Supplementary Figures

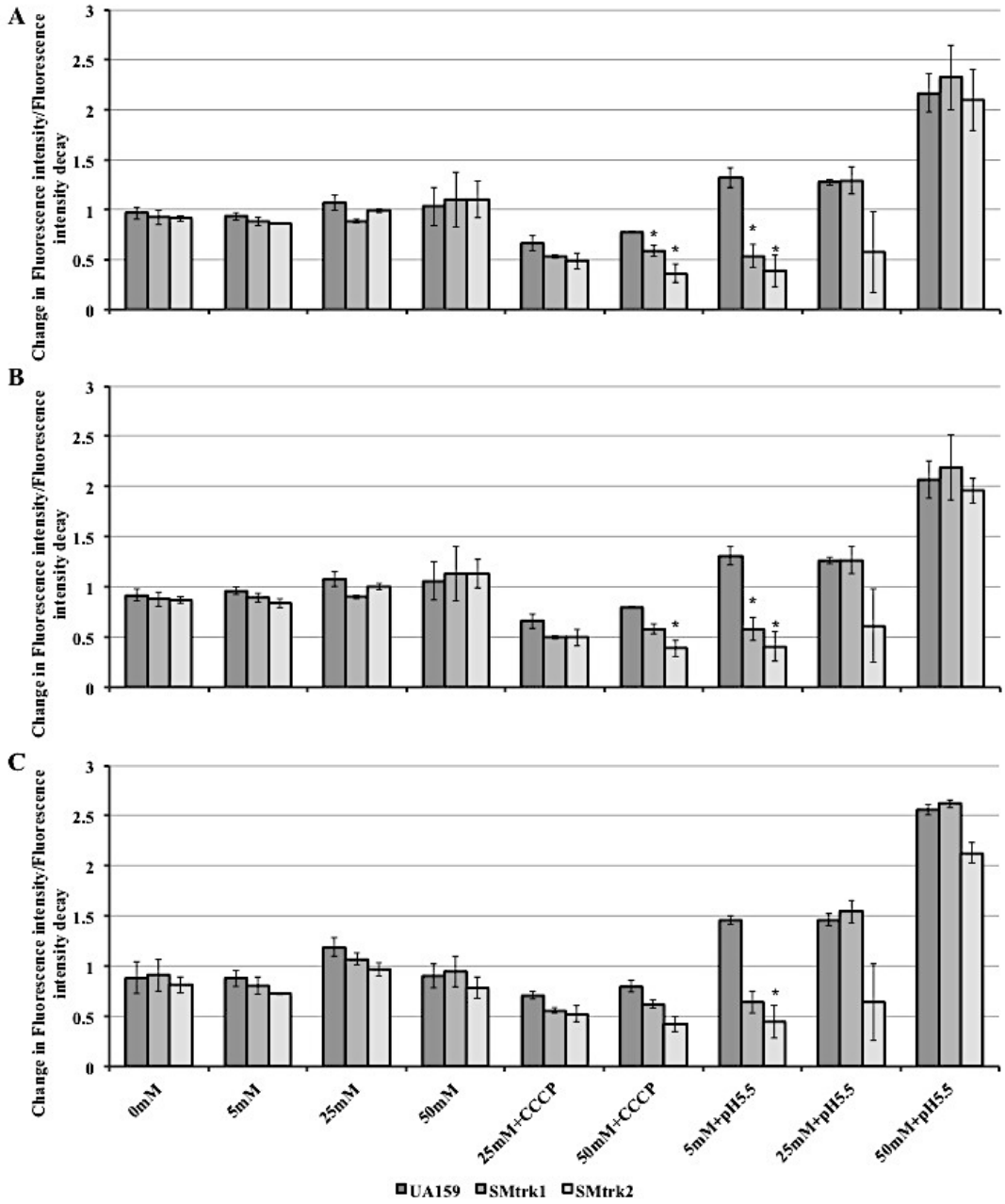

Figure S1: Membrane potential analysis of UA159, Trk1 null, and Trk2 null strains. Cells were grown in THYE to mid-exponential phase before washing and re-suspending in MMGK in the presence of 0, 5, 25, or 50 mM KCl. Aliquots of cell suspensions were incubated with $DiSBAC_2$ fluorescence dye. The effect of membrane stressors such as low pH (5.5) and introduction of potential dissipater carbonyl cyanide-m-chlorophenylhydrazone (CCCP) were also tested as described. Alterations in the membrane potential were calculated by measuring the changes in fluorescence intensity

after (A) 15 minutes, (B) 30 minutes, and (C) and 2 hours, and normalized with the fluorescence intensity decay of the dye. Asterisk (*) represents a statistical significance of p-value < 0.05 for mutant strains in comparison to wtUA159; calculation was done using student's t-test for a total of 6 replicates; bars represent mean ± SE values.

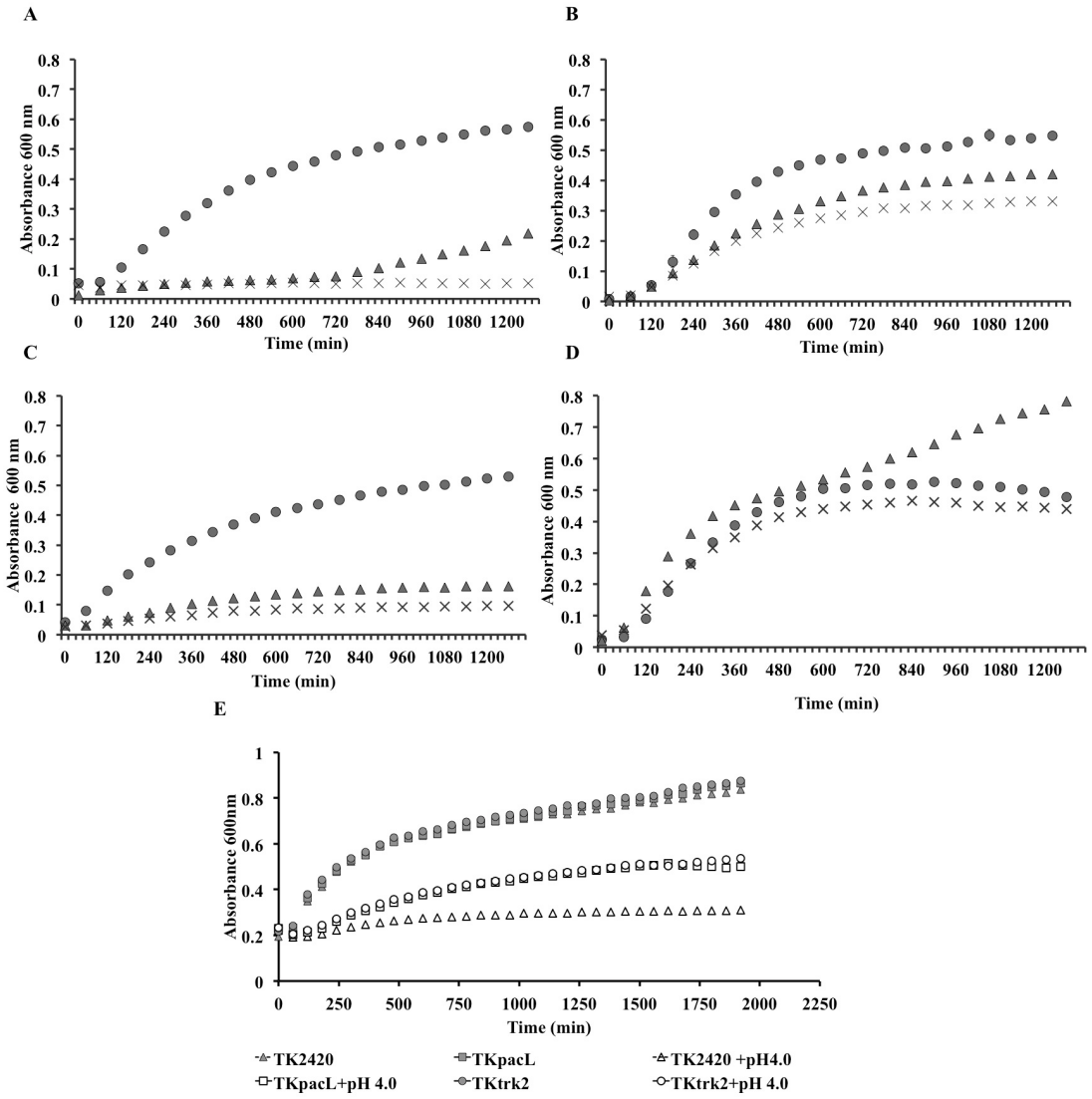

Figure S2: Growth curves for *E. coli* TK2420 mutant and its complemented strains. Cells are: (A) *E. coli* TK2420 mutant, and its complemented counter strains with (B) GlnQHMP, (C) Trk1, and (D) Trk2 systems. Strains were grown in HKLM medium (●), LKLM (Δ) and LKLM with 400 mM NaCl (✖) for 16 hours. Growth curves are representative of at least three independent experiments conducted with five replicates each. The lower panel (E) shows the effect of pH 4.0 on the growth of TK2420 *E. coli* mutant and TK2420 strains complemented with pacL or Trk2 systems as indicated.

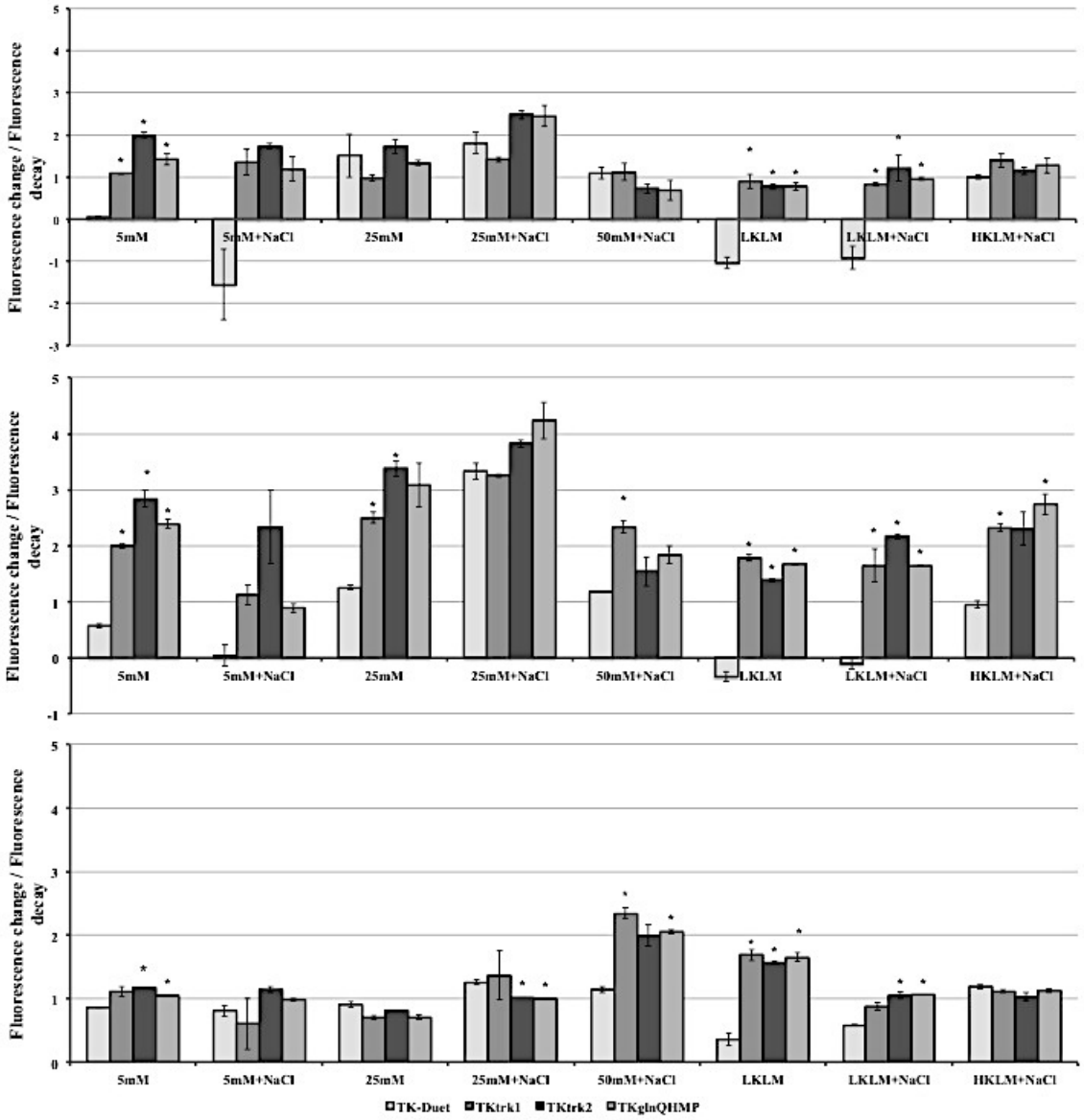

Figure S3: Change in membrane potential for complemented TK2420 strains: TK-DUET, TKtrk1, TKtrk2 and TKglnQHMP. All strains were grown in K^+-rich medium to OD_{600} ~0.4. Aliquots of cells were then incubated with $DiSBAC_2(3)$ fluorescent dye and change in fluorescence intensity was measured and normalized against decay of dye intensity was calculated for each sample at the following time points: (A) 2 minutes, (B) 4 minutes, and (C) 10 minutes. Asterisk (*) represents a statistical significance of p-value < 0.05 for all complemented strains in comparison to TK-Duet; calculation was done using student's t-test for a total of 6 replicates; bars represent mean ± SE values.

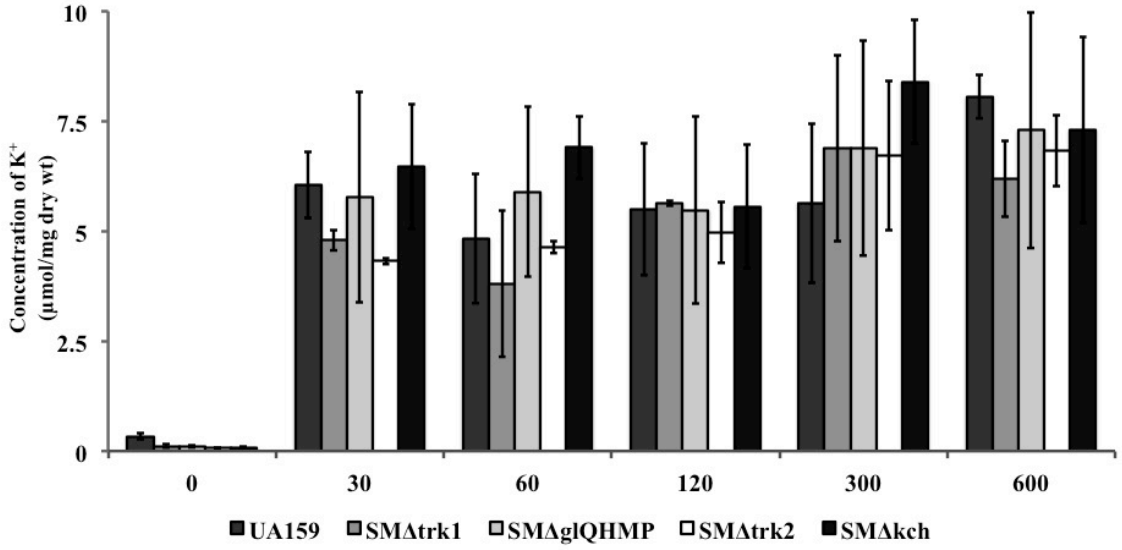

Figure S4: Potassium uptake assay. All strains were grown in K^+-rich medium to OD_{600} ~0.4. To deplete cells of K^+ prior to uptake assays, mid-exponential phase cells were incubated in K^+-deficient MMGK growth medium for 2 hours. Cells were then supplemented with 50 mM KCl and aliquots were sampled over time to measure intracellular K^+ content using ICP-OES. The graph represents mean ± SD values.

Table S1: Putative K^+ transport proteins and their predicted functions in *Streptococcus mutans* UA159.

Protein/ Locus ID	Conserved Domain SMART Search	NCBI Closest Homolog (%identity/%positive)	STRING Predicted Functional Partners	Function
TrkB / SMU_1561	N-terminal - Pfam domain Trk_N, Trk_C	*Roseiflexus sp.* RS-1 (38/64)	Trk, TrkA, PacL	Putative potassium uptake system protein TrkB
Trk / SMU_1562	N-terminal - Pfam domain Trk_N, Trk_C	*Roseiflexus castenholzii* DSM 13941 (39/66)	TrkB, TrkH, PacL, TrkA	Putative potassium uptake protein TrkA
PacL / SMU_1563	N-terminal - Pfam domain Cation	*Streptococcus agalactiae* 2603V/R (64/78)	Trk, TrkB	Putative cation-transporting

	ATPase_N, E1-E2 ATPase, putative hydrolase of Na^+/K^+ ATPase alpha-subunit, haloacid dehalogenase-like hydrolase, Cation ATPase_C			P-type ATPase
TrkA / SMU_1708	N-terminal - Pfam domain Trk_N, Trk_C, Trk, Trk_C	*S. agalactiae* strain COH12603V/R (74/88)	TrkH, TrkB	Potassium transporter peripheral membrane component
TrkH / SMU_1709	N-terminal signal peptide, 3 transmembrane domains, Pfam domain TrkH	*S. thermophilus* strain LMG 18311 (73/85)	TrkA, TrkB, Trk	Putative potassium uptake protein TrkH
GlnQ / SMU_1519	N-terminal Pfam AAA domain	*Streptococcus ratti FA-1* (92/98)	GlnH, GlnM, GlnP, SMU_935, SMU_567, PhoU	Substrate transport e.g. amino acids, inorganic
GlnH / SMU_1520	N-terminal – Pfam domain PBPb domain or PRK11917 domain	*Streptococcus ratti FA-1* (84/94)	GlnQ, SMU_1521, GlnP, SMU_934, SMU_1179c, SMU_567	Substrate transport e.g. amino acids, inorganic
GlnM / SMU_1521	N-terminal – transmembrane PBP2 domain	*Streptococcus ratti FA-1* (88/97)	GlnQ, GlnH, GlnP, SMU_817, SMU_815, SMU_936, SMU_568, SMU_805c	Substrate transport e.g. amino acids, inorganic

	GlnP / SMU_1522	N-terminal – transmembrane PBP2 domain	*Streptococcus ratti FA-1* (92/96)	GlnQ, GlnH, GlnM, SMU_568, SMU_805c, SMU_1177c	Substrate transport e.g. amino acids, inorganic
	Kch / SMU_1848	N-terminal - Voltage gated calcium channel IQ domain, 5 transmembrane domains, Pfam domain E1-E2 ATPase	*Streptococcus macacae* NCTC 11558 (83/94)		Hypothetical protein; K10716 voltage-gated potassium channel

64 **Table S2A: Strains and their isogenic variants**.

***S. mutans* strain**	**Relevant Characteristics and Source**	**Targeted Gene**
UA159	Wild type, Erm^S, provided by J. Ferretti, University of Oklahoma	
SMΔtrk1	UA159 trk1::ermAM, Erm^R, created for this study	SMU_1561-63
SMΔtrk2	UA159 trk2::ermAM, Erm^R, created for this study	SMU_1708-09
SMΔtrk12	UA159 trk1::ermAM, trk2::kanAM Ermr Kan^R, created for this study	SMU_1561-63 + SMU_1708-09
SMΔkch	UA159 Kch::ermAM, Erm^R, created for this study	SMU_1848
SMΔpacL	UA159 pacL::ermAM, Erm^R, created for this study	SMU_1563
SMΔtrk1a	UA159 trk1a::ermAM, Erm^R, created for this study	SMU_1561-62
SMΔglnQHMP	UA159 glnQHMP::ermAM, Erm^R, Krastel *et al*. (2010)	SMU_1519-1523
SMΔtrkB	UA159 trkB::ermAM, Erm^R, created for this study	SMU_1561
SMΔtrk	UA159 trk::ermAM, Erm^R, created for this study	SMU_1562
SMΔtrkA	UA159 trkA::ermAM, Erm^R, created for this study	SMU_1708
SMΔtrkH	UA159 trkH::ermAM, Erm^R, created for this study	SMU_1709

***E. coli* strains**		
TK2420	F⁻ *thi rha lacZ nagA* Δ(*kdpFAB*)*5* Δ(*trkA-mscL′*) *trkD1*, provided by Epstein (Epstein & Kim, 1971)	-
TKtrk1a	TK2420 + (pET-DUET + trk1a), created for this study	SMU_1561-62
TKpacL	TK2420 + (pET-DUET + pacL), created for this study	SMU_1563
TKtrk2	TK2420 + (pET-DUET + trk2), created for this study	SMU_1708-1709
TKglnQHMP	TK2420 + (pET-DUET +glnQHMP), created for this study	SMU_1591-1522
TKkch	TK2420 + (pET-DUET + kch), created for this study	SMU_1848
TK-DUET	TK2420 + (pET-DUET), created for this study	-
TK2420	F−thi rha lacZ nagA Δ(kdpFAB)5 Δ(trkA-mscL′) trkD1, provided by Epstein (Epstein & Kim, 1971)	-

Table S2B: Primers used for constructing mutants. KO: knockout primers used PCR ligation mutagenesis; CP: cloning primers used for cloning and expression in *E. coli* TK2420.

Gene-specific primer	Primer number and type	Primer Sequence
kch f1	KO P1	5'-CAACTTTACTAAGACTATCCGTCAG-3'
kch r1	KO P2	5'-GGCGCGCCTGTATTTCCATTCGCT-3'
kch f2	KO P3	5'-GGCCGGCCTGGCATCATTACAGCA-3'
kch r2	KO P4	5'-ATGGCAAGTATGGCGTTCG-3'
ERM	P1	5'-GGCGCGCCCCGGGCCCAAAATTTGTTTGAT-3'

ERM	P2	5'-GGCCGGCCAGTCGGCAGCGACTCATAGAAT-3'
trk1 f1	KO P1	5’-GAAGTTGTCTTTGCCCGTGTAG-3’
trk1 r1	KO P2	5’-GGCGCGCCACCTAAACGACCACATCCGAC-3’
trk1 f2	KO P3	5’-GGCCGGCCTGGCTATTTGATTCTCTG-3’
trk1 r2	KO P4	5’-ATAAGCAACGGCATTCGCTTG-3’
trk2 f1	KO P1	5’-CTGGGAGTCCTTTATCTTCACGTC-3’
trk2 r1	KO P2	5’-GGCCGGCCTTCTTCCACCAGTGAGCGACAGAG-3’
trk2 f2	KO P3	5’-GGCCGGCCCATGTTGTTGCTATTTATTCC-3’
trk2 r2	KO P4	5’-TGCCCGCTATCGTATTGTC-3’
pacL	CP P1	5’ATAAGAATGCGGCCGCACCAATGAATTAGTGTTTTC-3’
pacL	CP P2	5’-CGGAATTCGGTATATGTTATGAGTAGCACAG-3’
trk1a	CP P1	5’-GGCCGGCCAAGTGCAAAACTAACGGAAC-3’
trk1a	CP P2	5’-GGAATTCCATATGGGTAGCTATGAAAATTATTATTG-3’
trk2	CP P1	5’-CGGGATCCGAGGTAAATCATGAAAATTATTG-3’
trk2	CP P2	5’-GAATGCGGCCGCAAAAGGACTTAAGTTTTAGACC-3’
kch	CP P1	5’-CGGGATCCTCGATATATTATGAGACAAACG-3’
kch	CP P2	5’-GAATGCGGCCGCAGAGCTTTTCCTATTTTTTATGG-3’
glnQHMP	CP P1	5’-CATGCCATGGGGAGAAGACTATGGCACTC-3’
glnQHMP	CP P2	5’-GAATGCGGCCGCACTGATTTTTTAGTCCGTTGC-3’

Table S3 – Two nutrient-rich media preparations, THYE and TYE, both at pH7.5, were subjected to ICP-OES to quantify cation content. Detected levels of cations were calibrated against standard curves generated for each analyte [wavelength listed after the element in brackets – Na (589.592 nm), K (766.49 nm), Mg (285.213 nm), Ca (317.933 nm), Fe (238.204nm), Mn (257.61), Cu (327.393 nm), Al (396.153 nm), Ni (231.604 nm) and Zn (206.2 nm)] using a serially diluted QC4 (SCP Science, Quebec) cation standard. Each standard curve had a minimum correlation coefficient of 99.9%. Cations below the detection limits of this assay [Fe(179 μM), Mn (182 μM), Cu (157 μM), Al (371 μM), Ni (170 μM), Zn (153 μM)] are excluded. Values represent analyte concentration mean ± SE for experiments repeated three times.

Cation	**THYE**	**TYE**
Sodium	65.62 ± 0.29 mM	37.46 ± 0.12 mM
Potassium	18.55 ± 0.047 mM	12.85 ± 0.043 mM
Magnesium	4.89 ± 0.036 mM	0.263 ± 0.00141 mM