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# Impact of improved potassium accumulation on pH homeostasis, membrane potential adjustment and survival of *Corynebacterium glutamicum*

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ABSTRACT

Metal ion uptake is crucial for all living cells and an essential part of cellular bioenergetic homeostasis. In this study the uptake and the impact of the most abundant internal cation, potassium, were investigated in *Actinobacteria*, a group of high G+C Gram-positives with a number of prominent biotechnologically and medically important members. Genome analyses revealed a variety of different potassium uptake systems in this monophyletic group ranging from potassium channels common in virtually all *Actinobacteria* to different active carriers that were present predominantly in pathogenic members able to cope with various stress conditions. By applying *Corynebacterium glutamicum* as model system we provide experimental evidence that under optimal conditions a potassium channel is sufficient in bacteria for the maintenance of internal pH and membrane potential ensuring survival of cells under stress conditions. Under potassium limitation, however, viability of *C. glutamicum* was increased under acidic stress or during desiccation when a functional KtrAB potassium transporter from the pathogen *Corynebacterium jeikeium* was heterologously expressed. We provide experimental evidence that the KtrAB mediated enhanced potassium accumulation improved maintenance of internal pH and membrane potential. The results indicate that the occurrence of active potassium transport systems correlates with an improved potassium-dependent bioenergetic homeostasis and survival of bacterial cells under stress conditions.



## 1. Introduction

Ion homeostasis is an essential part of life. Since the cell membrane is impermeable for ions, transport systems are mandatory for ion uptake and extrusion and carrier proteins regulate the internal ion content. The alkali metal ion potassium plays an outstanding role in this respect [1]. With concentrations between 0.1 and 0.6 M potassium is the most abundant cation in bacteria, while in general only traces are available in the environment (0.1–10 mM). It is involved in membrane potential adjustment and required for the activity of the respiratory chain [2]. Potassium acts as second messenger for stress signalling and as regulatory element for transcription control as well as counter ion for glutamate and other compounds during osmotic stress response [3]. The impact of potassium for bacterial life is indicated by the diversity of transport systems present for this particular ion. In many enteric bacteria primary active P-type ATPases of the Kdp-type are present in addition to secondary carriers of the Trk-type. Ktr-type systems are related to Trk transporters and are present in many Gram-positive bacteria. A third type of uptake system is the secondary Kup-type transporter that is present in many Gram-positive and Gram-negative bacteria. All secondary active carriers are supposed to use the membrane potential for uptake of potassium [3]. Additionally, several ligand-gated channel proteins are involved in potassium uptake [4,5].

The distribution of potassium transport systems in bacteria was proposed to be correlated with the potassium availability in their natural habitat [6]. Bacteria like *Escherichia coli* or *Bacillus subtilis* harbor different types of potassium carriers and maintain high internal potassium levels under both stress and standard cultivation conditions [7,8], while *Corynebacterium glutamicum*, *Klebsiella pneumoniae* and *Bacillus stearothermophilus* do not require potassium under optimal growth conditions and can be propagated in its virtual absence [1,2]. However, under acidic stress *C. glutamicum* accumulates high cytoplasmic potassium concentrations as well. A Kup-type transporter and a potassium channel encoding gene were found in the *C. glutamicum* genome, respectively. Interestingly, no activity was observed for the secondary active potassium carrier Kup and the potassium channel CglK was found to represent the main uptake system under standard conditions [2]. CglK represents the first example of a bacterial potassium channel for which the activity was proven in its natural membrane environment. Additionally, the analysis of CglK in *C. glutamicum* unravelled for the first time the physiological function of a bacterial potassium channel, namely, the

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decease of the membrane potential and the maintenance of a neutral internal pH under conditions of acidic stress. The CglK channel is sufficient for potassium accumulation in presence of high potassium concentrations or at conditions of low potassium demand. However, if the potassium availability is limited or the requirement for potassium is increased the sole presence of a potassium channel might restrict growth and consequently active carriers might be required [6]. Taxonomically, *C. glutamicum* belongs to the *Actinobacteria* that are widespread in nature and live as soil bacteria, saprophytes, members of the microflora of humans or animal and plant pathogens. Medically important members are *Mycobacterium tuberculosis*, *Nocardia farcinica*, *Propionibacterium acnes*, *Corynebacterium diphtheriae* and *Corynebacterium jeikeium*, while *C. glutamicum*, *Corynebacterium efficiens*, *Bifidobacterium longum* as well as *Streptomyces coelicolor* are important in biotechnological processes [9].

In this study we analyzed the monophyletic group of *Actinobacteria* regarding the equipment with potassium transport systems. We show that all pathogenic strains, supposed to be exposed to challenging environmental limitations and stress conditions, harbor active potassium transport systems in addition to potassium channels. We addressed the impact of the mode of potassium transport by active carriers and/or channels on growth and survival from a bioenergetic point of view. Improvement of potassium transport of *C. glutamicum* harboring a functional potassium channel by additional presence of the transporter KtrAB from *C. jeikeium* led to an increased potassium content and was beneficial for the maintenance of membrane potential and internal pH accompanied by improved growth and survival of *C. glutamicum* under stress conditions.

## 2. Materials and methods

### 2.1. Bacterial strains, growth conditions and construction of mutants

*C. glutamicum* strain ATCC 13032 served as wild type and was grown either in brain heart infusion (BHI) medium (Becton-Dickenson, Heidelberg, Germany) or in minimal medium MMI [10] at 30 °C in Erlenmeyer flasks shaken at 130 rpm or in microtiter plates sealed with a gas-permeable membrane in a volume of 200 µl shaken at 1200 rpm. Plates were prepared by the addition of 15 g $l^{-1}$ agar to the medium. For all experiments precultivation was performed as described [2] and experiments were performed with cultures entered the exponential growth phase. Whereas BHI contains 10 mM potassium, MMI contains 37 mM potassium. The pH was adjusted by appropriate buffer substances (250 mM) as described [11]. If necessary, the medium was supplemented with kanamycin (25 µg $ml^{-1}$) and for induction of expression with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Growth was followed by measuring the $OD_{600}$. Standard molecular cloning techniques were applied using *E. coli* DH5αMCR cells grown in Luria-Bertani (LB) medium at 37 °C.

For amplification of the *ktr* locus of *C. diphtheriae* ISS3319 or *C. jeikeium* K411, primers listed in Table 1 were applied. The genomic fragments, harboring both *ktrB* and *ktrA* as well as 500 bp upstream of *ktrB*, including the putative promoter region and the ribosome binding site (Fig. S1), were cloned into the plasmid pDRIVE (Qiagen, Hilden, Germany) and the sequence of the resulting vectors pDRIVE_*ktrBA_Cd* and pDRIVE_*ktrBA_Cj* was confirmed (GATC, Konstanz, Germany). The PCR primers were designed in order to introduce a Strep-tag coding sequence at the 3′ end of the *ktrA* gene. Subsequently, the *ktrBA* fragments were cloned into the vector pEKEX2 mediating the IPTG inducible expression of genes under the control of the lac promoter [12], resulting in plasmids pEKEX2_*ktrBA_Cd* and pEKEX2_*ktrBA_Cj*. After electroporation of the *C. glutamicum* cells, the presence of the plasmids was proven by cultivation on kanamycin-containing plates, as well as by PCR. The resulting strains are listed in Table 1. Additionally, expression of the Strep-tagged KtrA protein was proven by Western blot analysis as described previously [2] using a Strep tag antibody (Sigma).

### 2.2. Measurement of external and internal potassium concentration as well as bioenergetic parameters

Monitoring of both external and internal potassium concentration was performed by flame photometry (ELEX 6361; Eppendorf, Germany) as described previously [2]. In short, after precultivation

**Table 1**
Strains, plasmids and primers used in this study.

| Strain or plasmid | Related genotype or description[a] | Reference |
|---|---|---|
| ***E. coli*** | | |
| DH5αMCR | *endA1 supE44 recA1 gyr96 relA1 deoR U169 ϕ80ΔlacZΔM15 mcrA* Δ (*mrr-hsdRMS-mcrBC*) | Grant et al. [22] |
| ***C. glutamicum* strains** | | |
| ATCC 13032 | Wild type | Abe et al. [23] |
| Δ*kup* | Deletion of *kup* (*cg0187*) in ATCC 13032 | Follmann et al. [2] |
| Δ*cglK* | Deletion of *cglK* (*cg0887*) in ATCC 13032 | Follmann et al. [2] |
| Δ*cglK*Δ*kup* | Deletion of *kup* and *cglK* in ATCC 13032 | Follmann et al. [2] |
| *Cg*_p | ATCC 13032 harboring plasmid pEKEX2 | This study |
| *Cg*_p*ktrBA* | ATCC 13032 harboring plasmid pEKEX2_*ktrBA_Cj* | This study |
| *Cg*_p*ktrBA* | ATCC 13032 harboring plasmid pEKEX2_*ktrBA_Cd* | This study |
| Δ*cglK*Δ*kup*_p*ktrBA_Cj* | Δ*kup*Δ*cglK* harboring plasmid pEKEX2_*ktrBA_Cj* | This study |
| **Plasmids** | | |
| pDRIVE | A-T cloning vector ($Km^R$, $Ap^R$) | Qiagen, Hilden, Germany |
| pDRIVE_*cglK_Cd* | pDRIVE harboring an internal fragment of gene DIP0724 of *C. diphtheriae* | This study |
| pDRIVE_*ktrB_Cd* | pDRIVE harboring an internal fragment of gene DIP1931 of *C. diphtheriae* | This study |
| pDRIVE_*ktrBA_Cj* | pDRIVE harboring the *ktrBA* genomic locus of *C. jeikeium* | This study |
| pDRIVE_*ktrBA_Cd* | pDRIVE harboring the *ktrBA* genomic locus of *C. diphtheriae* | This study |
| pEKEX2 | *E. coli*–*C. glutamicum* shuttle expression vector ($Km^R$) | Eikmanns et al. [12] |
| pEKEX2_*ktrBA_Cj* | pEKEX2 harboring the *ktrBA* genomic locus of *C. jeikeium* | This study |
| pEKEX2_*ktrBA_Cd* | pEKEX2 harboring the *ktrBA* genomic locus of *C. diphtheriae* | This study |
| **Primer**[b] | | |
| Pre500_*ktrB*_Cjeik_5` | 5′-GC**CTGCAG**GGAGACTCAGCCCGTGCTGCGTTTGC-3′ (PstI) | This study |
| *ktrA*_CStrep_Cjeik_3′ | 5′-GC**GAATTC**GCCTA*TTTTTCGAACTGCGGGTGGCTCCA*GGAATCCGCAAACTTATCCAGGT-3′ (EcoRI) | This study |
| Pre500_*ktrB*_Cdiph_5′ | 5′-GC**CTGCAG**GAAACTCAGGCGGTGTTGGCGTTGCTGC-3′ (XhoI) | This study |
| *ktrA*_CStrep_Cdiph_3′ | 5′-C**GAATTC**GCCTA*TTTTTCGAACTGCGGGTGGCTCCA*-ACCAATGATCAGCCTTTCTACAGG-3′ (EcoRI) | This study |

[a] Abbreviations: $Ap^R$, ampicillin resistance; $Km^R$, kanamycin resistance.
[b] Letters in bold indicate the recognition site for the restriction enzyme given in parentheses, the stop codon is underlined and the Strep tag-coding sequence is shown in italic letters.