部位容易形成功能性的K⁺选择性通道，无论在所有组织中都是非常低的。10 只有极少数质膜TPKs在功能上被表征。6,11,12

AtTPK1和NtTPK1亚单位的同源性研究显示，AtTPK1和NtTPK1亚单位形成同源二聚体。9 后者表明AtTPK1和NtTPK1亚单位形成同源二聚体。14

关键词：AtTPK1, K⁺, 质膜TPK, 质膜, 协解

研究背景

植物的质膜是细胞内可形成水和矿物质的储库，能提供细胞张力，在细胞膜内存储养分。此外，质膜还能成

功能K⁺内流路径。在这种情况下，我们利用Escherichia coli

导电性和在细胞中的存储方式。

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nous and in heterologous systems.6,11-13 The latter suggests AtTPK1 and NtTPK1 subunits form homodimers.11 Channel properties include inward rectification and modulation of channel activity by Ca²⁺, 14-3-3 proteins and cytosolic pH.6,12,14 However, no functional data are available on any of the other tonoplast TPK isoforms from Arabidopsis. Whereas homologous and heterologous expression of AtTPK1 and its orthologs readily results in clearly identifiable channel activity, expression of AtTPK2, 3 and 5 in various systems does not appear to produce channel currents. This suggests that AtTPK2, 3 and 5 may encode “silent” subunits incapable of forming active channels when expressed on their own. Such silent subunits could associate with subunits from AtTPK1 to form various heterodimer combinations with slightly different channel properties. However, GUS reporter expression studies show little overlap in expression patterns of different TPK isoforms whereas bimolecular fluorescence complementation studies indicate TPKs express as homodimers.9 Thus, an important question remains concerning potential physiological roles of AtTPK2, 3 and 5 and this is closely connected to the question whether AtTPK2, 3 and 5 subunits can form functional proteins. Previous reports showed that NtTPK1 was capable of complementing the K⁺ uptake deficiency phenotype of the E. coli strain LB2003 which lacks both Trk and Kdp K⁺-transporters.15 We used a similar complementation strategy to test whether AtTPK2, 3 and 5 channel subunits can rescue the low K⁺ growth phenotype of these bacteria. Our data show that, apart from AtTPK1, TPK2 and TPK5 are competent in restoring E. coli growth on low K⁺ media. Furthermore, K⁺ uptake assays show expression of the same three isoforms significantly increases bacterial K⁺ uptake.
Growth of *E. coli* LB2003 on low K+ media. Growth as a function of external K+ we grew all genotypes in liquid K+ uptake transporters Trk (TrkG and TrkH), Kup (TrkD) strain transformed with empty vector (WT-EV) (an exceedingly slow growth rate compared with the wild type test whether this defect could be overcome by plant K+ channels.

![Figure 1](image.png)

**Figure 1.** *E. coli* growth. *E. coli* wildtype cells (WT-EV), the LB2003 mutant (EV) and bacteria transformed with AtTPK1, AtTPK2, AtTPK3 and AtTPK5 were grown in liquid yeast extract/tryptone medium containing either 0.1 mM (A) or 3 mM (B) K+ in the medium. OD 600 values were obtained for each genotype for a period of 8 h (0.1 mM K+) or 6 h (3 mM K+).

Results

Growth of *E. coli* LB2003 on low K+ media. *E. coli* strain LB2003 is incapable of growing on media that contain less than around 0.1 mM K+, due to the lack of the high and medium affinity K+ uptake transporters Trk (TrkG and TrkH), Kup (TrkD) and Kdp. It has been used previously to characterize plant ion channels. It was observed that with ambient K+ levels of 0.1 mM K+ strain LB2003 transformed with empty vector (EV) has an exceedingly slow growth rate compared with the wild type strain transformed with empty vector (WT-EV) (Fig. 1A). To test whether this defect could be overcome by plant K+ channels, LB2003 was transformed with various *Arabidopsis* TPK cDNAs. RT-PCR analyses showed that in each case full length TPK transcript was present after bacterial transformation (Fig. S1). *E. coli* strains transformed with AtTPKs showed growth curves that were intermediate between WT-EV and EV respectively (Fig. 1A). These qualitative differences in growth rates are clearly dependent on K+ in the growth medium: The differences in growth rates largely disappear when external K+ is raised to 3 mM (Fig. 1B).

To get a more detailed picture of how AtTPKs affect *E. coli* growth as a function of external K+ we grew all genotypes in liquid media without added K+ (“0K”), with 0.1 mM K+, 3 mM K+ and 150 K+ mM in the solution. The growth doubling time in minutes, determined during the exponential growth phase, was substantially larger for EV and TPK3 transformed *E. coli* in the presence of 0 and 0.1 mM K+ in the medium when compared with WT-EV, TPK2 or TPK5 (Fig. 2). When external K+ is 3 mM, there is no significant difference between WT-EV and EV indicating that transport mechanisms other than Trk and Kdp mediate sufficient K+ uptake for growth. Not surprisingly, in this condition all four strains expressing *Arabidopsis* TPKs showed growth rates comparable to that of WT-EV and EV. Although the doubling times of TPK1, TPK2 and TPK5 expressing strains were larger than that of WT-EV, statistical analysis did not show significant differences between samples (Fig. 2).

K+ uptake in *E. coli* LB2003 transformants. To test whether the AtTPK dependent growth complementation was due to actual K+ uptake we performed 1 h K+ uptake assays in cells previously starved for K+. Our results show that with 0.1 mM K+ in the assay buffer the net K+ influx in WT-EV cells is around 2 nmol/gFW/h (Fig. 3). In the EV cells uptake is “negative”, i.e., cells are actually loosing K+ during the assay. Net K+ flux in TPK3 expressing cells is similar to that in EV cells with a loss of around 0.5 nmole per hour. Cells that express AtTPK1 or TPK2 show net uptake albeit smaller than that observed for WT-EV cells. With around 3 nmole per hour, the largest uptake was recorded for cells that expressed AtTPK5. The pattern of net K+ flux changed greatly when assays were performed with 3 mM K+ in the external buffer. At this external concentration, a net influx was recorded for EV cells but it was significantly smaller than that of WT-EV cells. In this condition there was also net influx for TPK3 expressing cells but this flux was not significantly different (p < 0.05) from that in EV cells. Net influx in TPK1 and TPK2 expressing cells mirrored that measured in WT-EV cells whereas the net influx in TPK5 expressing cells was not significantly different (p < 0.05) from EV (Fig. 3).

Materials and Methods

Plasmids and *E. coli* Strains. *E. coli* strain LB2003 [ΔtrkA kup1 (trkD1) ΔkdpABC5 rpsL metE thr ha gal] was kindly provided by Evert Bakker (University of Osnabruck). The cDNA from RNA isolated from *Arabidopsis* seedlings and buds was used to obtain full length clones of AtTPK1, AtTPK2, AtTPK3 and AtTPK5. Clones were amplified with the following primers:

- AtTPK1BamHI_for GCG GAT CCT GAT GTC GAG TGA TGC AGC CAT GCA AAG CCA AGG AAG TGA and AtTPK1SmaI_rev GCC CCG GGC CTT TGC AGC TCG CAT GGA ACC ACT CAT
- AtTPK2BamHI_for GCG GAT CCT GAT GTC GAG TGA TGC AGC CAT GCA AAG CCA AGG AAG TGA and AtTPK2SmaI_rev GCC CCG GGA ATA GAA GTT GCA GTG
- AtTPK3BamHI_rev GCG AGC TCG CAT GGA ACC ACT CAT
- AtTPK4SmaI_for GCC CGG GCA AAG AGG TGA and AtTPK3KpnI_rev GCG GAT CCT GAT GTC GAG TGA TGC AGC CAT GCA AAG CCA AGG AAG TGA and AtTPK4SmaI_rev GCC CGG GCA AAG AGG TGA and AtTPK3KpnI_rev GCG GAT CCT GAT GTC GAG TGA TGC AGC CAT GCA AAG CCA AGG AAG TGA and AtTPK4SmaI_rev GCC CGG GCA AAG AGG TGA and AtTPK3KpnI_rev GCG GAT CCT GAT GTC GAG TGA TGC AGC CAT GCA AAG CCA AGG AAG TGA

The plasmids were digested with *SacI* to remove the promoter sequence and subsequently inserted into the *E. coli* expression vector pET-30. The cDNAs from *Arabidopsis* were then amplified with the following primers:

- AtTPK1Rev GCC TGC AGG CCA AAG
- AtTPK2Rev GCC CCG GGC CTT TGC AGC TCG CAT GGA ACC ACT CAT
- AtTPK3Rev GCC CCG GGA ATA GAA GTT GCA GTG
- AtTPK4Rev GCC CGG GCA AAG AGG TGA and AtTPK2SmaI_rev GCC CCG GGA ATA GAA GTT GCA GTG

The PCR product was subcloned into the pET-30 vector using the *SacI* and *XhoI* restriction sites. The plasmids were transformed into *E. coli* DH5α competent cells. The resulting transformants were grown on LB agar plates containing 100 μg/mL of ampicillin and 3 μg/mL of chloramphenicol. The plasmids were purified from the transformants using a plasmid isolation kit. The plasmids were then transformed into *E. coli* DH5α competent cells.

The cDNAs from *Arabidopsis* were then amplified with the following primers:

- AtTPK1Rev GCC TGC AGG CCA AAG
- AtTPK2Rev GCC CCG GGC CTT TGC AGC TCG CAT GGA ACC ACT CAT
- AtTPK3Rev GCC CCG GGA ATA GAA GTT GCA GTG
- AtTPK4Rev GCC CGG GCA AAG AGG TGA and AtTPK2SmaI_rev GCC CCG GGA ATA GAA GTT GCA GTG

The PCR product was subcloned into the pET-30 vector using the *SacI* and *XhoI* restriction sites. The plasmids were transformed into *E. coli* DH5α competent cells. The resulting transformants were grown on LB agar plates containing 100 μg/mL of ampicillin and 3 μg/mL of chloramphenicol. The plasmids were purified from the transformants using a plasmid isolation kit. The plasmids were then transformed into *E. coli* DH5α competent cells.

For the K+ uptake assays, *E. coli* cells were grown in liquid media and harvested when the optical density at 600 nm was around 0.1. The cells were washed with 10 mM potassium phosphate buffer (pH 7.0) and resuspended in the same buffer at a concentration of 10^9 cells/mL. The K+ uptake was measured by incubating 200 μl of the cell suspension with 100 μl of 10 mM potassium phosphate buffer (pH 7.0) containing 30 μM NADPH and 100 μM ATP. The K+ concentration in the medium was monitored by measuring the absorbance at 280 nm. The net K+ influx was calculated by subtracting the K+ concentration in the medium before the incubation from the K+ concentration after the incubation.
subcloned into the pQE-32 (Qiagen) expression vector using different restriction sites: AtTPK1 and AtTPK2 by BamH I and SmaI; AtTPK3 by BamH I and KpnI; AtTPK5 by SacI and KpnI.

**Bacterial growth assays.** Bacteria were transformed with pQE32-AtTPKs and pQE32-EV plasmids and grown overnight in high K⁺ medium (KLM) containing 5 g/liter yeast extract, 10 g/liter tryptone, 150 mM KCl and 100 mg/liter ampicillin. From the overnight culture, 100 μl was diluted in 5 ml of KLM and grown to OD 600 = 0.5. Channel expression was induced by the addition of 1 mM IPTG. After 2 h of induction, the cell density was measured and the culture was diluted (normalized) to an OD 600 of 0.5. Five-μl drops of the diluted cell suspension were spotted on ampicillin plates containing 5 g/liter yeast extract, 10 g/liter tryptone and either 0, 0.1, 3 or 150 mM KCl with IPTG and ampicillin. Reproducibility of complementation assays was confirmed in independent replicate experiments and data are shown for 3–5 assays for each condition and genotype. In order to assay bacterial growth in liquid medium, the culture density of induced transformants was normalized as described above. After centrifugation for 2 min at 10,000× g and wash with fresh medium, cells were inoculated into liquid medium containing 5 g/liter yeast extract, 10 g/liter tryptone, with IPTG and ampicillin and different concentrations of K⁺ (0, 0.1, 3 or 150 mM KCl). Experiments were repeated 3–5 times and data are expressed as means ±SD. RT-PCR RNA from pelleted samples of induced transformants was isolated with the NucleoSpin RNA II kit (Macherey-Nagel) according to manufacturer’s instructions. cDNA obtained from RNA preps was used as template for PCR amplification of full length AtTPKs clones with the same set of primers described above.

**K⁺ uptake assay.** Bacteria were grown on liquid medium containing 5 g/liter yeast extract, 10 g/liter tryptone, 10 mM KCl with IPTG and ampicillin to an OD600 = 0.5. After centrifugation for 5 sec at 4,000 RPM, bacteria were resuspended in liquid medium with 0 mM KCl and IPTG and left for 3 h for K⁺ starvation. After 3 h of K⁺ starvation the cultures were split in four and spun down for 5 sec at 4,000 RPM. One fraction was incubated on ice as reference. Another three fractions of bacterial cultures were resuspended in 10 ml of liquid medium containing either 0.1 mM or 3 mM KCl and IPTG, and left for 1 h for K⁺ uptake. After measuring OD 600 of each tube, the samples (including reference samples) were washed with 0 mM KCl liquid medium. After spinning of samples for 5 sec at 4,000 RPM and removing supernatant, bacterial pellets were resuspended in 2 ml of 5% TCA (Trichloroacetic acid) and left for 0.5 h. The K⁺ measurement of bacterial samples were performed by flame photometer. Net K⁺ uptake (μmol/gFW/h) was calculated from the difference between K⁺ content in each sample minus that found in the reference sample. Experiments were repeated 3 times and data are expressed as means ±SD.

**Statistical analyses.** Statistical differences between measurements on different times and variants were analyzed by on unpaired two-tailed t-tests. Differences were considered significant at a probability level of p < 0.05.
Concluding Remarks

Taken together our data show that several Arabidopsis TPKs can complement the E. coli LB2003 phenotype. Growth data (Figs. 1 and 2) show that in particular TPK1, 2 and 5 are competent in restoring growth to almost wild type levels in conditions where EV cells grow only very slowly (0 and 0.1 mM K\(^+\)). In contrast, expression of TPK3, as confirmed by RT-PCR (Fig. S1), did not have a substantial impact on growth in these conditions. Expression of TPK1, 2 and 5 led to a substantially larger K\(^+\) uptake in short-term assays (Fig. 3) compared with EV cells. This occurred irrespective of using 0.1 or 3 mM K\(^+\) in the uptake buffer. Again, no or only a very small increment in net K\(^+\) uptake was found in cells expressing AtTPK3. Clearly, AtTPK3 does not complement the LB2003 growth defect, nor does it augment K\(^+\) uptake in these cells and this leads us to conclude that AtTPK3 does not form functional K\(^+\) channels when expressed in E. coli. In contrast to AtTPK3, AtTPK1, AtTPK2 and TPK5 not only complement the LB2003 growth defect but their expression leads to substantially increased K\(^+\) influx (Figs. 1–3). Our results therefore strongly suggest that in addition to TPK1, AtTPK2 and AtTPK5 can form functional K\(^+\) transport systems in E. coli and as such may also form functional channels in planta.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Materials

Supplemental materials may be found here: www.landesbioscience.com/journals/psb/article/24665

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Figure 3. K\(^+\) uptake in E. coli expressing Arabidopsis TPKs. K\(^+\) starved E. coli cells were exposed to 0.1 or 3 mM K\(^+\) for one hour and the difference in K\(^+\) content before and after was measured to calculate net uptake for wildtype E. coli (WT), the LB2003 mutant (EV) and bacteria transformed with AtTPK1, AtTPK2, AtTPK3 and AtTPK5. Negative values denote net K\(^+\) loss from cells. Asterisks indicate significant differences based on unpaired two-tailed t-tests (p < 0.05).
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