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## Requirement for $K^+$ ions at conidial germination of *Neurospora crassa*

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### Abstract

Turian G., Löffel R. and M. Michéa-Hamzehpour 1984, Requirement for  $K^+$  ions and conidial germination of *Neurospora crassa*. Bot. Helv. 94:301–305.

$K^+$  ions are required for the germination of conidia of *Neurospora crassa* incubated in an ammonium nitrate synthetic medium. This promoting effect can also be obtained, but less efficiently, with  $Rb^+$  ions while the ionophore valinomycin can counteract it. It is suggested that  $K^+$  ions are implicated in both changes of internal pH and turgor pressure required for germ tube outgrowth.

A need for certain ions has been emphasized for general processes of fungal spore germination (Gottlieb 1978). Potassium ions are required for zoospore germination of *Blastocladiella emersonii* (Soll and Sonneborn 1972). In this species  $K^+$  is the first signal for encystment to begin which involves depolarization of the ATP-energized plasma membrane (van Brunt and Harold 1980, Jen and Haug 1981).

In *Neurospora crassa* the lack of  $Mg^{2+}$  was shown to delay maximally the emergence of abnormally enlarged germ tubes from macroconidia while a delay consecutive to a lack of  $K_2HPO_4$  could not clearly be ascribed to either  $K^+$  or phosphate ions in the experimental conditions set by Farach et al. (1979). It was therefore important to reassess the role of  $K^+$  at the germination of macroconidia of *N. crassa* with reference to the stage involved, as our finding of a localized acidification at germ tube outgrowth had led us to suggest a possible plasmalemmal  $H^+/K^+$  exchange at this stage (Turian 1980).

### Material and methods

Conidia of *N. crassa* (strain STA<sub>4</sub>) were produced on solid nitrate minimal medium (Difco) in Fernbach flasks maintained for 2 days in darkness followed by 3 days in the light at 25 °C. Conidia were then collected with sterile water, filtered through gauze and centrifuged 5 min at 5000 × g. Erlenmeyer flasks containing 30 ml of medium were inoculated with  $1.2 \times 10^8$  conidia. The Vogel's synthetic medium (1956) was used either in its standard composition (with  $NH_4NO_3$ ) or modified by equimolar substitution of  $NaH_2PO_4$  for the usual  $KH_2PO_4$ ; the final pH was 5.8. The germination rates were determined for 1 to 6 h in shaken cultures at 25 °C, with either freshly harvested conidia, or with conidia maintained for 14–24 h at 4 °C.

Counting of germinated conidia (outgrown germ tubes) has been made on slides receiving each a drop of conidial suspension stained with cotton blue in lactophenol (Langeron 1945). For each condition of germination, 500 conidia have been rated as germinated (emergent tube) versus non-germinated in each of four repeated experiments.

All our chemicals were puriss products and the  $K^+$ -ionophore valinomycin was obtained from Sigma products.

Photomicrography has been made on a microscope Wild M-20 with Fujicolor-400 film.

## Results and discussion

In all experiments in which  $KH_2PO_4$  of Vogel's medium has been equimolarly replaced by  $NaH_2PO_4$  (52 mM  $Na^+$ ), there was no significant percentage of germ tube emergence before 6 h of incubation at 25 °C (average 8%).

However, the addition of 1 mM  $K^+$  to such Vogel-Na medium strongly enhanced the emergence rate (Fig. 1). The stimulation followed immediately the addition of  $K^+$  into the  $Na^+$  medium. A minimum of 2 h incubation, which corresponds to the normal period of so-called "swelling" stage of the inoculated conidia (Schmit and Brody 1976), was required to obtain the stimulation effect of  $K^+$  ions. After 2 h incubation, the out-

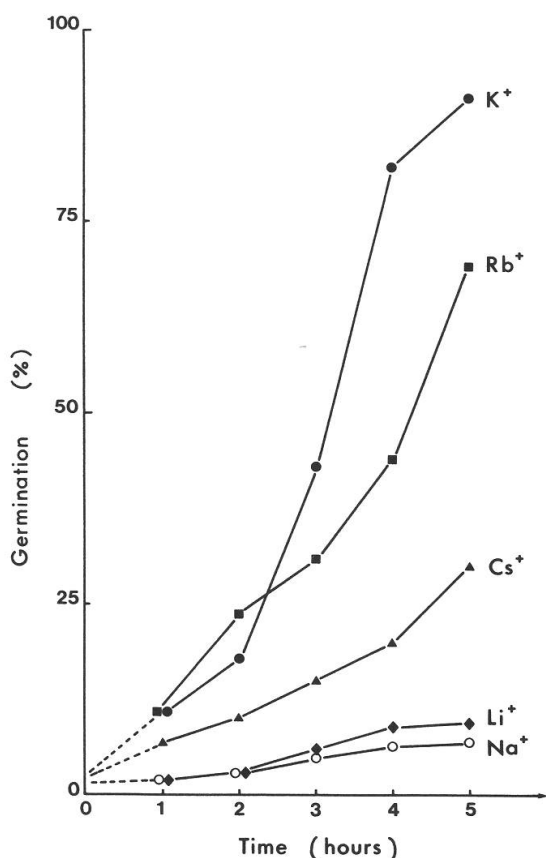


Fig. 1. Percentage conidial germination at 25 °C in  $Na^+$ -Vogel's liquid synthetic medium complemented with 1 mM of the alkali metal ions indicated. Each curve is representative of one among four repeated experiments.

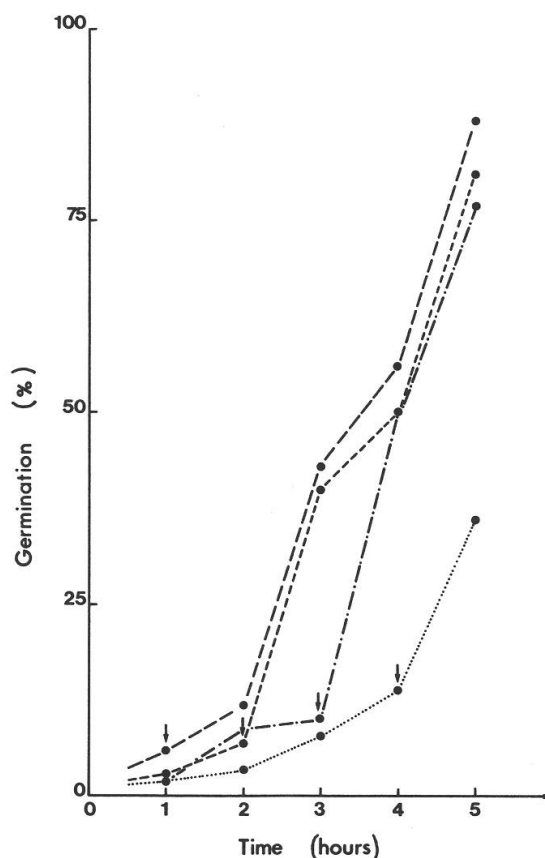


Fig. 2. Conidial germination at 25 °C induced by 1 mM  $K^+$  sequentially (1–4 h) added into  $Na^+$ -Vogel's liquid synthetic medium.

growth rate follows immediately the sequential addition of  $K^+$  (Fig. 2). The "swelling" stage can therefore proceed with the exogenous  $Na^+$  only while the process of internal polarization leading to the built-up of an outgrowth site at the surface of the conidium appears to be contingent upon the exogenous presence of  $K^+$  ions.

As an alternative efficient ion to the outgrowth need for  $K^+$ , only  $Rb^+$  was found to be efficient. However, the concentration of  $Rb^+$  had to be increased to 25 mM to bring the rate of germ tube outgrowth at the closest with that obtained with 1 mM  $K^+$  (Fig. 1). Comparatively to  $K^+$ -induced outgrown tubes, those emitted in the presence of  $Rb^+$  were wider and elongated at a slower pace.

The addition of  $CsCl$  to the  $Na^+$  medium also improved the outgrowth rate but in a lesser degree than with  $RbCl$  (Fig. 1). Here also 25 mM  $Cs^+$  were needed for an optimal outgrowth rate, and the tubes emerged from unequally swollen conidia were again wider than those emitted in the presence of  $K^+$ .

Lithium ions (1–48 mM  $Li^+$ ) offered as  $LiCl$  or  $Li$ -citrate were totally ineffective. With 1 mM  $Li^+$ , only 6% of germ tubes outgrew by 6 h but with no apparent abnormality of the emerged tubes.

Stringent  $K^+$  requirement for conidial germination only occurred with  $NH_4NO_3$  from which "ammonia" is known to be transported and metabolized preferentially to nitrate and internalized  $K^+$  required for  $NH_4^+$  exchange (MacMillan 1956). When incubated in Vogel- $Na^+$  medium with  $NaNO_3$  as nitrogen source, conidia could slowly germinate reaching 50–60% after only 6 h at 25 °C while that percentage was already attained after 3 h with  $KNO_3$ . This suggests that some process in the transport and metabolization of  $NO_3^-$  could substitute somehow but less efficiently for the role of  $K^+$  at conidial germination.

If  $K^+$  influx is requested to drive inversely directional  $H^+$  extrusion from the cytoplasm of germinating conidia, then it is expected that such process could be disturbed in the presence of a ionophore antibiotic such as valinomycin known to permeate cellular membranes to  $K^+$  (Moore and Pressman 1964). We have indeed observed an efficient prevention of germ tube outgrowth in the presence of 250–1000 nM valinomycin in the standard Vogel's medium. The process was still delayed with 50 nM of valinomycin and 10 nM of the ionophore were sufficient to slow the fast rate of germination induced by 1 mM  $K^+$  in the Vogel- $Na^+$  medium.

In *N. crassa*, an electrogenic ATP driven  $K^+-H^+$  plasmalemmic exchange system has been well characterized (Slayman et al. 1973). It produces an electrical current carried by protons which are extruded into the external medium in exchange for  $K^+$  (Slayman 1970). The necessary pumps at the plasmic membrane are  $H^+ (Na^+)/K^+$  ATPases, the number of which was found to drop as one moves to the hyphal tip (Jennings 1973) thus explaining the decrease in membrane potential measured at such level (Slayman and Slayman 1962).

On such basis, we can suggest that  $K^+$  ions are required at conidial germination of *N. crassa* to extrude the protons dissipated in excess into the cytoplasm of conidia during their semidormancy and thereby bring it to a more relative alkaline status compatible with the resumption of mitotic activity – as known in yeasts and animal cells, see Nucitelli and Deamer, 1983 – after emergence of the acidified germ tube (Turian 1983 a). Such interpretation is strengthened by our recent observation that pH-indicators such as alizarin yellow S or bromocresol purple turn to their dark purple alkaline-side color in the "mother cell" while still remaining relatively acid in the early swelling conidia as shown by their reddish tinge turning more and more acidic (yellow color) in the emerging tubes (Fig. 3). We can therefore presume that the deacidification process by  $K^+-H^+$

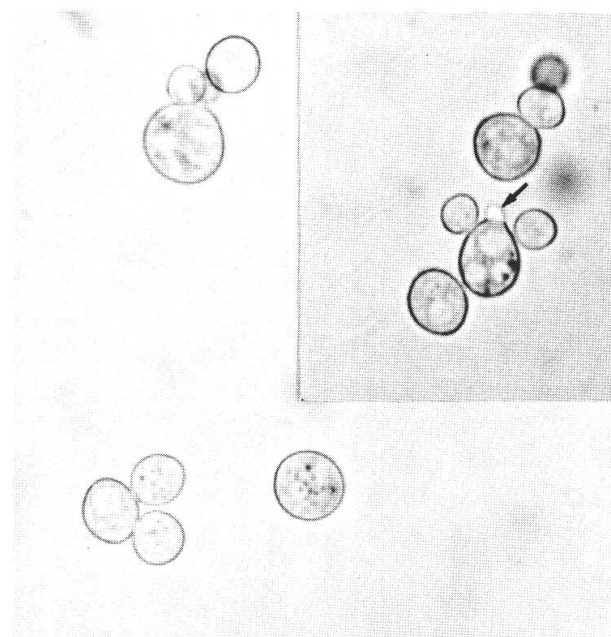


Fig. 3. Early germinating conidia of *Neurospora crassa* briefly stained in a dilute aqueous solution ( $10^{-4}$ ) of alizarin yellow S and color microphotographed for pH-indicative contrasts: "swollen" conidia with the darkened (purple red = pH >) cytoplasm contrasting with the more fair staining (pinkish) of the still "unactivated" conidia; sharper contrast between one mother conidium and its fair (yellowish = pH <) outgrowing germ tube (arrow).  $\times 1000$ .

exchange is active all along the periphery of the conidial "mother cell" at the exclusion of the outgrowing membrane site depolarized from inside by the protons vectorially dissipated from the frontally positioned mitochondria; such protons would be scavenged in the cytosolic exclusion zone, sorted at the tip acting as proton sink (Turian 1980) probably through the negatively-charged content of its accumulated vesicles (Turian 1983 b). An additional, and parallel, explanation for the relative alkalinization of the conidial cytoplasm could also be found in the reintrusion of protons inside the mitochondria (except in those positioned in front of the prospective germ tube) at the "swelling" stage preceding outgrowth, as shown by the recovery of an efficient cytochromic respiration to initiate that stage, while heat-imposition and maintenance of the alternate oxidase respiration only allows isometric growth (Michéa-Hamzehpour and Turian 1984).

Outside their presumed role in that relative alkalinization of the conidial cytoplasm,  $K^+$  ions can be expected to play a role as osmolite in increasing the turgor pressure of the cytoplasm in the "mother cell" thereby pressing against what might be the weak plasticized point in the wall at the prospective site of germ tube outgrowth. The finding of a local acidification and consequent charge positivity at that topocytological level, initiated by vectorial dissipation of protons from frontal mitochondria (Turian 1980) and further sustained by locally reintruded protons producing an inward electrical current (Harold and Harold 1980) could thus be combined with the proposal of a standing-flow osmotic gradient of water toward the apex (Jennings 1979, Gooday 1983) into an electro-osmotical model as driving force to polarized germ tube outgrowth.

We thank Miss A. Cattaneo for her efficient technical help.

## Résumé

Les ions  $K^+$  sont requis pour la germination des conidies de *Neurospora crassa* incubées en milieu synthétique au nitrate d'ammonium. L'effet promoteur peut aussi être obtenu, avec moins d'efficacité, avec les ions  $Rb^+$  alors que le ionophore valinomycine s'y oppose. Suggestion est faite que les ions  $K^+$  sont impliqués à la fois dans les changements différentiels du pH interne et de pression de turgescence requis pour l'émergence du tube germinatif.

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