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Diverse genes of alkaliphilic *Bacillus firmus* OF4 that complement K^+-uptake-deficient *Escherichia coli* include an *ftsH* homologue

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Abstract Seven clones isolated from libraries of DNA from alkaliphilic *Bacillus firmus* OF4 restored the growth of a K^+-uptake-deficient *Escherichia coli* mutant on only 10 mM K^+. None of the clones contained genes with apparent homology to known K^+ transport systems in other organisms. Based on sequence homologies, the newly isolated alkaliphile loci included: *ftsH*; a dipeptide transport system; a *gerC* locus with hydrophobic open reading frames not found in the comparable locus of *Bacillus subtilis*; a sugar phosphotransferase enzyme; and a *capBC* homologue. The *ftsH* gene provided a new and striking example of a recognized property of extracellular and external regions of polytopic alkaliphile proteins: a significant paucity of basic amino acid residues relative to neutrophile counterparts. The alkaliphile *ftsH* gene was able to complement a mutant of *E. coli* with a temperature-sensitive *ftsH* gene product.

Key words Alkaliphile · *Bacillus firmus* · *ftsH* · K^+ transport

Introduction

A central physiological challenge confronting extremely alkaliphilic *Bacillus* species at the upper edge of their pH range for growth is maintenance of a cytoplasmic pH that is below the external pH. This has been illustrated by studies of alkaliphilic *Bacillus firmus* OF4 growing on malate in continuous culture at various carefully controlled pH values (Sturr et al. 1994; Krulwich 1995). As the growth pH was elevated from 7.5 to 9.5, the cytoplasmic pH remained close to pH 7.5, representing a transmembrane pH gradient (ΔpH), acid in, of approximately 2 units at pH 9.5. A ΔpH of 2.0–2.3 units, acid in, was maintained at values of the growth pH up to 11.2, above which a diminished capacity to maintain this gradient was evident. Importantly, since the magnitude of the ΔpH did not increase substantially above 2 units as the external pH rose above 9.5, the cytoplasmic pH began to rise at values of the growth pH above 9.5 and there was a concomitant decrease in the growth rate. Other energetic parameters, e.g., the transmembrane electrical potential or total electrochemical proton gradient, did not correlate with the growth rate in a comparable fashion.

In other studies in *B. firmus* OF4, as well as other alkaliphilic *Bacillus* species, the capacity for maintaining a cytoplasmic pH significantly below that of the medium was shown to be dependent upon Na^+ (Krulwich et al. 1985,1996; Kitada and Horikoshi 1992). Several lines of evidence implicate electrogenic Na^+/H^+ antiporters in Na^+-dependent pH homeostasis in alkaliphilic *Bacillus* species (Krulwich and Guffanti 1992; Kitada and Horikoshi 1992). In alkaliphilic *Bacillus* C-125, a gene apparently encoding an Na^+/H^+ antiporter has been identified and shown to restore pH homeostasis to a nonalkaliphilic mutant lacking this capacity (Hamamoto et al. 1994). The specificity for Na^+ in the pH homeostasis of alkaliphilic *Bacillus* species is in marked contrast to the finding that *Bacillus subtilis* can use either Na^+ or K^+ to achieve pH homeostasis in its more modest upper range of growth pH (Cheng et al. 1994; Krulwich et al. 1996). The basis for the specificity in the alkaliphile is of interest. One possibility is that a detrimental loss of cytoplasmic K^+ might occur were K^+/H^+ antiporters involved in ongoing, active accumulation of protons during nonfermentative growth at extremely alkaline pH. K^+ is generally maintained at high concentrations in the cytoplasm of prokaryotes, where it is required for optimal activity of certain enzymes (Suelter 1970) and plays a role in cell turgor control (Epstein 1986). Perhaps the systems for K^+ accumulation are inadequate to sustain cytoplasmic K^+ under conditions of rapid K^+ efflux. *B. firmus* OF4 may lack the specialized mechanisms that allow the more moderate alkaliphile or alkaline-tolerant *Bacillus pasteurii* to substitute high cytoplasmic NH_4^+ for K^+, but such substitution in any event requires special growth conditions that are not routinely used for *B. firmus* OF4 (Jahns 1996).

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To gain insights into the nature of the K^+ transport systems in *B. firmus* OF4, we characterized seven clones of alkaliphile DNA that could complement the phenotype of an *Escherichia coli* mutant that is deficient in three of its K^+ uptake systems. As reported here, none of these clones contains genes predicted to encode an obvious homologue of any of the K^+ uptake systems that have been characterized in other organisms. Perhaps such homologues exist in the alkaliphile, and are used at high pH, but cannot functionally complement at the neutral pH of the screen. The newly characterized clones were found, however, to encode diverse hydrophobic proteins of interest, and include several candidates for stress-involved activities.

Materials and methods

Bacterial strains, plasmids, and growth conditions

The bacterial strains and plasmids used are listed in Table 1. LB medium (Sambrook et al. 1989), LBK medium (Ivey et al. 1991), and minimal medium supplemented with 10 mM or 30 mM K^+ (Epstein and Kim 1971) were used. Ampicillin at 100 μg/ml, 20 μg/ml of chloramphenicol, and 50 μg/ml of kanamycin were added for growing plasmid-bearing cells as well as for selecting transformants. Plasmid pQEF1 carried *ftsH*-his_6 without its own promoter and with its expression strictly controlled by the *lac* promoter-operator. It was constructed as follows. Polymerase chain reactions with *B. firmus* OF4 chromosomal DNA as a template were primed by two synthetic mutagenesis primers. The upstream primer (5′-ATAAACGAAGCTAGCATGCCCGTC-3′) contained the *SphI* recognition sequence followed by nucleotides 714–737 of pC22 (Accession No. U61844), and the downstream primer (5′-CCTCCCTTTCATCAAAAAG-ATGGATCC-3′) contained the *Bgl*II recognition sequence corresponding to nucleotides 2815–2789. The polymerase chain reaction products were digested with *Sph*I and *Bgl*II, and a 2.0-kb fragment was ligated with *Sph*I- and *Bgl*II-digested pQE70 (QIAGEN, Chatsworth, CA, USA). The *ftsH* gene and the his_6 sequence on the resulting plasmid were fused in frame.

Preparation of DNA libraries from *B. firmus* OF4

Chromosomal DNA isolation and preparation of the *Cla*I–pGEM3zf(+) (Promega, Madison, WI, USA) library (Library 1) and *Mbo*I–pSPT19 (Boehringer-Mannheim, Indianapolis, IN, USA) library (Library 2) were done as described previously (Ivey et al. 1991,1992). A novel library (Library 3) was prepared by completely digesting chromosomal DNA from *B. firmus* OF4 with *Hind*III and ligating it into *Hind*III-digested and dephosphorylated pBK36.

Table 1. Strains and plasmids used in this study

Strain or plasmid	Genotype or characteristics	Reference or source
Escherichia coli		
DH5αMCR	F⁻ *mcrA* Δ(*mrr-hsd, RMS-mcrBC*) ϕ*80dlacZ* Δ*M15* Δ(*lacZYA-argF*) *U169 deoR recA1 endA1 supE44* λ⁻ *thi-1 gyrA96 relA1*	Gibco, Grand Island, NY, USA
JM109	*endA1 recA1 gyrA96 thi-1 hsdR17 relA supE44* [F' *traD36 lac1*^{q} Δ*(lacZ)M15 proAB*⁻]	Stratagene, La Jolla, CA, USA
TK2420	Δ(*kdpABC*) *trkD*1 Δ*trkA*	Epstein et al. 1993
EP432	*melB*Lid Δ*nhaA*::Km Δ*nhaB*::Cm Δ*lacZY thr*1	Pinner et al. 1994
AR432	*met gal supE hsdR sfiC* Δ(*srl-recA*) 306::Tn10 Δ*ftsH3*::Km [pAR171 *ftsH rep*^{TS} Cm^R]	Akiyama et al. 1994a
Bacillus firmus		
OF4	Wild-type	Sturr et al. 1994
Plasmids		
pGEM3zf(+)	Cloning vector (Ap^R)	Promega, Madison, WI, USA
pSTP19	Cloning vector (Ap^R)	Boehringer-Mannheim, Indianapolis, IN, USA
pBK36	Shuttle vector (Ap^R in Gram⁻ and Km^R in Gram⁺)	Obtained from Dr. Kevin Zen
pAR171	*ftsH rep*^{TS} Cm^R	Akiyama et al. 1994a
pQE70	Expression vector (Ap^R)	QIAGEN, Chatsworth, CA, USA
pQEF1	pQE70 with a 2.0 kb *Sph*I–*Bgl*II fragment (*ftsH* gene from *B. firmus* OF4)	This work
pC22	Isolated from library 1	This work
pC23	Isolated from library 1	This work
pC24	Isolated from library 1	This work
pT26	Isolated from library 2	This work
pBK154	Isolated from library 3	This work
pBK379	Isolated from library 3	This work
pBK3711	Isolated from library 3	This work

Km, kanamycin; Ap, ampicillin; Cm, chloramphenicol.

Complementation screening

Transformation of *E. coli* strains and all recombinant DNA manipulations were carried out by standard methods (Sambrook et al. 1989). Screening of *E. coli* TK2420 transformants for complementing clones was accomplished by transferring colonies initially obtained on LBK + 100 µg/ml ampicillin (Ap) medium to a minimal medium supplemented with only 10 mM K^+ at pH 7.0 (Epstein and Kim 1971). The seven plasmids isolated from *E. coli* TK2420 were used to transform kanamycin- and chloramphenicol-resistant *E. coli* EP432 (Δ*nhaA*Δ*nhaB* Km^R Cm^R) and each transformant was tested for Na^+ resistance on LBK plus 0.4 M NaCl at pH 7.5. Strain EP432 is deficient in Na^+/H^+ antiporters and cannot grow in medium containing 0.4 M NaCl (Pinner et al. 1993).

Measurement of intracellular K^+ concentration of the cells and generation times

Cells in the late log phase of growth in minimal medium supplemented with 10 mM or 30 mM K^+ were harvested by centrifugation (3000 *g*, 10 min, 25°C), washed in 300 mM sucrose, and then resuspended in 5% (w/v) trichloroacetic acid. The cell suspensions were incubated at 100°C for 10 min to destroy the cell membrane. After centrifugation (3000 *g*, 10 min, 25°C), the supernatant was used for K^+ measurement. The K^+ concentration of each sample was determined with a flame photometer (Coleman Model 51 Ca, Bacharach, Pittsburgh, PA, USA) calibrated with standard K^+ solutions of known concentration. Protein was determined by the Lowry method (Lowry et al. 1951) with lysozyme as a standard. Generation times of each strain were calculated from increases in turbidity measured by absorbance at 600 nm.

Complementation of Δ*ftsH3*::Km in *E. coli* with *ftsH* of *B. firmus* OF4

E. coli AR423 shows temperature-sensitive growth, because the replication of the plasmid pAR171, containing the essential *ftsH* gene and a Cm resistance (Cm^R) marker, is defective at 42°C (Akiyama et al. 1994a). The plasmids pQEF1 and pQE70 were used to transform strain AR423, and transformants were selected by Ap^R at 30°C. The transformants obtained were incubated on LB plates at 42°C overnight, and then plated on LB plates containing Km at 30°C. The colonies were tested for antibiotic resistance by streaking on LB plates containing Ap or Cm.

DNA sequence analysis

The nucleotide sequence of both strands of the insert DNA from the newly isolated complementing plasmids was determined by the dideoxy nucleotide method by using an automatic sequencer (Applied Biosystems model 373A; Foster City, CA, USA). Universal primers from Stratagene (La Jolla, CA, USA) or customized primers were used as sequencing primers. Sequence analysis was done with both FASTA (Devereux et al. 1984) and BLAST (Altschul et al. 1990) programs and utilized the Genetics Computer Group software package run on a VAX 4000–300 computer. Estimation of molecular masses, isoelectric points, and hydropathy profiles were done with the Gene Runner software package version 3.0 (Hastings Software, Hastings, NY, USA).

Results and discussion

Seven clones were isolated from a screen for enhancement of the ability of *E. coli* TK2420 to grow in the presence of 10 mM K^+. The plasmids isolated contained apparently distinct inserts and were able to complement TK2420 upon retransformation. The intracellular K^+ content of transformant cells and generation times are shown in Table 2. Strain TK2420 carrying only the vector was not able to grow in minimal medium with 10 mM K^+, and grew very slowly in minimal medium with 30 mM K^+. The wild type (DH5αMCR) and each transformant carrying a complementing clone clearly grew better than strain TK2420 carrying the vector alone. The osmolarity of minimal medium with 30 mM K^+ is about 0.2 osmolar. The intracellular K^+ content of *E. coli* cells grown under these conditions is about 220 mmol K^+ per l of cytoplasmic H_2O (Bakker 1993). The concentration obtained for the wild type in this study (234 mM) is in good agreement with this previously published value. The intracellular K^+ content of TK2420 grown in minimal medium with 30 mM K^+ was only 68% of that of the wild type. This low K^+ content is presumably why the generation times for TK2420 are so long. All complemented transformants exhibited an intracellular K^+ content of at least 160 mM when grown on 10 mM K^+ media, a concentration very similar to the internal $[K^+]$ for TK2420 grown on 30 mM K^+. Consistently, the generation times of all but one of the complemented transformants was in a range close to

Table 2. Intracellular K^+ concentration of TK2420 transformants and generation times

Strain	10 mM $[K^+]_{out}$		30 mM $[K^+]_{out}$	
	$[K^+]_{in}$ (mM)	*g* (min)	$[K^+]_{in}$ (mM)	*g* (min)
DH5αMCR(pGM3zf)	219 ± 2	73	234 ± 0	72
TK2420(pGEM3zf)	ND	NG	160 ± 6	149
(pC22)	154 ± 2	87	194 ± 10	74
(pC23)	195 ± 4	94	224 ± 3	76
(pC24)	161 ± 6	315	178 ± 7	92
(pT26)	209 ± 7	70	207 ± 4	57
(pBK154)	160 ± 6	167	211 ± 4	60
(pBK379)	181 ± 7	87	222 ± 3	58
(pBK3711)	211 ± 3	120	245 ± 1	63

Values are the averages of at least two independent experiments in which triplicate measurements were made; they are shown ± standard deviations.
ND, not done; NG, no growth; *g*, generation time.

that of TK2420 growing on 30 mM K^+. The exception, TK2420/pC24, may have a growth rate representing a composite of enhanced growth due to a higher internal [K^+] and growth inhibition due to the genes overexpressed from the clone. When grown on 30 mM K^+ all complemented transformants had a higher internal K^+ concentration than TK2420 grown under the same condition, and again, the pC24 transformant had a longer generation time than the other transformants. None of the plasmids complemented the Na^+-sensitive phenotype of *E. coli* strain EP432, indicating that a gene encoding a TetK-like transporter that has both the capacity for Na^+ efflux and K^+ uptake (Guay et al. 1993; Cheng et al. 1996) had not been identified.

The deduced arrangement of open reading frames (ORFs) on the inserts of the seven complementing clones is shown in Fig. 1. A summary of the search for similar gene products in the gene databases, which formed the basis for the arrangements shown in the figure, is provided in Table 3. None of the clones was predicted to encode a protein that was a strong candidate for a regulatory gene, i.e., one that might directly enhance the production of an *E. coli* product that would cause the increased K^+ accumulation. All of the alkaliphile clones were predicted to encode at least one hydrophobic protein, although in four of the plasmids, pBK379, pBK3711, pT26, and pBK154, those products would be truncated. In one of those same clones, pBK379, the putative hydrophobic products had no homologues in the gene databases and there were no additional ORFs that provided a hypothetical relationship to K^+ accumulation. The remaining six clones contained one or more ORFs expected to encode one or more hydrophobic proteins that had significant sequence similarity to known proteins.

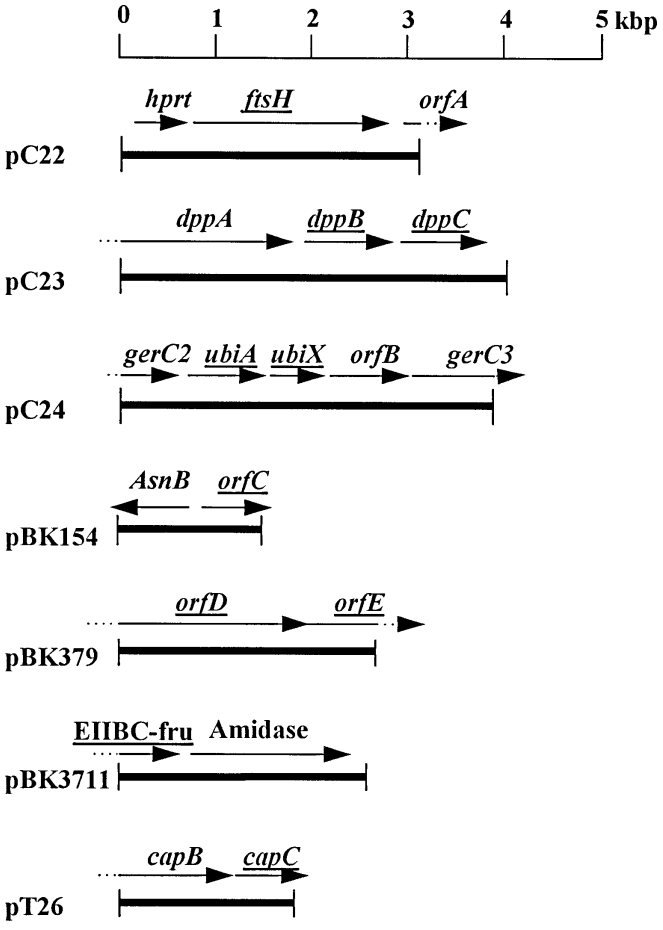

Fig. 1. Diagrammatic presentations of the deduced arrangement of open reading frames (ORFs) in seven cloned alkaliphile DNA fragments that complemented the *E. coli* TK2420 for growth on 10 mM K^+. ORFs were named based on the sequence comparisons summarized in Table 2. ORFs that are likely to encode membrane proteins are *underlined*. *Arrows* indicate the direction of transcription of the ORF, and *dotted arrows* indicate expected directions of extension of apparently truncated ORFs

A homologue of *ftsH* was found, on pC22, in a region that bore overall similarity to that in which *ftsH* genes had earlier been found in *Lactococcus lactis* and *B. subtilis* (Nilsson et al. 1994; Ogasawara et al. 1994). A derivative of the alkaliphile *ftsH* gene was therefore constructed to express a hexa-histidine-tagged FtsH under an inducible promoter. This gene complemented the strain, AR423, of *E. coli* (data not shown) that has a temperature-sensitive *ftsH* gene derived from *E. coli* (Akiyama et al. 1994a). The *ftsH* genes encode adenosine triphosphatases (ATPases) of the AAA-protein family (Erdmann et al. 1991) that are hypothesized to have both protease and chaperone activities (Akiyama et al. 1994a, b). Previous work has implicated the *ftsH* genes of other bacteria in stress responses that include high temperature and hypersaline stress (Ogura et al. 1991; Geisler and Schumann 1993; Nilsson et al. 1994; Deuerling et al. 1995; Hecker et al. 1996). These stresses, respectively, pose the challenges of potential loss of K^+ from the cytoplasm accompanying heat-induced membrane leakiness and the need for enhanced K^+ uptake to restore cell turgor. The complementation by multicopy expression of a heterologous *ftsH* gene might involve an indirect effect upon proper assembly of a requisite transport system. Interestingly, a different heat-shock protein, a member of the clp/HSP104 family, was found to suppress the defect of a yeast potassium transport mutant (Perier et al. 1995).

The deduced sequence of the alkaliphile *ftsH* gene product provides a striking example of a generalization that has been noted for these extremophiles, i.e., that extracellular enzymes or the hydrophilic loops of polytopic proteins, in this case only one, predicted to face the external milieu present an extreme version of the "basic in" rule (Von Heijne 1994). That is, the alkaliphile versions have an even more exaggerated reduction in basic amino acids (Van der Laan et al. 1991; Kang et al. 1992; Quirk et al. 1993). As indicated in Fig. 2 and Table 4, the alkaliphile FtsH is predicted to have numerous substitutions of acidic or neutral amino acid residues for residues that are basic in other homologues of FtsH (Ogasawara et al. 1994; Tomoyasu et al. 1993; Nilsson et al. 1994; Fraser et al. 1995). The overall loop is strikingly low in basic amino acids and far more acidic than its homologues from neutrophiles. It has been suggested (S. Lazar and R.G. Kolter, unpublished results) that even in *E. coli*, moderately alkaline pH promotes the need for enhanced expression of the periplasmic chaperone *surA* to avoid protein misfolding; perhaps in the extreme alkaliphiles, folding and/or function of proteins in the alka-

Table 3. Analysis of open reading frames (ORF) from plasmids complementing strain TK2420

Plasmid (Accession no)#	ORF	Possible protein length (amino acids)	Homology to	Function	Identity % (amino acid overlap)	Similarity % (amino acid overlap)	Species	Reference
pC22 (U61844)	1	199	Hprt (180aa)	Hypoxanthine-guanine phosphoribosyltransferase	75.4 (179aa)	88.8 (179aa)	*B. subtilis*	Ogasawara et al. 1994
	2	679	FtsH (637aa)	Chaperone	74.9 (648aa)	85.1 (648aa)	*B. subtilis*	Ogasawara et al. 1994
	3 (*orfA*)	56*	Hypothetical protein (233aa)		56.4 (55aa)	74.5 (55aa)	*B. subtilis*	Ogasawara et al. 1944
pC23 (U64514)	1	170*	DppA (535aa)	Dipeptide transport system	32.7 (170aa)	53.7 (170aa)	*E. coli*	Sofia et al. 1994
	2	333	DppB (339aa)	Dipeptide transport system	48.9 (333aa)	71.2 (333aa)	*E. coli*	Sofia et al. 1994
	3	304	DppC (300aa)	Dipeptide transport system	49.0 (296aa)	70.5 (296aa)	*E. coli*	Sofia et al. 1994
pC24 (U61168)	1	221*	GerC2 (233aa)	Spore germination protein	63.3 (218aa)	76.6 (218aa)	*B. subtilis*	Yazdi and Moir. 1990
	2	277	UbiA (290aa)	4-hydroxybenzoate octaprenyltransferase	22.7 (276aa)	52.7 (276aa)	*E. coli*	Nishimura et al. 1992
	3	200	UbiX (189aa)	3-octaprenyl-4-hydroxy-benzoate-carboxy-lyase	44.8 (194aa)	66.1 (194aa)	*E. coli*	Nonet et al. 1987
	4 (*orfB*)	281	unknown					
	5	288*	GerC3 (384aa)	Spore germination protein	63.1 (244aa)	79.5 (244aa)	*B. subtilis*	Yazdi and Moir. 1990
pBK154 (U64314)	1	159*	AnsB (348aa)	L-Asparaginase II	36.3 (159aa)	56.7 (159aa)	*E. coli*	Sun and Setlow. 1991
	2 (*orfC*)	213*	a. Hypothetical protein (218aa)		48.6 (213aa)	74.5 (213aa)	*B. subtilis*	Sorokin et al. 1993
			b. Na^+/H^+ antiporter system (804aa)		23.2 (213aa)	49.3 (213aa)	*Bacillus* sp. C-125	Hamamoto et al. 1994
pBK379 (U64515)	1 (*orfD*)	508*	unknown					
	2 (*orfE*)	141*	unknown					
pBK3711 (U64312)	1	208*	FruA (304aa)	PTS system, fructose specific IIBC component	46.1 (208aa)	68.4 (208aa)	*E. coli*	Prior and Kornberg 1988
	2	481	Amidase (462aa)		30.2 (474aa)	52.6 (474aa)	*Rhodococcus sp.*	Mayaux et al. 1991
pT26 (U60883)	1	300*	CapB (397aa)	Encapsulation protein	67.5 (299aa)	78.9 (299aa)	*B. anthracis*	Makino et al. 1989
	2	138*	CapC (149aa)	Encapsulation protein	69.8 (138aa)	86.3 (138aa)	*B. anthracis*	Makino et al. 1989

aa, amino acids; PTS, phosphotransferase system.
* Incomplete ORF.

line medium is dependent upon the extreme paucity of residues with pK values in the range of the growth medium.

Plasmid pC23 contains one incomplete and two complete ORFs that are likely to encode a dipeptide transport system of the ABC-type by analogy with homologues (Sofia et al. 1994). Possibly, this category of transporter possesses some capacity for catalyzing K^+ uptake when overexpressed, inasmuch as a mutation in another oligopeptide transport system in K^+-uptake-deficient *E. coli* has been noted by Epstein et al. (1993) to allow growth of the strain on otherwise insufficient K^+.

Plasmid pC24 contained an insert that is predicted to encode homologues to the *B. subtilis* germination genes *gerC2* and *gerC3* (Yazdi and Moir 1990). The apparent gene organization of the locus is different, however, in *B. firmus* OF4. In the alkaliphile locus there are three ORFs between the two putative *gerC* genes (Fig. 1). Two of them are predicted to encode hydrophobic products that show similarity to membrane-associated proteins in the ubiquinone

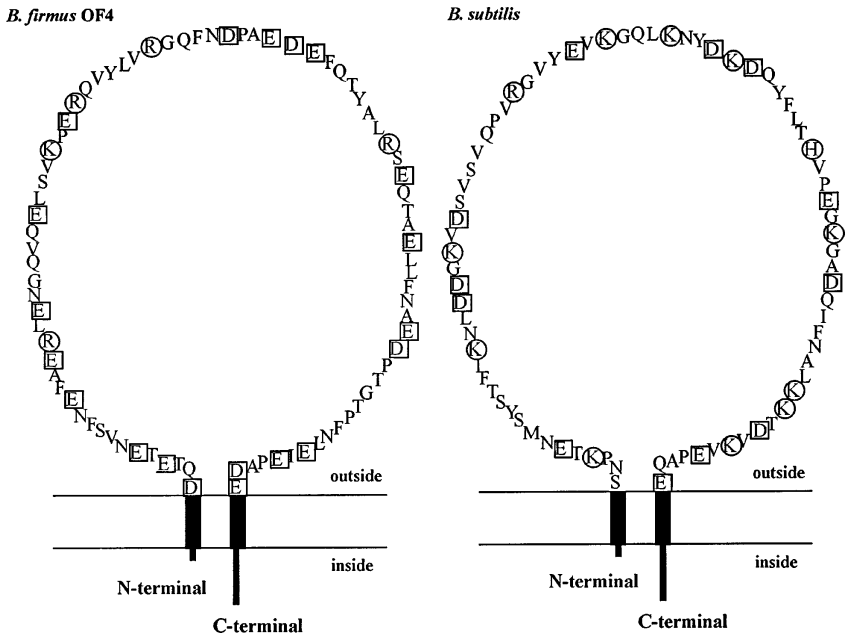

Fig. 2. Comparison of the deduced sequence of the putative extracytoplasmic loop of the *ftsH* gene products of *B. firmus* OF4 and *B. subtilis*. Acidic residues are in *squares* and basic residues are in *circles*

Table 4. Comparison of extracellular loop region of FtsH from *B. firmus* OF4 and other microorganisms

FtsH all	Number of acidic and basic amino acids		Overcharge
	D + E	H, K, + R	
B. firmus OF4	20	5	–15
B. subtilis	12	12	±0
E. coli	12	11	–1
M. genitalium	4	6	+2
L. lactis	10	9	–1

biosynthetic pathway of *E. coli* (Nishimura et al. 1992; Nonet et al. 1987). How overexpression of such proteins mediates the complementation is unclear. However, it is notable that evidence has recently been presented (Tani et al. 1996) that a spore germination gene of *Bacillus megaterium* encodes a transporter with sequence similarity to a K^+- and Na^+-involved transporter from *Enterococcus hirae* (Waser et al. 1992). Given the long-standing recognition of a role for K^+ in at least some spore germination pathways (Moir and Smith 1990), it is possible that the *B. megaterium* gene and one of the genes in pC24 encode K^+ transporters of some kind. A second such candidate in the current screen was *orfC* of pBK154 which has a homologue in *B. subtilis* and also shows sequence similarity to a putative monovalent cation/H^+ antiporter from alkaliphilic *Bacillus* C-125 (Hamamoto et al. 1994).

The final two complementing plasmids, pBK3711 and pT26, each encode homologues of known hydrophobic proteins, a fructose-specific Phosphotransferase system (PTS): hexose phosphotransferase component and encapsulation proteins, respectively (Table 3). There is no basis for hypothesizing about the specific mechanism for the complementation, which could well be a physiologically unrelated side-product overexpression. While multicopy suppression of mutant phenotypes, especially with heterologous genes, can provide initial approaches to genes of physiological interest, the limitations of this approach are also clear (Ueguchi and Ito 1992).

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References

Akiyama Y, Ogura T, Ito K (1994a) Involvement of FtsH in protein assembly into and through the membrane. I. Mutations that reduce retention efficiency of a cytoplasmic reporter. J Biol Chem 269: 5218–5224

Akiyama Y, Shirai Y, Ito K (1994b) Involvement of FtsH in protein assembly into the membrane. II. Dominant mutations affecting FtsH function. J Biol Chem 269:5225–5229

Altschul SF, Gissh W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Bakker EP (1993) Cell K^+ and K^+ transport systems in prokaryotes. In: Bakker EP (ed) Alkali cation transport systems in prokaryotes. CRC Press, Boca Raton

Cheng J, Guffanti AA, Krulwich TA (1994) The chromosomal tetracycline-resistance locus of *Bacillus subtilis* encodes a Na^+/H^+ antiporter that is physiologically important at elevated growth pH. J Biol Chem 269:27365–27371

Cheng J, Baldwin K, Guffanti AA, Krulwich TA (1996) Na^+/H^+ antiport activity conferred by *Bacillus subtilis tetA*(L), a 5′ truncation product of *tetA*(L), and related plasmid genes upon *Escherichia coli*. Antimicrob Agents Chemother 40:852–857

Deuerling E, Paeslack B, Schumann W (1995) The *ftsH* gene of *Bacillus subtilis* is transiently induced after osmotic and temperature upshift. J Bacteriol 177:4105–4112

Devereux J, Haeberli P, Smithies O (1984) A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res 12:387–395

Epstein W (1986) Osmoregulation by potassium transport in *Escherichia coli*. FEMS Microbiol Rev 39:73–78

Epstein W, Kim BS (1971) Potassium transport loci in *Escherichia coli* K-12. J Bacteriol 108:639–644

Epstein W, Buurman E, McLaggan D, Naprstek (1993) Multiple mechanisms, roles and controls of K^+ transport in *Escherichia coli*. Biochem Soc Trans 21:1006–1010

Erdmann R, Wiebel FF, Flessau A, Rytka J, Beyer A, Frohlich K, Kunau W (1991) *PAS1*, a yeast gene required for peroxisome biogenesis, encodes a member of a novel family of putative ATPases. Cell 64:499–510

Fraser CM, Gocayne JD, White O, Adams MD, Clayton RA, Fleischmann RD, Bult CJ, Kerlavage AR, Sutton G, Kelly JM, Frichman JL, Weidman JF, Small KV, Sandusky M, Fuhrmann J, Nguyen D, Utterback TR, Saudek DM, Phillips CA, Merrick JM, Tomb J, Dougherty BA, Bott KF, Hu P, Lucier TS, Peterson SN, Smith HO, Hutchison CA, Venter JC (1995) The minimal gene complement of *Mycoplasma genitalium*. Science 270:397–403

Geisler U, Schumann W (1993) Isolation of stress mutants of *Bacillus subtilis* by a novel genetic method. FEMS Microbiol Lett 108:251–254

Guay GG, Tuckman P, McNicholas P, Rothstein DM (1993) The *tet*(K) gene from *Staphylococcus aureus* mediates the transport of potassium in *Escherichia coli*. J Bacteriol 175:4927–4929

Hamamoto T, Hashimoto M, Hino M, Kitada M, Seto Y, Kudo T, Horikoshi K (1994) Characterization of a gene responsible for the Na^+/H^+ antiporter system of alkalophilic *Bacillus* species strain C-125. Mol Microbiol 14:939–946

Hecker M, Schumann W, Voker U (1996) Heat-shock and general stress response in *Bacillus subtilis*. Mol Microbiol 19:417–428

Ivey DM, Guffanti AA, Bossewich JS, Padan E, Krulwich TA (1991) Molecular cloning and sequencing of a gene from alkaliphilic *Bacillus firmus* OF4 that functionally complements an *Escherichia coli* strain carrying a deletion in the *nhaA* Na^+/H^+ antiporter gene. J Biol Chem 266:23483–23489

Ivey DM, Guffanti AA, Shen Z, Kudyan N, Krulwich TA (1992) The *cadC* gene product of alkaliphilic *Bacillus firmus* OF4 partially restores Na^+ resistance to an *Escherichia coli* strain lacking an Na^+/H^+ antiporter (NhaA). J Bacteriol 174:4878–4884

Jahns T (1996) Ammonium/urea-dependent generation of a proton electrochemical potential and synthesis of ATP in *Bacillus pasteurii*. J Bacteriol 178:403–409

Kang S-K, Kudo T, Horikoshi K (1992) Molecular cloning and characterization of an alkalophilic *Bacillus* sp. C125 gene homologous to *Bacillus subtilis secY*. J Gen Microbiol 138:1365–1370

Kitada M, Horikoshi K (1992) Kinetic properties of electrogenic Na^+/H^+ antiport in membrane vesicles from an alkalophilic *Bacillus* sp. J Bacteriol 174:5936–5940

Krulwich TA (1995) Alkaliphiles: "basic" molecular problems of pH tolerance and bioenergetics. Mol Microbiol 15:403–410

Krulwich TA, Guffanti AA (1992) Proton-coupled bioenergetic processes in extremely alkaliphilic bacteria. J Bioenerg Biomembr 24:587–599

Krulwich TA, Federbush J, Guffanti AA (1985) Presence of a nonmetabolizable solute that is translocated with Na^+ and enhances Na^+-dependent pH homeostasis in an alkalophilic *Bacillus*. J Biol Chem 260:4055–4058

Krulwich TA, Ito M, Gilmour R, Sturr MG, Guffanti AA, Hicks DB (1996) Energetic problems of extremely alkaliphilic aerobes. Biochim Biophys Acta 1275:21–26

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Makino S, Uchida I, Terakado N, Sasakawa C, Yoshikawa M (1989) Molecular characterization and protein analysis of the *cap* region, which is essential for encapsulation in *Bacillus anthracis*. J Bacteriol 171:722–730

Mayaux JF, Cerbelaud E, Soubrier F, Yeh P, Blanche F, Petre D (1991) Purification, cloning and primary structure of a new enantiomer-selective amidase from a *Rhodococcus* strain: structural evidence for a conserved genetic coupling with nitrile hydratase. J Bacteriol 173:6694–6704

Moir A, Smith DA (1990) The genetics of bacterial spore germination. Annu Rev Microbiol 44:531–553

Nilsson D, Lauridsen AA, Tomoyasu T, Ogura T (1994) A *Lactococcus lactis* gene encodes a membrane protein with putative ATPase activity that is homologous to the essential *Escherichia coli ftsH* gene product. Microbiology 140:2601–2610

Nishimura K, Nakahigashi K, Inokuchi H (1992) Location of the *ubiA* gene on the physical map of *Escherichia coli*. J Bacteriol 174:5762

Nonet ML, Marvel CC, Tolan DR (1987) The *hisT–purF* region of the *Escherichia coli* K-12 chromosome. Identification of additional genes of the *hisT* and *purF* operons. J Biol Chem 262:12209–12217

Ogasawara N, Nakai S, Yoshikawa H (1994) Systematic sequencing of the 180 kilobase region of the *Bacillus subtilis* chromosome containing the replication origin. DNA Res 1:1–14

Ogura T, Tomoyasu T, Yuki T, Morimura S, Begg KJ, Donachie WD, Mori H, Niki H, Hiraga S (1991) Structure and function of the *ftsH* gene in *Escherichia coli*. Res Microbiol 142:279–282

Perier F, Radeke CM, Raab-Graham KF, Vandenberg CA (1995) Expression of a putative ATPase suppresses the growth defect of a yeast potassium transport mutant: identification of a mammalian member of the Clp/HSP104 family. Gene 152:157–163

Pinner E, Kotler Y, Padan E, Schuldiner S (1993) Physiological role of *nhaB*, a specific Na^+/H^+ antiporter in *Escherichia coli*. J Biol Chem 268:1729–1734

Plunkett G, Burland V, Daniels DL, Blattner FR (1993) Analysis of the *Escherichia coli* genome. III. DNA sequence of the region from 87.2 to 89.2 minutes. Nucleic Acids Res 21:3391–3398

Prior TI, Kornberg HL (1988) Nucleotide sequence of *furA*, the gene specifying enzyme IIfru of the phosphoenolpyruvate-dependent sugar phosphotransferase system in *Escherichia coli* K12. J Gen Microbiol 134:2757–2768

Quirk PG, Hicks DB, Krulwich TA (1993) Cloning of the *cta* operon from alkaliphilic *Bacillus firmus* OF4, and characterization of the pH-regulated cytochrome caa_3 oxidase it encodes. J Biol Chem 268:678–685

Sambrook J, Frisch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Sofia HJ, Burland V, Daniels DL, Plunkett G III, Blattner FR (1994) Analysis of the *Escherichia coli* genome. V. DNA sequence of the region from 76.0 to 81.5 minutes. Nucleic Acids Res 22:2576–2586

Sorokin A, Zumstein E, Azevedo V, Ehrlich SD, Serror P (1993) The organization of the *Bacillus subtilis* 168 chromosome region between the *spoVA* and *serA* genetic loci, based on sequence data. Mol Microbiol 10:385–395

Sturr MG, Guffanti AA, Krulwich TA (1994) Growth and bioenergetics of alkaliphilic *Bacillus firmus* OF4 in continuous culture at high pH. J Bacteriol 176:3111–3116

Suelter CH (1970) Enzymes activated by monovalent cations. Science 168:789–795

Sun D, Setlow P (1991) Cloning, nucleotide sequence, and expression of the *Bacillus subtilis ans* operon, which codes for L-asparaginase and L-aspartase. J Bacteriol 173:3831–3845

Tani K, Watanabe T, Matsuda H, Nasu M, Kondo M (1996) Cloning and sequencing of *Bacillus megaterium* ATCC 12872: similarities to the NaH-antiporter gene of *Enterococcus hirae*. 40:99–105

Tomoyasu T, Yuki T, Morimura S, Yamanaka K, Niki H, Hiraga S, Ogura T (1993) The *Escherichia coli* FtsH protein is a prokaryotic member of a protein family of putative ATPases involved in membrane functions, cell cycle control, and gene expression. J Bacteriol 175:1344–1351

Ueguchi C, Ito K (1992) Multicopy suppression: an approach to understanding intracellular functioning of the protein export system. J Bacteriol 174:1454–1461

Van der Laan JC, Gerritse G, Mulleners LJSM, Van der Hock RAC, Quax WJ (1991) Cloning, characterization, and multiple chromosomal integration of a *Bacillus* alkaline protease gene. Appl Environ Microbiol 57:901–909

Von Heijne G (1994) Membrane protein: from sequence to structure. Annu Rev Biophys Biomol Struct 23:167–192

Waser M, Hess-Bienz D, Davies K, Solioz M (1992) Cloning and disruption of a putative NaH-antiporter gene of *Enteroccocus hirae*. J Biol Chem 267:5396–5400

Yazdi MA, Moir A (1990) Characterization and cloning of the *gerC* locus of *Bacillus subtilis* 168. J Gen Microbiol 136:1335–1342