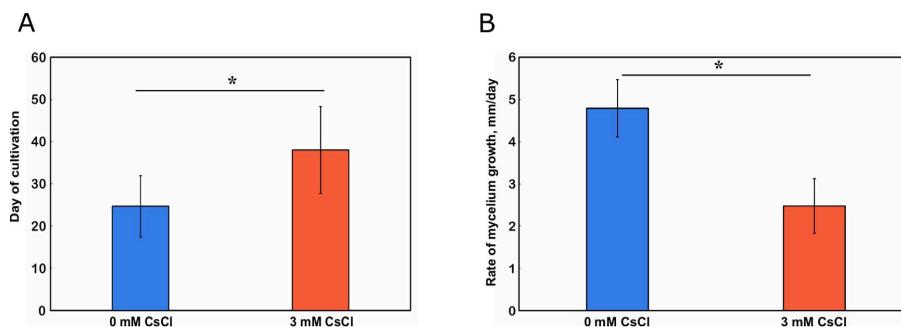
Fig. 1. Effect of CsCl on *Ph. infestans* growth on agar plates. A − A day when mycelia growth in 0 and 3 mM of CsCl was detected (mean ±SD); B − rate of mycelium growth in 0 and 3 mM CsCl (mean ±SD). *Asterisks* indicate statistically significant difference (unpaired two-tailed t-test, n = 6, p < 0,05).

dish (Eppendorf, Germany). Thus, the final concentrations of CsCl were 24, 12, 6, and 3 mM. In control replicates, zoospore suspension was mixed with a modified Petri solution without CsCl. Experimental and control replicates were incubated in a cold chamber (10 °C) for 1 h. Since experiments were performed on different days and different mycelia were used for each concentration of CsCl, each experiment possesses its own control replicate (0 mM CsCl). All experiments were repeated three times.

ZOOSPORE TRACKING

After the end of the incubation, cell imaging dishes with zoospores were transferred quickly to the stage of the inverted microscope Micromed I (Micromed, Saint Petersburg, Russia) equipped with a ToupCam 9.0 MP digital camera and Toup View 3.7 software (Hangzhou ToupTek Photonics Co., Ltd, Zhejiang, P.R. China, https://touptek. com/). For each replicate, a series of 80 consecutive photographic frames at a speed of 8 frames per second was taken. In order to minimize the effect of temperature and light on zoospore swimming, all recordings were made in the interval from the 1st to the 15th second from the moment the light was turned on.

Individual zoospores were tracked (Supplementary Videos) automatically with the help of TrackMate Fiji plugin (Tinevez et al., 2017). All tracks with duration lower than 1,25 s were removed and the remaining ones were adjusted manually if it was necessary (e.g. to remove tracks of debris accidentally taken by the software). For each track,

a mean speed (*Vt*) was calculated as an average of the instantaneous velocities between each two successive points of this track. Then, a mean *Vt* was calculated for a given replicate.

STATISTICS

Statistical analysis was performed by means of MaxStat Lite 3.60 software (https://maxstat.de/ en/home-en/). In the case of growth experiments, mean values were compared using the unpaired two-tailed t-test. In the case of zoospore tracking experiments, mean values were compared using paired two-tailed t-test.

Results

EFFECT OF CsCL ON THE GROWTH OF *PH. INFESTANS* MYCELIUM

After 62 days of observations we detected growth of *Ph. infestans* in Rye A Agar plates with 0 and 3 mM CsCl. The growth of the oomycete was not detected in the plates with 6, 12, and 24 mM CsCl. At the same time, the addition of 3 mM CsCl to the medium lead to a delay of the mycelium growth: the growth was detected after 24,7±7,3 (mean ± SD, n = 6) days incubation at 0 mM CsCl and after 38,0±10,3 (mean ±SD, n = 6) days incubation at 3 mM CsCl (Fig. 1, A). Moreover, the mean rate of *Ph. infestans* growth was almost two times lower at 3 mM CsCl (2,5±0,6) compared to the control (4,8 ±0,7) (Fig. 1, B).
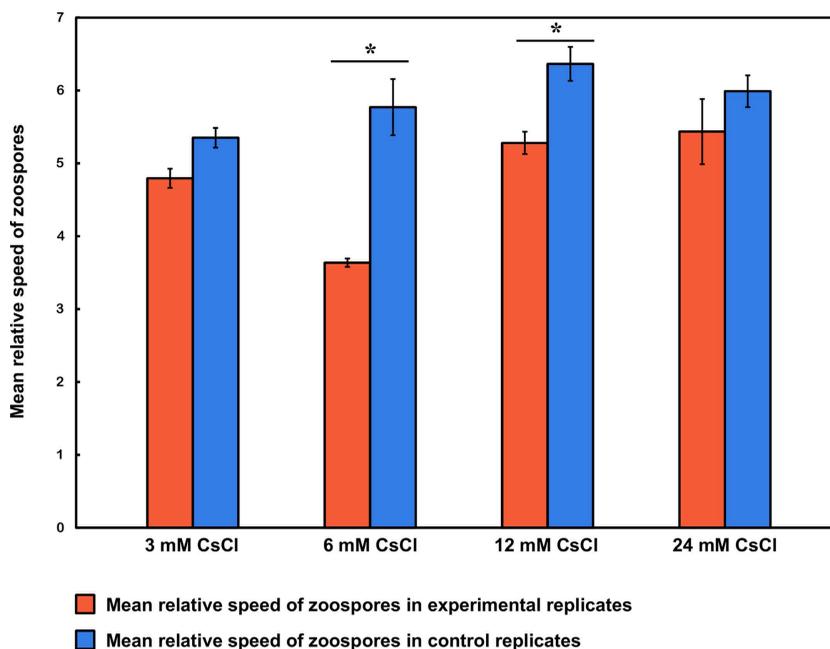
Fig. 2. Effect of CsCl on mean relative speed of *Ph. infestans* zoospores. Standard errors are indicated. *Asterisks* indicate statistically significant differences (paired two-tailed t-test, n = 3, p < 0,05).

INFLUENCE OF CSCL CONCENTRATION ON ZOOSPORE MOTILITY

The addition of CsCl to the medium has led to a decrease in the mean relative swimming speed of zoospores (Fig. 2). However, only in the Petri solutions with 6 and 12 mM CsCl the relative swimming speed of zoospores was significantly lower than in the control replicates (paired t-test, p < 0,05, n = 3). Surprisingly, the most substantial decrease in speed was observed in the 6-mM-series (1,6 times lower than in control). The mean relative swimming speed of zoospores was 1,2 times lower in 12-mM-experiments than in the control, and there was no significant difference between mean relative speeds in the experimental and control replicates in the case of 24-mM-experiments.

Discussion

The application of CsCl at concentrations of 3, 6, 12, and 24 mM to the Rye A Agar medium has led to both the delay and inhibition of the mycelium growth of *Ph. infestans*. It is possible that 62 days of observation were not enough to detect a start of mycelia growth in CsCl concentrations higher than 3 mM. The obtained data clearly showed an inhibiting effect of cesium ions on the oomycete growth indicating an essential role of the potassium transport in this process. Previously, Garrill with co-authors demonstrated the participation of Ca^{2+}-activated K^+ channels in the turgor maintenance and tip growth of the oomycete Saprolegnia ferax (Garrill et al., 1992; Garrill et al., 1993). Most of metazoan potassium channels, including Ca^{2+}-activated K^+ channels BK, are blocked by cesium ions (Cecchi et al., 1987). Hence, the negative effect of Cs^+ on the phytophthora growth can be mediated by a homolog of *S. ferax* Ca^{2+}-activated K^+ channel or by the other classes of potassium channels. Recently we found the homologs of metazoan voltage-gated potassium channels (K_v) and hyperpolarization activated cation channels (HCN) in *Ph. infestans* and other oomycetes (Pozdnyakov et al., 2020). These channels are known to be sensitive to Cs^+ also in animal cells (Cecchi et al., 1987). Since the oomycetan homologs possess the same amino acid sequence of the selectivity filter as the animal ones (Pozdnyakov et al., 2020), it is possible that these ion channels are potassium-permeable and sensitive to cesium ions. Thus, it can be assumed that the inhibition of *Ph. infestans* mycelium growth can be due to the cesium blockage of K_v- and HCN-like channels.

Previously it was shown that, depending on concentration, cesium caused encystment or lysis of zoospores of at least two *Phytophthora* species (Byrt et al., 1982; Grant et al., 1986); moreover, it decreased the viability of cysts of the oomycete *Aphanomyces astaci* (Svensson and Unestam, 1975). Therefore, we assumed that the motility of zoospores would more or less steadily decline with the increasing CsCl concentration. However, the result was unexpected: the slowest zoospores were detected in 6 mM-experiments, while in the solutions with higher concentrations of CsCl (12 and 24 mM), the relative swimming speed was higher. Moreover, in 24 mM-experiments, the speed did not differ significantly from the control value. The same effect was observed for the 3 mM-experiments.

There is a hypothetical explanation for the pecu-liar pattern described above. As already mentioned, a correlation between Cs^+ concentration and the percentage of encystment and lysis of zoospores was previously described. According to the research conducted by Byrt and co-authors (1982), the viability of *Ph. cinnamomi* zoospores dropped below 20% in 20 mM CsCl solution after 20 min of incubation. A similar pattern was demonstrated for *Ph. palmivora*: the proportion of lysed zoospores after 1 hour of incubation in 10 mM CsCl solution reached 80%; at the same time, encystment was induced by lower (0,3–3 mM) concentrations (Grant et al., 1986). Although we did not determine the concentration of zoospores in the studied suspensions in the course of this research, we noticed that experimental replicates with a high concentration of CsCl contained fewer swimming zoospores (12 mM and especially in 24 mM) than in 3 mM, 6 mM, and in control replicates. Therefore, it is possible that at higher concentrations of CsCl solutions, a significant part of the zoospores underwent lysis, which thus was the main effect caused by Cs^+, while a minor part endured the treatment, remained motile, and was tracked. Zoospores resistant to CsCl likely were able to maintain their normal swimming speed. It seems that in the case of 6-mM-experiments, the concentration of CsCl was enough to slow zoospores down but not enough to cause a significant damage. Finally, the 3 mM concentration of Cs^+ was probably too low to decrease the swimming speed of zoospores significantly.

Thus, our experiments showed that potassium transport is essential for normal activity of mycelium and zoospores of the plant pathogen *Ph. infestans.*

This information is important for understanding the physiology of oomycetes and should be taken into account during the development of the oomycete-inhibiting agents.

Acknowledgements

References

Appiah A.A., van West P., Osborne M.C. and Gow N.A.R. 2005. Potassium homeostasis influences the locomotion and encystment of zoospores of plant pathogenic oomycetes. Fungal Genet. Biol. 42, 213–223.

Barrero-Gil A., Garciadeblбs B. and Benito B. 2005. Sodium, potassium-ATPases in algae and oomycetes. J. Bioenerg. Biomembr. 37, 269–278.

Beakes G.W., Honda D. and Thines M. 2014. 3 Systematics of the Straminipila: Labyrinthulomycota, Hyphochytriomycota, and Oomycota. In: Systematics and Evolution: Part A (Eds.: McLaughlin D.J. and Spatafora J.W.). Springer, Berlin, Heidelberg, pp. 39–97.

Byrt P.N., Irving H.R. and Grant B.R. 1982. The effect of cations on zoospores of the fungus *Phytophthora cinnamon*. J. Gen. Microbiol. 128, 1189–1198.

Caten C.E. and Jinks J.L. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. Can. J. Bot. 464, 329–348.

Cecchi X., Wolff D., Alvarez O. and Latorre R. 1987. Mechanisms of Cs^+ blockage in a Ca^{2+}-activated K^+ channel from smooth muscle. Biophys. J. 52, 707–7016.

Edgerton B.F., Henttonen P., Jussila J., Mannonen A., Paasonen P., Taugbøl T., Edsman L. and Souty-Grosset C. 2004. Understanding the causes of disease in European freshwater crayfish: disease in European crayfish. Conservat. Biol. 18, 1466–1474.

Galiana E., Cohen C., Thomen Ph., Etienne C. and Noblin X. 2019. Guidance of zoospore by potassium gradient sensing mediates aggregation. J. R. Soc. Interface. 16, 20190367.

Garrill A., Jackson S.L., Lew R.R. and Heath I.B. 1993. Ion channel activity and tip growth: tip-

localized stretch-activated channels generate an essential Ca^{2+} gradient in the oomycete *Saprolegnia ferax*. Eur. J. Cell Biol. 60, 358−365.

Garrill A., Lew R.R. and Heath I.B. 1992. Stretch-activated Ca^{2+} and Ca^{2+}-activated K$^+$ channels in the hyphal tip plasma membrane of the oomycete *Saprolegnia ferax*. J. Cell Sci. 101, 721−730.

Grant B.R., Griffith J.M. and Irving H.R. 1986. A model to explain ion-induced differentiation in zoospores of *Phytophthora palmivora*. Exp. Microbiol. 10, 89−98.

Haverkort A.J., Boonekamp P.M., Hutten R., Jacobsen E., Lotz L.A.P, Kessel G.J.T., Visser R.G.F. and van der Vossen E.A.G. 2008. Societal costs of late blight in potato and prospects of durable resistance through cisgenic modification. Potato Res. 51, 47−57.

Lew R.R., Garrill A., Covic L., Heath I.B. and Serlin B.C. 1992. Novel ion channels in the protists, *Mougeotia* and *Saprolegnia*, using sub-gigaseals. FEBS Letters. 310, 219−222.

Nowicki M., Foolad M.R., Nowakowska M. and Kozik, E.U. 2012. Potato and tomato late blight caused by *Phytophthora infestans*: an overview of pathology and resistance breeding. Plant Dis. 96, 4−17.

Post R.L., Hegyvary C. and Kume S. 1972 Activation by adenosine triphosphate in the phosphorylation kinetics of sodium and potassium ion transport adenosine triphosphatase. J. Biol. Chem. 247, 6530−6540.

Pozdnyakov I., Safonov P. and Skarlato S. 2020. Diversity of voltage-gated potassium channels and cyclic nucleotide-binding domain-containing channels in eukaryotes. Sci. Rep. 10, 17758.

Ratheal I.M., Virgin G.K., Yu H., Roux B., Gatto C. and Artigas P. 2010. Selectivity of externally facing ion-binding sites in the Na/K pump to alkali metals and organic cations. Proc. Natl. Acad. Sci. U.S.A. 107, 18718−18723.

Sternlichi J.P. and Vassalle M. 1995. Cesium, Na$^+$-K$^+$ pump and pacemaker potential in cardiac Purkkinje fibers. J. Biomed. Sci. 2, 366−378.

Sutton J.C., Sopher C.R., Owen-Going T.N., Weizhong L., Grodzinski B., Hall J.C. and Benchimol R.L. 2006. Etiology and epidemiology of *Pythium* root rot in hydroponic crops: current knowledge and perspectives. Summa Phytopathol. 32, 307−321.

Svensson E. and Unestam T.1975. Differential induction of zoospore encystment and germination in *Aphanomyces astaci*, Oomycetes. Physiol. Plant. 35, 210−216.

Thines M. and Choi Y.-J. 2016. Evolution, diversity, and taxonomy of the Peronosporaceae, with focus on the genus *Peronospora*. Phytopathology. 106, 6−18.

Tinevez J.Y., Perry N., Schindelin J., Hoopes G.M., Reynolds G.D., Laplantine E., Bednarek S.Y., Shorte S.L. and Elicieri K.W. 2017. TrackMate: An open and extensible platform for single-particle tracking. Methods. 115, 80−90.

van West P. 2006. *Saprolegnia parasitica*, an oomycete pathogen with a fishy appetite: new challenges for an old problem. Mycologist. 20, 99−104.

van Wyk P.S., Jones B.L., Vilgoen A. and Rong I.H. 1995. Early lodging, a novel manifestation of *Albugo tragopogonis* infection on sunflower in South Africa. Helia. 18, 83−90.

Yoshida K., Schuenemann V.J., Cano L.M., Pais M., Mishra B., Sharma R., Lanz C., Martin F.N., Kamoun S., Krause J., Thines M., Weigel D. and Burbano H.A. 2013. The rise and fall of the *Phytophthora infestans* lineage that triggered the Irish potato famine. ELife. 2, e00731.

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