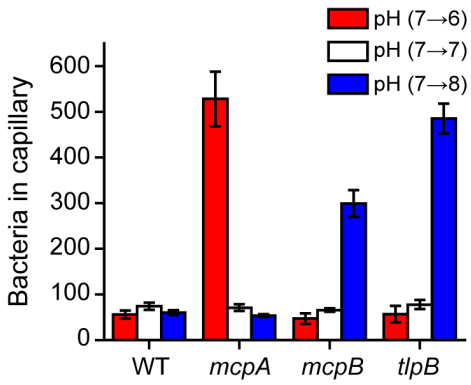

Figure S1. Wild-type *B. subtilis* prefers neutral regions (pH 7) as no accumulation towards acidic buffer (pH 6) or basic buffer (pH 8) is observed when cells were originally suspended in neutral buffer (pH 7). McpA mediates an attractant response to acidic buffer (pH 6) while McpB and TlpB mediate attractant responses to basic buffer (pH 8) when cells expressing them as their sole chemoreceptors were originally suspended in neutral buffer (pH 7). Error bars denote the standard deviation of three biological replicates performed on three separate days.

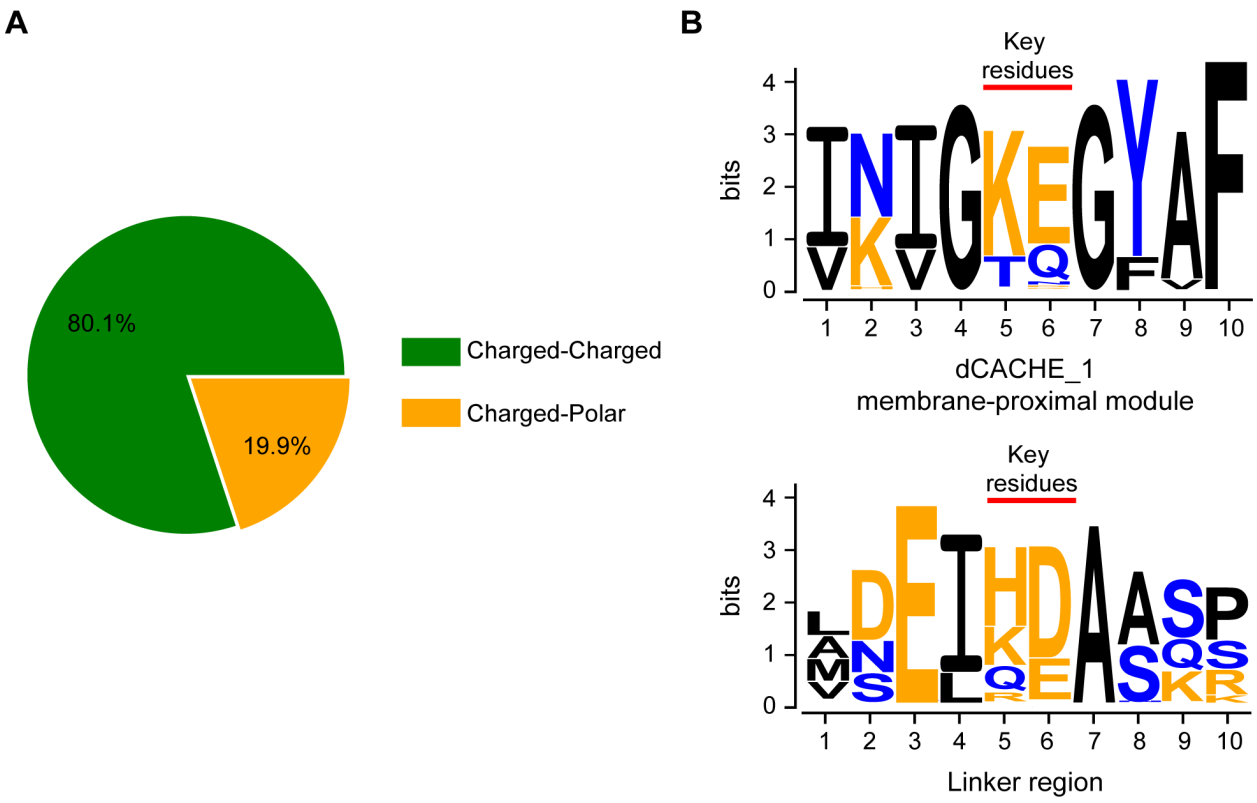

Figure S3. Conservation of pH-sensing amino-acid residues among *B. subtilis* species. (**A**) Fraction of identified domains that contain charged amino acid(s) in positions equivalent to T199/Q200 in the membrane-proximal module and H273/E274 in the linker region of McpA (green), fraction that contain charged amino acid(s) in corresponding positions in the membrane-proximal module and polar amino acid(s) in corresponding positions in linker region of McpA or vice versa (orange). (**B**) Amino-acid sequence logos depicting conservation of the key pH-sensing and the neighboring residues in membrane-proximal module (top) and in liker region (bottom).

Table S2. Oligonucleotides used in this study

Name	Sequence	Purpose
MP090	5'TCAACCAGCATAGTAAGAATACTAGTCTTACTGGACATCGGCAGTTCATA	Construction of pJSpe
MP091	5'GAACTGCCGATGTCCAGTAAGACTAGTATTCTTACTATGCTGGTTGACGT	
PT108F	5'GTCGACGGCCAACGAGGCCCCATATAAAGCTGCACAGCTTG	PCR of homology templates for *cheV* gene deletion
PT108R	5'CTGTTTTTATTTCAATCCCTCGCTATTC	
PT109F	5'AGGGATTGAAATAAAAACAGCCGTTGCC	
PT109R	5' TAGAAAGGCCTTATTGGCCCAGTTTGTTTCAAACATTGATTATC	
PT110F	5'TACGCACGTATAATATGCATACCG	Target sequence for *cheV* gene deletion
PT110R	5'AAACCGGTATGCATATTATACGTG	
PT123F	5'AAGGCCAACGAGGCCAAGGGTTTGCGGTATATATC	PCR of homology templates for *cheB* gene deletion
PT123R	5'AAGGCCATGTTGGCCATTAAGCCCTCCTCATTAAAAAAG	
PT124F	5'AAGGCCAACATGGCCATGGATATGAATCAGTATTTAGATG	
PT124R	5'AAGGCCTTATTGGCCGAATTGTTTTGGCTACTTCT	
PT125F	5'TACGTGTGTTTACATAGCACCGGG	Target sequence for *cheB* gene deletion
PT125R	5'AAACCCCGGTGCTATGTAAACACA	
PT120F	5'AAGGCCAACGAGGCCATCTGACAGGAGACTCTTC	PCR of homology templates for *cheR* gene deletion
PT120R	5'AAGGCCATGTTGGCCCAGCCGATACATTCTTTTATC	
PT121F	5'AAGGCCAACATGGCCCGTTGTAAATACGCTGTATG	
PT121R	5'AAGGCCTTATTGGCCTCGAACGTAAGGGCTTAC	
PT122F	5'TACGGGAGTGCCGCCTGCTCAACG	Target sequence for *cheR* gene deletion
PT122R	5'AAACCGTTGAGCAGGCGGCACTCC	
PT222F	5'ACCAGCATAGTAAGAATAAAGACATTTTGCAAACGC	PCR of homology templates for *cheC* gene deletion
PT222R	5'AAAGAGCTTTTAAGCTTGATCGGGCAC	
PT223F	5'CAAGCTTAAAAGCTCTTTGTCGCCTTAG	
PT223R	5'CCGATGTCCAGTAAGAGAGGCGTAGGTATCAAATTTTAAG	
PT221F	5'TACGAACTGGGCAATATTTTAGCG	Target sequence for *cheC* gene deletion
PT221R	5'AAACCGCTAAAATATTGCCCAGTT	
PT185F	5'CGGCCAACGAGGCCCATCGTTAATATACTGCTCGGCTTC	PCR of homology templates for 4*mcp* gene deletion
PT185R	5'CAAGTGACTGAAACGAGAAAGCGGCATATC	
PT186F	5'CTCGTTTCAGTCACTTGCTCCTTCAGG	
PT186R	5'AGGCCTTATTGGCCCTCAGCTTATCAATAATTTGCTTTC	
PT184F	5'TACGATAAGCGCTAACCGAAAGGG	Target sequence for 4*mcp* gene deletion
PT184R	5'AAACCCCTTTCGGTTAGCGCTTAT	
PT111F	5'ACCAGCATAGTAAGAATATTGGCTCAAAATAAAAGCATAG	PCR of homology templates for *mcpB* gene deletion
PT111R	5'CAAGTGACAAACCGAAAAACAGCGCTATC	
PT112F	5'TTTCGGTTTGTCACTTGCTCCTTCAGG	
PT112R	5'CCGATGTCCAGTAAGATCAGCTTATCAATAATTTGCTTTC	

Table S2. Oligonucleotides used in this study (continued)

PT113F	5'TACGCTGTCATTCGCATACATTTT	Target sequence for *mcpB* gene deletion
PT113R	5'TACGCTGTCATTCGCATACATTTT	
PT250F	5'ACCAGCATAGTAAGAATAATCGTTAATATACTGCTCGGCTTC	PCR of homology templates for *tlpB* gene deletion
PT250R	5'CAACGAGATGAAACGAGAAAGCGGCATATC	
PT251F	5'CTCGTTTCATCTCGTTGACTCCTCCTTTAAG	
PT251R	5'CCGATGTCCAGTAAGATCGACAGAAATTGCAAGTG	
PT249F	5'TACGAAGCCCGGTGCTGAACACTG	Target sequence for *tlpB* gene deletion
PT249R	5'AAACCAGTGTTCAGCACCGGGCTT	
PT323F	5'ACCAGCATAGTAAGAATATGTCCAATCGCCGGAACC	PCR of homology templates for *mcpA* gene deletion
PT323R	5'GAAATGAATGTAAGCCTTAACACCCAAGCTTGTTG	
PT324F	5'TAAGGCTTACATTCATTTCTCCTTTTTTATG	
PT324R	5'CCGATGTCCAGTAAGAAGCAAAGGGGAGACACTC	
PT322F	5'TACGGAAGCGGAGCAAGTCGTACG	Target sequence for *mcpA* gene deletion
PT322R	5'AAACCGTACGACTTGCTCCGCTTC	
PT327F	5'ACCAGCATAGTAAGAATACAGTTCCTGATTGTTCATTAG	PCR of homology templates for *tlpA* gene deletion
PT327R	5'TTCAAAAGGAATAGGTCTAAAATACTATTTATTTATGGG	
PT328F	5'TTAGACCTATTCCTTTTGAACCTTCTTTC	
PT328R	5'CCGATGTCCAGTAAGAGAACTCGATTAATCAATCTGTTC	
PT326F	5'TACGCGTCAAACGGTGGATGGGTC	Target sequence for *tlpA* & *mcpA-tlpA* genes deletion
PT326R	5'AAACGACCCATCCACCGTTTGACG	
PT354F	5'CAACCAGCATAGTAAGAATAATATACTTGGTTTGTCCAATCG	PCR of homology templates for *mcpA-tlpA* genes deletion
PT354R	5'TCAAAAGGAATAAGCCTTAACACCCAAG	
PT355F	5'TTAAGGCTTATTCCTTTTGAACCTTCTTTC	
PT355R	5'ACTGCCGATGTCCAGTAAGAATTAATCAATCTGTTCAGCAG	
tlpA-F	5'GTAGAATTCGCAGATGGCAGAAGAGCTTCG	Construction of pAIN750*tlpA*
tlpA-R	5'TGAGGATCCGATCGACAGAAAGGAAACGAG	
tlpB-F	5'CGCGAATTCAAGAAATCAGCTCGTCTGC	Construction of pAIN750*tlpB*
tlpB-R	5'TATGGATCCCTCTACGCCTTTAGGTAGCTG	
tlpC-F	5'GGCGAATTCAACTATGTTCACTCACCACG	Construction of pAIN750*tlpC*
tlpC-R	5'TGAGGATTCGATGTCCGTCGTGATGTT	
yfmS-F	5'CTGGAATTCGAAACGCATTGAAACAGG	Construction of pAIN750*yfmS*
yfmS-R	5'ACTGGATCCGCTCATGGTTCCTATCAATC	
yvaQ-F	5'CTGGAATTCGGCACTAGCACCCATATTAGG	Construction of pAIN750*yvaQ*
yvaQ-R	5'TGAGGATCCAAACGCAACAAACGGAACTG	
hemAT-F	5'CAGGAATTCATCTGGCGAAGTTGTAGAGG	Construction of pAIN750*hemAT*
hemAT-R	5'ATAGGATCCCACCCGTTCCGATTACATT	

Table S2. Oligonucleotides used in this study (continued)

yoaH-F	5'CTGAATGCAGAATTCCTATAAAACTTGATAACACGTGTCGAT	Construction of pAIN750*yoaH*
yoaH-R	5'ACGTTGTGCGGATCCATTAGAAAAAGGATTGGCTGAAACTCA	
PT233F	5'CATTCATTTCTCCTTTTTTATGCTACC	Long PCR for construction of pPT065
PT237R	5'AATGTTGCCGCTTCCTCTG	
PT238F	5'AGGAAGCGGCAACATTTTCTACAGACGTTTGAATAACG	PCR of insert for construction of pPT065
PT234R	5'TAAAAAAGGAGAAATGAATGATGGGAAAATTCATACAATG	
PT233F	5'CATTCATTTCTCCTTTTTTATGCTACC	Long PCR for construction of pPT063
PT233R	5'ATTGTCTTGGCCGCAGCTATTATC	
PT234F	5'CTGCGGCCAAGACAATAACCGCAGTGTTCAGCAC	PCR of insert for construction of pPT063
PT234R	5'TAAAAAAGGAGAAATGAATGATGGGAAAATTCATACAATG	
PT359F	5'CATTCATTTCTCCTTTTTTATG	Long PCR for construction of pPT143
PT359R	5'AAGATCGGCGGCACCATG	
PT360F	5'TACATGGTGCCGCCGATCTTCCAGCCAGTCAGTTTATTTG	PCR of insert for construction of pPT143
PT360R	5'TAAAAAAGGAGAAATGAATGATGGGAAAATTCATACAATG	
PT548F	5'CATTCATTTCTCCTTTTTTATG	Long PCR for construction of pPT224
PT548R	5'GATTTTCAATACACCATGGATG	
PT549F	5'CCATGGTGTATTGAAAATCGCCTTCTTTTTTGGAATATAC	PCR of insert for construction of pPT224
PT549R	5'AAAAAAGGAGAAATGAATGATGGGAAAATTCATACAATG	
PT556F	5'TGCGATAACGCCTGAACCATC	Long PCR for construction of pPT233
PT556R	5'ATTGTCTTGGCCGCAGCTATTATC	
PT557F	5'CTGCGGCCAAGACAATAACCGCAGTGTTCAGCAC	PCR of insert for construction of pPT233
PT557R	5'TTCAGGCGTTATCGCACTAGATTTAAACCTGGATGAAGTG	
PT376F	5'CATTCATTTCTCCTTTTTTATGCTAC	Long PCR for construction of pPT234
PT561R	5'ATCAACATGACGATTGAAAATCTG	
PT562F	5'TTTCAATCGTCATGTTGATCGCTATAACCCCTGAACC	PCR of insert for construction of pPT234
PT562R	5'AAAAAAGGAGAAATGAATGATGGGAAAATTCATACAATGGATC	
PT563F	5'GATGTTGACTTTTTTGGTTGTTTTCAG	Long PCR for construction of pPT236
PT563R	5'ATTGTCTTGGCCGCAGCTATTATC	
PT564F	5'CTGCGGCCAAGACAATAACCGCAGTGTTCAGCAC	PCR of insert for construction of pPT236
PT564R	5'CAACCAAAAAAGTCAACATCGGAAAAGAAGGCTTTGCATTTATTAC	
PT565F	5'AGTTCCTGATTGTTCATTAGGGTG	Long PCR for construction of pPT237
PT565R	5'ATTGTCTTGGCCGCAGCTATTATC	
PT566F	5'CTGCGGCCAAGACAATAACCGCAGTGTTCAGCAC	PCR of insert for construction of pPT237
PT566R	5'AATGAACAATCAGGAACTACCGGTTCCGGCGATTGG	
PT341F	5'ACTGACAAAATATGTGCCCGC	Construction of pPT129
PT341R	5'GAATTGCATGAAGCTTCAAGC	
PT569F	5'TGATTCTTTTTGATGCAGTAAGC	Construction of pPT162 and pPT202
PT569R	5'AAATCGGAACACAAGGCTTTGC	

Table S2. Oligonucleotides used in this study (continued)

PT520F	5'ATGAAATCCAAGACGCAGCCCAG	Construction of pPT196
PT520R	5'AGTACGCTGCAAAATAAATTAG	
PT570F	5'GTAGCCTTCTTTGCCGATGTTG	Construction of pPT163 and pPT222
PT570R	5'GCATTTATCATGACGAAGGAC	
PT304F	5'ACCGGGCTTGAAGCATTTTG	Construction of pPT101
PT304R	5'GCTGAACACTGCGGTTATCATTC	
PT314F	5'AAATCCATCAGGCAGCCCAG	Construction of pPT107
PT314R	5'CATCCAAATACATGGTGCCG	
GB006F	5'CATTCATTTCTCCTTTTTTATG	Long PCR for construction of pGB45
GB006R	5'TAAGCCTTAACACCCAAG	
GB007F	5'TTGGGTGTTAAGGCTTATTATTTGTCTACTTTAAATTGTTTTG	PCR of *tlpA* for construction of pGB45
GB007R	5'TAAAAAAGGAGAAATGAATGATGAAAAAAACACTCACCAC	