supplementary information

A novel type bacterial flagellar motor that can use divalent cations as a coupling ion

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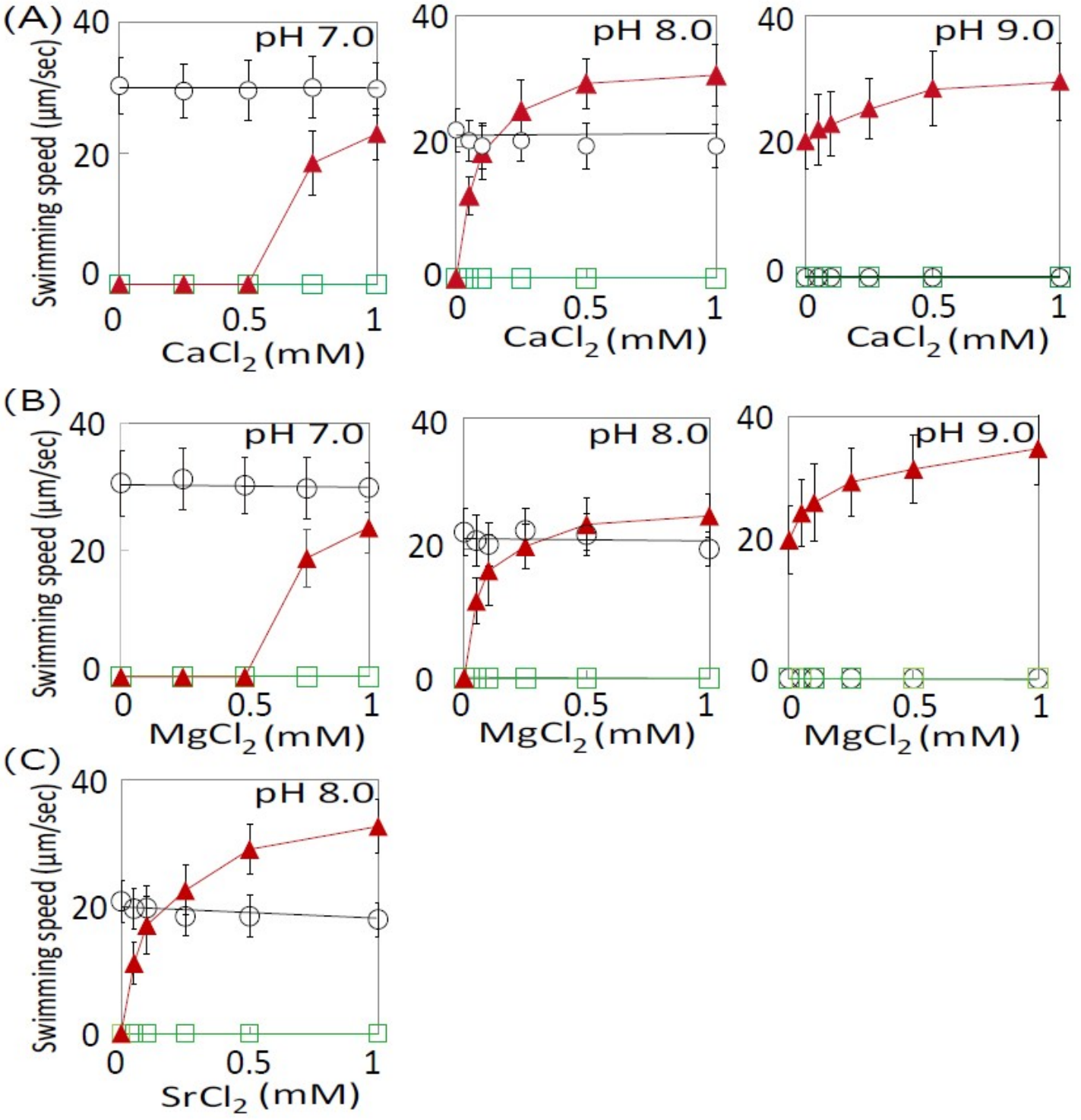

Fig. S1. Effect of lower concentration of divalent cations on swimming speed of *Paenibacillus* sp. TCA20, *E. coli*, and *B. pseudofirmus* OF4. Swimming speeds of *Paenibacillus* sp. TCA20, *E. coli*, and *B. pseudofirmus* OF4 cells were measured in 30 mM Tris-HCl containing less than 1 mM $CaCl_2$ (A), $MgCl_2$ (B), or $SrCl_2$ (C) concentrations. The results represent the average swimming speed of 30 independent cells of three independent experiments. The error bars indicate standard deviations.

Table S1. Bacterial strains and plasmids used in this study.

Strains or plasmid	Description	Source or reference
Strain		
Escherichia coli		
DH5αMCR	F- *mcrA*Δ1 (*mrr-hsd RMS-mcrBC*) Φ80*dlacZ* Δ(*lacZYAargF*) *U169 deoR recA1 endA1 supE44* λ*thi-1 gyr-496 relA*1	Stratagene
Paenibacillus sp.		
TCA20	Wild type	This study
Bacillus pseudofirmus		
OF4	Wild type	(1)
Bacillus subtilis		
BR151MA	*lys3 trpC2* (wild type)	(2)
ΔABΔPS	*lys3 trpC2* Δ*motAB* Δ*motPS*	(3)
BS-AB	ΔABΔPS *lacA*::P_{xylA}-*motAB* from BR151MA	(4)
BS-PS	ΔABΔPS *lacA*::P_{xylA}-*motPS* from BR151MA	(4)
OF4PS	ΔABΔPS *lacA*::P_{xylA}-*motPS* from OF4	This study
TCA-AB1	ΔABΔPS *lacA*::P_{xylA}-*motAB1* from TCA20	This study
TCA-AB2	ΔABΔPS *lacA*::P_{xylA}-*motAB2* from TCA20	This study
ΔABPSΔKQ	ΔABΔPS Δ*ykoK* Δ*yfjQ*	This study
ΔΔTCA-AB1	ΔABPSΔKQ *lacA*::P_{xylA}-*motAB1* from TCA20	This study
Plasmid		
pGEM-7zf(+)	Cloning vector; Ap^R	Promega
pAX01	*lacA* integration vector with Em^R gene and P_{xylA} promoter upstream of multiple cloning site	(5)
pGEM-AB1	pGEM-7zf(+) + *motAB1* from TCA20	This study
pGEM-AB2	pGEM-7zf(+) + *motAB2* from TCA20	This study
pAX-P_{xylA}-AB1	pAX01 + P_{xylA}-*motAB1* from TCA20	This study
pAX-P_{xylA}-AB2	pAX01 + P_{xylA}-*motAB2* from TCA20	This study
pUC18Tc	Cloning vector, Ap^R::Tc^R	(3)
pUC18Tc-ΔykoK	pUC18Tc+Δ*ykoK* fragment	This study
pUC18Tc-ΔyfjQ	pUC18Tc+Δ*yfjQ* fragment	This study

Table S2. Oligonucleotides used in this study.

Primer	Sequence (5′→3′) [a]	Accession number and corresponding sequence [b]
PUmotAB1-SacII-F	gttcccgCGGattatactcggttcatg	BBIW01000007.1 (13736-13762)
PUmotAB1-SacII-R	ccatcCcgcGGtaaaaatcaggatgg	BBIW01000007.1 (15458-15483)
PUmotAB2-SacII-F	aacCCgCggatatcttgaaaggattcag	BBIW01000023.1 (33114-33130)
PUmotAB2-SacII-R	caaagccGcGGacaggattggaggc	BBIW01000023.1 (34800-34824)
BS-YkoK-CM-1	GAAATTTCCGCAAAAGATGGACGC	CP010052.1 (1395250-1395273)
BS-YkoK-CM-2	GGCTCGCAGTTGAGACGGACGTACCTCCTCTACGGAGACG	CP010052.1 (1395998-1396017) (1397391-1397410)
BS-YkoK-CM-3	CGTCTCCGTAGAGGAGGTACGTCCGTCTCAACTGCGAGCC	CP010052.1 (1395998-1396017) (1397391-1397410)
BS-YkoK-CM-4	CGGTATTGTCCGTTTTGAACCG	CP010052.1 (1398073-1398094)
BS-YfjQ-CM-1	CGAACATGAGGACGTTTTGCACGG	CP010052.1 (873101-873124)
BS-YfjQ -CM-2	GGCTTACAACAAAAAAGAACCCTCCACCTGCCATTATATC	CP010052.1 (872323-872342) (871322-871340)
BS-YfjQ -CM-3	GATATAATGGCAGGTGGAGGGTTCTTTTTTGTTGTAAGCC	CP010052.1 (872323-872342) (871322-871340)
BS-YfjQ -CM-4	GCCCTAAAGACATTTTGAAGCCG	CP010052.1 (870568-870546)

[a] Nucleotides that were added to introduce point mutations are shown by a capital letter. [b] Minus strand is underlined.

References

1. Guffanti AA, *et al.* (1986) Isolation and characterization of new facultatively alkalophilic strains of *Bacillus* species. *J. Bacteriol.* 167(3):766-773.
2. Grundy FJ & Henkin TM (1991) The *rpsD* gene, encoding ribosomal protein S4, is autogenously regulated in *Bacillus subtilis*. *J Bacteriol* 173(15):4595-4602.
3. Takahashi Y, Koyama K, & Ito M (2014) Suppressor mutants from MotB-D24E and MotS-D30E in the flagellar stator complex of *Bacillus subtilis. J. Gen. Appl. Microbiol.* 60(4):131-139.
4. Takahashi Y & Ito M (2014) Mutational analysis of charged residues in the cytoplasmic 542 loops of MotA and MotP in the *Bacillus subtilis* flagellar motor. *J. Biochem.* 156(4):211-220.
5. Ireton K, Rudner DZ, Siranosian KJ, & Grossman AD (1993) Integration of multiple developmental signals in *Bacillus subtilis* through the Spo0A transcription factor. *Genes Dev.* 7(2):283-294.