Brief Report

A first response to osmostress in *Acinetobacter baumannii*: transient accumulation of K⁺ and its replacement by compatible solutes

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Summary

The extraordinary desiccation resistance of the opportunistic human pathogen *Acinetobacter baumannii* is a key to its survival and spread in medical care units. The accumulation of compatible solute such as glutamate, mannitol and trehalose contributes to the desiccation resistance. Here, we have used osmolarity as a tool to study the response of cells to low water activities and studied the role of a potential inorganic osmolyte, K⁺, in osmostress response. Growth of *A. baumannii* was K⁺-dependent and the K⁺-dependence increased with the osmolarity of the medium. After an osmotic upshock, cells accumulated K⁺ and K⁺ accumulation increased with the salinity of the medium. K⁺ uptake was reduced in the presence of glycine betaine. The intracellular pools of compatible solutes were dependent on the K⁺ concentration: mannitol and glutamate concentrations increased with increasing K⁺ concentrations whereas trehalose was highest at low K⁺. After osmotic upshock, cells first accumulated K⁺ followed by synthesis of glutamate; later, mannitol and trehalose synthesis started, accompanied with a decrease of intracellular K⁺ and glutamate. These experiments demonstrate K⁺ uptake as a first response to osmostress in *A. baumannii* and demonstrate a hierarchy in the time-dependent accumulation of K⁺ and different organic solutes.

Introduction

Fluctuation in the environmental osmolarity and water availability is one of the biggest challenges for living cells (Kempf and Bremer, 1998; Ventosa et al., 1998; Wood, 1999). This is also true for the opportunistic human pathogen *Acinetobacter baumannii*. Its natural habitat is still unknown but it is frequently found in health care institutions worldwide (Dijkshoorn et al., 2007; Peleg et al., 2008). One key to its success in the hospital environment is its enormous potential to withstand long times of dryness; *A. baumannii* is known to survive on abiotic surfaces for month or even years (Antunes et al., 2011). The unravelling of the molecular basis of drought- and osmo-resistance has just begun (Zeidler and Müller, 2019). High salinity and drought have in common a low water activity, although they have individual stress components. *Acinetobacter baumannii* takes up glycine betaine or its precursor choline from the environment (Zeidler et al., 2017; Breisch et al., 2019). In the absence of solutes in its environment, it synthesizes glutamate or mannitol, and, to a smaller extent, trehalose (Zeidler et al., 2017, 2018). Whether or not inorganic solutes such as the commonly taken up K⁺ is also taken up by *A. baumannii* remained to be established and was addressed in this study.

Results and discussion

**K⁺-dependence of growth of *A. baumannii***

To elucidate the minimal K⁺ concentration required for growth of *A. baumannii* and to deplete cells of K⁺ for further experiments, cells were grown in minimal media with 20 mM succinate as carbon and energy source and subsequently transferred in K⁺-free media (Fig. 1A). After the first transfer, the growth rate as well as final yield was not affected but after the second transfer, a drastic reduction in growth rate from 0.64 to 0.19 h⁻¹ was observed. At the same time, the yield was reduced by 80%. To restore exponential growth, K⁺ was added back to the medium. As can be seen in Fig. 1B, the growth rate increased exponentially with increasing
K+ concentration and maximal growth was reached at 200 μM K+.

Acinetobacter baumannii accumulates K+ as a response to osmotic stress

When cells were transferred twice in K+-free medium and then transferred to minimal medium containing 300 mM NaCl and increasing amounts of K+, the growth rates were reduced by more than 50% compared to NaCl-free media (Fig. 1B). The amount of K+ required for optimal growth increased fivefold to 1 mM K+. The higher K+ requirement for growth at high NaCl concentrations is in line with the hypothesis that K+ is involved in osmostress response. To analyse the effect of NaCl on intracellular K+ concentrations, cells were grown in minimal medium to mid-exponential growth phase and then subjected to an osmotic upshock by the addition of 300 mM NaCl. Cells were separated via filtration from excess media and washed to remove bound K+, followed by cell lysis and determination of the K+ concentration by atomic absorption flame spectroscopy. At low salinities (no NaCl added), the intracellular K+ concentration was 0.56 ± 0.08 μmol mg−1 protein (Fig. 2A). If one assumes an internal volume of 2 μlm g−1 protein (Dinnbier et al., 1988), this would calculate roughly to 0.4 M intracellular K+. This is plausible given the osmolarity of the minimal medium. After addition of NaCl to a final concentration of 300 mM, the intracellular K+ concentration increased within 3–4 min threefold and then declined slowly over time. The transient increase in intracellular K+ after an osmotic upshock has been seen before with other bacteria (Dinnbier et al., 1988; Whatmore et al., 1990; McLaggan et al., 1994). The intracellular K+ concentration was a clear function of the external salinity (NaCl concentration) (Fig. 2B).

K+ accumulation at high NaCl concentration may be the result of the action of an Na+/K+ antiport driven by the membrane potential to expel cytotoxic Na+ from the cell or the result of a general osmotic effect. To address this question, cells were grown in minimal medium to mid-exponential growth phase; then the osmolarity in the medium was increased by addition of sucrose or glucose. As can be seen in Fig. 3A, non-ionic osmolytes also induced K+ influx with the same rate and to the same extent as with 300 mM NaCl. This experiment clearly demonstrates that uptake of K+ is a response to general osmotic stress.

If K+ is taken up as an osmolyte in a first, fast response to osmостress, the presence of organic osmolytes taken up fast by the cells in response to osmostress such as glycine betaine should repress or reduce K+ uptake. This was addressed by incubating cells in the presence of 1 mM glycine betaine prior to addition of NaCl. The presence of glycine betaine indeed resulted in a drastically reduced K+ accumulation (Fig. 3B). While a minor amount of K+ was taken up in the first minutes, K+ was exported fast.

The intracellular pool of compatible solutes is a function of the external K+ concentration

The experiments described so far are consistent with the notion that K+ is accumulated by the cells as a first response to osmostress. To elucidate whether the K+ concentration affects the pool size of compatible solutes, cells were grown in minimal medium on 20 mM succinate in the presence of 300 mM NaCl and different K+ concentrations. Strict K+ limitation lead to a significant reduction of intracellular glutamate (0.2 ± 0.02 μmol mg−1 protein)
and mannitol (0.04 ± 0.01 μmol mg⁻¹ protein) concentrations (Fig. 4). Increasing K⁺ concentrations restored glutamate and mannitol levels. Surprisingly, the trehalose concentration was extraordinarily high at low K⁺ concentrations and reached 0.26 ± 0.06 μmol mg⁻¹ protein, which is roughly 13-fold than what was observed before (0.02 μmol mg⁻¹ protein; Zeidler et al., 2017). With increasing K⁺ concentrations, the trehalose content decreased and reached a minimum at 1 mM K⁺; at this K⁺ concentration, the trehalose concentration was only 0.02 μmol mg⁻¹ protein, or 10% of the maximal value which is in the range of the trehalose concentration determined before (0.02 μmol mg⁻¹ protein; Zeidler et al., 2017).

**Dynamics of intracellular pools of K⁺ and compatible solutes after osmotic upshock**

To address the time-dependence of the accumulation of K⁺ and organic solutes, cells were grown to mid-exponential growth phase. After the addition of NaCl to a final concentration of 300 mM, the intracellular K⁺ concentration increased by 65% within 2–3 min (Fig. 5).

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Uptake of K+ was accompanied by synthesis of glutamate, delayed by only a few minutes. Ten minutes after the upshock, the synthesis of mannitol and trehalose started. At the same time, K+ and glutamate concentrations decreased again. This experiment clearly shows a temporal order of solute accumulation: K+ ! glutamate ! mannitol and trehalose. The same has been observed before for *Escherichia coli* and *Bacillus subtilis* (Strøm et al., 1986; Larsen et al., 1987; Dinnbier et al., 1988; Whatmore et al., 1990).

**Conclusion**

Like other bacteria such as *E. coli* (Dinnbier et al., 1988; McClaggan et al., 1994) and *B. subtilis* (Whatmore et al., 1990), *A. baumannii* transiently accumulates K+, followed by the synthesis of compatible solutes. Glutamate is produced to counterbalance the positive charges (Booth and Higgins, 1990; Kempf and Bremer, 1998). Uptake of K+ can be mediated by one or more of the primary and secondary active transporter encoded in the genome of *A. baumannii*. The transiently high concentration of K+ (and glutamate) is often discussed to induce the synthesis of compatible solutes (Sutherland et al., 1986; Gowrishankar and Manna, 1996; Gralla and Vargas, 2006) and there is good evidence for this hypothesis. On the other hand, other data (Balaji et al., 2005) are not in line with this hypothesis. Anyway, in the absence of K+, synthesis of compatible solutes is largely reduced in *A. baumannii* and maximal growth is observed at 0.2 mM K+, but this value is increased at high NaCl concentrations. This is of physiological significance. In the urinary tract, an ecological niche for *A. baumannii*, the K+ concentration is 40–70 mM and the NaCl concentration is 400 mM (Culham et al., 2001; Meyer et al., 2019). In addition, the human blood (4 mM) and different types of host cells (100–160 mM) supply enough K+ to sustain growth of *A. baumannii* (Su et al., 2009; Xue et al., 2011). To answer the question whether K+ uptake is important for survival in or infection of the host remains a challenging task for future studies.

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**Conflict of interest**

The authors declare no conflict of interest.

**References**

Antunes, L.C.S., Imperi, F., Carattoli, A., and Visca, P. (2011) Deciphering the multifactorial nature of *Acinetobacter baumannii* pathogenicity. *PLoS One* 6: e22674.

Balaji, B., O’Connor, K., Lucas, J.R., Anderson, J.M., and Csonka, L.N. (2005) Timing of induction of osmotically controlled genes in *Salmonella enterica serovar*...
Typhimurium, determined with quantitative real-time reverse transcription-PCR. *Appl Environ Microbiol* 71: 8273–8283.

Booth, I.R., and Higgins, C.F. (1990) Enteric bacteria and osmotic stress: intracellular potassium glutamate as a secondary signal of osmotic stress? *FEMS Microbiol Rev* 6: 239–246.

Breisch, J., Waclawska, I., and Averhoff, B. (2019) Identification and characterization of a carnitine transporter in *Acinetobacter baumannii*. *Microbiology* 8: e00752.

Culham, D.E., Lu, A., Jishage, M., Krogfelt, K.A., Ishihama, A., and Wood, J.M. (2001) The osmotic stress response and virulence in pyelonephritis isolates of *Escherichia coli*: contributions of RpoS, ProP, ProU and other systems. *Microbiology* 147: 1657–1670.

Dijkshoorn, L., Nemec, A., and Seifert, H. (2007) An increase in threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat Rev Microbiol* 5: 939–951.

Dinnbier, U., Limpinsel, E., Schmid, R., and Bakker, E.P. (1988) Transient accumulation of potassium glutamate and its replacement by trehalose during adaptation of growing cells of *Escherichia coli* K-12 to elevated sodium chloride concentrations. *Arch Microbiol* 150: 348–357.

Gowrishankar, J., and Manna, D. (1996) How is osmotic regulation of transcription of the *Escherichia coli* proU operon achieved? *Genetica* 97: 363–378.

Gralla, J.D., and Vargas, D.R. (2006) Potassium glutamate as a transcriptional inhibitor during bacterial osmoregulation. *EMBO J* 25: 1515–1521.

Kempf, B., and Bremer, E. (1998) Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. *Arch Microbiol* 170: 319–330.

Larsen, P.I., Sydnes, L.K., Landfald, B., and Strem, A.R. (1987) Osmoregulation in *Escherichia coli* by accumulation of organic osmolytes: betaines, glutamic acid, and trehalose. *Arch Microbiol* 147: 1–7.

McLaggan, D., Naprstek, J., Buurman, E.T., and Epstein, W. (1994) Interdependence of K⁺ and glutamate accumulation during osmotic adaptation of *Escherichia coli*. *J Biol Chem* 269: 1911–1917.

Meyer, H.E., Johansson, L., Eggen, A.E., Johansen, H., and Holvik, K. (2019) Sodium and potassium intake assessed by spot and 24-h urine in the population-based Tromsø study 2015–2016. *Nutrients* 11: 1619.

Peleg, A.Y., Seifert, H., and Paterson, D.L. (2008) *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev* 21: 538–582.

Sand, M., Mingote, A.I., Santos, H., Müller, V., and Averhoff, B. (2013) Mannitol, a compatible solute synthesized by *Acinetobacter baylyi* in a two-step pathway including a salt-induced and salt-dependent mannitol-1-phosphate dehydrogenase. *Environ Microbiol* 15: 2187–2197.

Schmidt, K. (1963) Carotenoids in thiorhodaceae. II. Carotenoid composition of *Thiospirillum jenense* winogradsky and *Chromatium vinosum* winogradsky. *Arch Mikrobiol* 46: 127–137.

Strøm, A.R., Falkenberg, P., and Landfald, B. (1986) Genetics of osmoregulation in *Escherichia coli*: uptake and biosynthesis of organic osmolytes. *FEMS Microbiol Rev* 2: 79–86.

Su, J., Gong, H., Lai, J., Main, A., and Lu, S. (2009) The potassium transporter Trk and external potassium modulate *Salmonella enterica* protein secretion and virulence. *Infect Immun* 77: 667–675.

Sutherland, L., Cairney, J., Elmore, M.J., Booth, I.R., and Higgins, C.F. (1986) Osmotic regulation of transcription: induction of the proU betaine transport gene is dependent on accumulation of intracellular potassium. *J Bacteriol* 168: 805–814.

Ventosa, A., Nieto, J.J., and Oren, A. (1998) Biology of moderately halophilic aerobic bacteria. *Microbiol Mol Biol Rev* 62: 504–544.

Whatmore, A.M., Chudek, J.A., and Reed, R.H. (1990) The effects of osmotic upshock on the intracellular solute pools of *Bacillus subtilis*. *Microbiology* 136: 2527–2535.

Wood, J.M. (1999) Osmosensing by bacteria: signals and membrane-based sensors. *Microbiol Mol Biol Rev* 63: 230–262.

Xue, T., You, Y., Hong, D., Sun, H., and Sun, B. (2011) The *Staphylococcus aureus* KdpDE two-component system couples extracellular K⁺ sensing and Agr signaling to infection programming. *Infect Immun* 79: 2154–2167.

Zeidler, S., and Müller, V. (2019) Coping with low water activities and osmotic stress in *Acinetobacter baumannii*: significance, current status and perspectives. *Environ Microbiol* 21: 2212–2220.

Zeidler, S., Hubloher, J., Schabacker, K., Lamosa, P., Santos, H., and Müller, V. (2017) Trehalose, a temperature- and salt-induced solute with implications in pathobiology of *Acinetobacter baumannii*. *Environ Microbiol* 19: 5088–5099.

Zeidler, S., Hubloher, J., König, P., Ngu, N.D., Scholz, A., Averhoff, B., and Müller, V. (2018) Salt induction and activation of MltD, the key enzyme in the synthesis of the compatible solute mannitol in *Acinetobacter baumannii*. *Microbiology* 7: e00614.