Supporting Information

The adhesive and cohesive properties of a bacterial polysaccharide adhesin are modulated by a deacetylase.

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SUPPORTING EXPERIMENTAL PROCEDURES

Formic acid treatment of HfaA and HfsD samples.

Cell fractionation and protein analysis were performed as previously described (Hardy *et al.*, 2010). Cell pellets from 20 ml of exponentially growing cells normalized to OD_{600}= 0.6 were resuspended in 1 ml of 20 mM Tris buffer pH = 8 suspended, and lysed by FastPrep®-24 Instrument (MP Biomedicals LLC) in 2.0 ml Lysing Matrix tube containing specialized Lysing Matrix beads for 45 sec. Unbroken cells were removed by centrifugation at 16,000 g at 4°C for 2 min. The supernatant was removed and centrifuged at 100,000 x g at 4°C for 30 min. The pellet was suspended in 500 µl 20 mM Tris, pH 8.0 and 1% sodium lauryl sarcosine, rocked at RT for 45 min, and centrifuged at 100,000 x g for 30 min. The pellet comprises the OM fraction which contains HfaA and HfaD and was treated with 90% formic acid for 2h at room temperature at dark prior to analysis by SDS-PAGE. After incubation with formic acid, all samples were lyophilized until dry. Two volumes of deionized water were added to each formic acid sample, which was lyophilized again to remove traces of formic acid. Samples were suspended in equal volumes of 1 M Tris, pH 8 and 2X SDS-PAGE sample buffer (0.125 M Tris, 4% w/v SDS, 25% v/v glycerol, 4% w/v dithiothreitol, 10% v/v β-mercaptoethanol, and 0.2% w/v bromophenol blue) and boiled for 5 min prior to electrophoresis where each loaded sample was equivalent to 25 ml cell culture at OD600=0.6.

Western Blot Analysis

Protein samples were resuspended in 50 µl 10 mM Tris pH 8.0, and 50 uL of 2x SDS sample buffer was added to the suspension. Samples were then boiled for 5 min before being run on a 12% w/v polyacrylamide gel, and transferred to nitrocellulose membranes. Membranes were blocked for 1 h in

5% w/v nonfat dry milk in TBST (20 mM Tris, pH 8, 0.05% v/v Tween 20), and incubated with M2-HRP at a concentration of 1:1000 overnight at 4ºC. Then, a 1:10,000 dilution of secondary antibody, horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin, was incubated with membranes at room temperature for 1h. Membranes were developed with SuperSignal West Dura Substrate (Thermo Scientific, Rockford, IL).

Epifluorescence microscopy and image analysis

Microscopy was performed on a Nikon Eclipse 90i equipped with Chroma 83000 filter set, a 100X (DIC or phase-contrast) oil objective, and a Photometrics Cascade 1K EMCCD camera. Images were captured using Nikon NIS Elements advance research version 4.0

Cell fractionation and protein analysis.

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SUPPORTING TABLES

Table S1. Comparison of the genes involved in holdfast biosynthetis in *C. crescentus* and their homologs in close relatives of *Caulobacter*.

C. crescentus CB15		*A. biprosthecum* C19		*H. baltica*		*B. diminuta*	
Holdfast gene	Predicted gene function	Locus tag	% Ident.*	Locus tag	% Ident.*	Locus tag	% Ident.*
hfsE (CC2425)	Glycosyltransferase	NA**	53	NA**	43	1681	49
hfsF (CC2426)	Flippase	42660	46	100	37	17320	52
hfsG (CC2427)	Glycosyltransferase	42650	59	1964	41	17330	60
hfsH (CC2428)	Polysaccharide deacetylase family protein	42640	51	1965	33	22530	37
hfsC (CC2429)	Polysaccharide polymerase	42530	45	1972	31	18490	43
hfsB (CC2430)	Polysaccharide autokinase-related protein	42620	44	1967	37	17340	47
hfsA (CC2431)	Chain length determinant family protein	42610	41	1968	34	17350	42
hfsD (CC2432)	Polysaccharide biosynthesis/export family proteins	42600	50	1969	41	17360	49

* Gene is found in the region without annotation.

** % identity to gene homolog in *C. crescentus* CB15.

TABLE S2. Bacterial strains and plasmids

	Derivation/phenotype/genotype	Reference/Source
E. coli		
Alpha select	F- *deoR endA1 recA*1 *rel*A1 *gyrA*96 *hsdR*17(rk-, mk+) *supE*44 *thi*-1 *phoA*Δ(*lacZYAargF*)U169 φ80*lacZ*ΔM15 λ-	Bioline
BL21(DE3)	F- *ompT hsdSB* (rB-mB-) *gal dcm* (λDE3)	
C. crescentus		
YB135	CB15 wild-type	(Poindexter, 1964)
YB2857	CB15 Δ*hfsDAB*	Brun lab
YB6364	CB15 Δ*hfsD-E*	June Javens
YB2198	CB15 Δ*hfsH*	Toh *et al*, 2008
YB4251	CB15 Δ*hfaB*	Hardy *et al*, 2010
YB4284	CB15 *hfaB*::pCHYC*hfaAB*	Hardy *et al*, 2010
YB6886	CB15 Δ*hfsH hfaB*::pCHYC*hfaAB*	This work
YB2578	CB15 *hfaA*::pJM23*hfaA*	Brun lab
YB5622	CB15 Δ*hfsH hfaA*::pJM23*hfaA*	This work
YB2579	CB15 *hfaD*::pJM23*hfaD*	Brun lab
YB5624	CB15 Δ*hfsH hfaD*::pJM23*hfaD*	This work
YB6887	CB15 Δ*hfsH* pMR10*hfsH*	This work
YB6888	CB15 Δ*hfsH* pMR10*hfsH*-D48A	This work
A. biprosthecum		
YB642	C19 wild type	(Larson and Pate, 1975)
YB5191	C19nal; parent strain of transposon mutants	This study
YB5649	C19nal *hfsH*::MarTn	This study
YB5650	C19nal *hfsE*::MarTn	This study
YB5651	C19nal *hfsA*::MarTn	This study
YB6593	C19nal *hfsD*::MarTn	This study
Hirschia baltica		
YB5842	ATCC49814	(Chertkov *et al.*, 2011)

Brevundimonas diminuta		
YB5193	ATCC11568	
Plasmids		
pET28a *hfsH*	Protein overexpression vector that carries the *hfsH* gene	This work
pET28a *hfsHD48A*	Protein overexpression vector that carries the *hfsH* gene with single amino acid mutation from Asp48 to Ala.	This work
pMR10	shuttle plasmid for *E. coli* and *Caulobacter*, Km^R	Roberts *et al*., 1996
pMR10 *hfsH*	Complementation vector that carries the native *hfsE* promoter and the *hfsH* gene	Toh *et al*, 2008
pMR10 *hfsHD48A*	Complementation vector that carries the native *hfsE* promoter and the *hfsH* gene with single amino acid mutation from Asp48 to Ala.	This study
pUJ142	High copy number plasmid that is a derivative of pBBR1MCS with a xylose inducible promoter. Cm^R	U. Jenal, unpublished
pUJ142 *hfsH*	Complementation vector that carries the *hfsH* gene	This study

SUPPORTING FIGURE LEGENDS

Figure S1. Alignment of *C. crescentus* HfsH and *A. biprosthecum* HfsH along with biochemically characterized CE4 esterase family members. The conserved motifs are indicated by squares. Motif 2 (black box) contains the zinc binding triad. The conserved acetate binding residues (blue triangles) are required for enzymatic activity and include the site of the point mutation (blue star). From the top to the bottom, the polysaccharide deacetylases compared are as follows: *C. crescentus* CB15 HfsH (accession number AAK24399.1), *A. biprosthecum* HfsH (accession number AAK24399.1), *Streptococcus pneumonia* PgdA (accession number CAB96552.1), *Bacillus subtilis* PdaA (accession number O34928.1), and *Colletotrichum lindemuthianum* CDA (accession number AY63365).

Figure S2. Circular dichroism spectra of wild-type HfsH-WT(wild-type) (pink) and HfsH-D48A (green) in 50 mM sodium phosphate pH 7.4. Samples have a concentration of 0.17 mg ml^{-1}, and were measured in a 0.1-cm cell.

Figure S3. Analysis of holdfast anchoring machinery in a Δ*hfsH* mutant. (A) Western Blots of outer membrane fractions. HfaA forms SDS/heat high-molecular weight (HMW) complexes in wild-type (WT) and Δ*hfsH* mutant cells expressing HfaA-M2 or HfaD-M2, and these HMW complexes disassociate into monomers after formic acid (FA) treatment. Blots were probed with M2-specific antibody. Lane 1) CB15 wild-type (WT) treated with SDS and heat; 2) CB15 treated with SDS, heat and formic acid; Lane 3) Δ*hfsH* wild-type treated with SDS and heat; 4) Δ*hfsH* treated with SDS, heat and formic acid; (B) HfaD forms HMW complexes in wild-type and Δ*hfsH* mutant cells. Blots are arranged in the same order as (A). (C) Overlay micrographs of differential interference contrast (DIC) and epifluorescence microscopy showing the localization of HfaBmCherry. The left panel is CB15

hfaB::pCHYC*hfaAB*, and the right panel is CB15 Δ*hfsH hfaB*::pCHYC*hfaAB*. The localization of HfaBmCherry (in red) in indicated by white arrows.

Figure S4. HfsH localizes to the soluble fraction of cells. Total whole cells (WC) were separated into soluble fraction (Soluble) and insoluble membrane fraction (Insoluble) fraction. McpA (membrane positive control), and CtrA (soluble fraction positive control) are shown.

SUPPORTING REFERENCES

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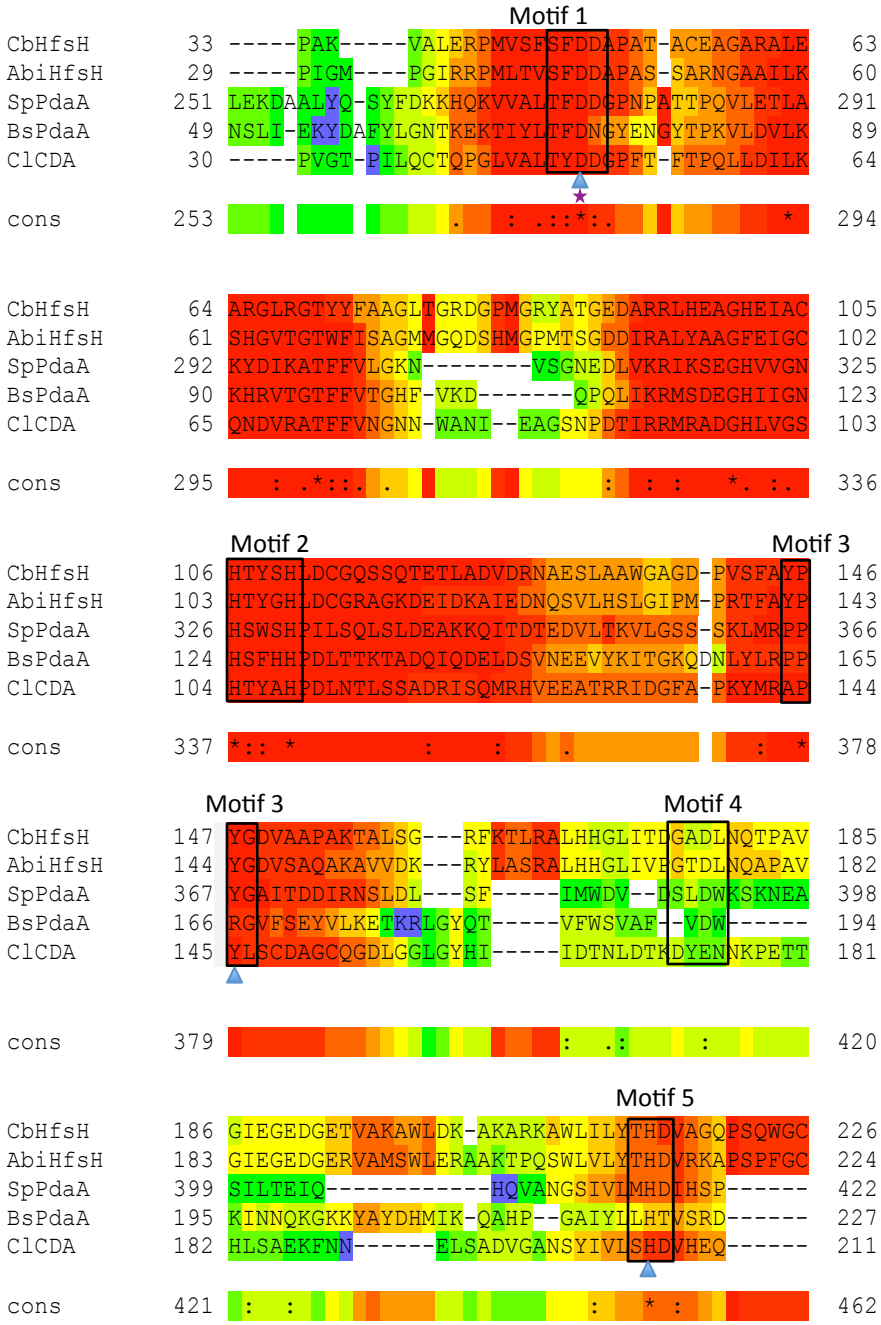
Larson, R. J. & J. L. Pate, (1975) Growth and morphology of *Asticcacaulis biprosthecum* in defined media. *Arch Microbiol* **106**: 147–157.

Poindexter, J. S., (1964) Biological Properties and Classification of the Caulobacter Group. *Bacteriological reviews* **28**: 231-295.

Fig. S1


Motif 1
CbHfsH 33 -----PAK-----VALERPMVSFSFDDAPAT-ACEAGARALE 63
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SpPdaA 251 LEKDAALYQ-SYFDKKHQKVVALTFDDGPNPATTPQVLETLA 291
BsPdaA 49 NSLI-EKYDAFYLGNTKEKTIYLTFDNGYENGYTPKVLDVLK 89
ClCDA 30 -----PVGT-PILQCTQPGLVALTYDDGPFT-FTPQLLDILK 64
cons 253 294
CbHfsH 64 ARGLRGTYYFAAGLTGRDGPMGRYATGEDARRLHEAGHEIAC 105
AbiHfsH 61 SHGVTGTWFISAGMMGQDSHMGPMTSGDDIRALYAAGFEIGC 102
SpPdaA 292 KYDIKATFFVLGKN--------VSGNEDLVKRIKSEGHVVGN 325
BsPdaA 90 KHRVTGTFFVTGHF-VKD-------QPQLIKRMSDEGHIIGN 123
ClCDA 65 QNDVRATFFVNGNN-WANI--EAGSNPDTIRRMRADGHLVGS 103
cons 295 336
Motif 2
Motif 3
CbHfsH 106 HTYSHLDCGQSSQTETLADVDRNAESLAAWGAGD-PVSFAYP 146
AbiHfsH 103 HTYGHLDCGRAGKDEIDKAIEDNQSVLHSLGIPM-PRTFAYP 143
SpPdaA 326 HSWSHPILSQLSLDEAKKQITDTEDVLTKVLGSS-SKLMRPP 366
BsPdaA 124 HSFHHPDLTTKTADQIQDELDSVNEEVYKITGKQDNLYLRPP 165
ClCDA 104 HTYAHPDLNTLSSADRISQMRHVEEATRRIDGFA-PKYMRAP 144
cons 337 378
Motif 3
Motif 4
CbHfsH 147 YGDVAAPAKTALSG---RFKTLRALHHGLITDGADLNQTPAV 185
AbiHfsH 144 YGDVSAQAKAVVDK---RYLASRALHHGLIVEGTDLNQAPAV 182
SpPdaA 367 YGAITDDIRNSLDL---SF------IMWDV--DSLDWKSKNEA 398
BsPdaA 166 RGVFSEYVLKETKRLGYQT-----VFWSVAF--VDW------ 194
ClCDA 145 YLSCDAGCQGDLGGLGYHI-----IDTNLDTKDYENNKPETT 181
cons 379 420
Motif 5
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AbiHfsH 183 GIEGEDGERVAMSWLERAAKTPQSWLVLYTHDVRKAPSPFGC 224
SpPdaA 399 SILTEIQ-------------HQVANGSIVLMHDIHSP------ 422
BsPdaA 195 KINNQKGKKYAYDHMIK-QAHP--GAIYLLHTVSRD------ 227
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cons 421 462

Fig. S2

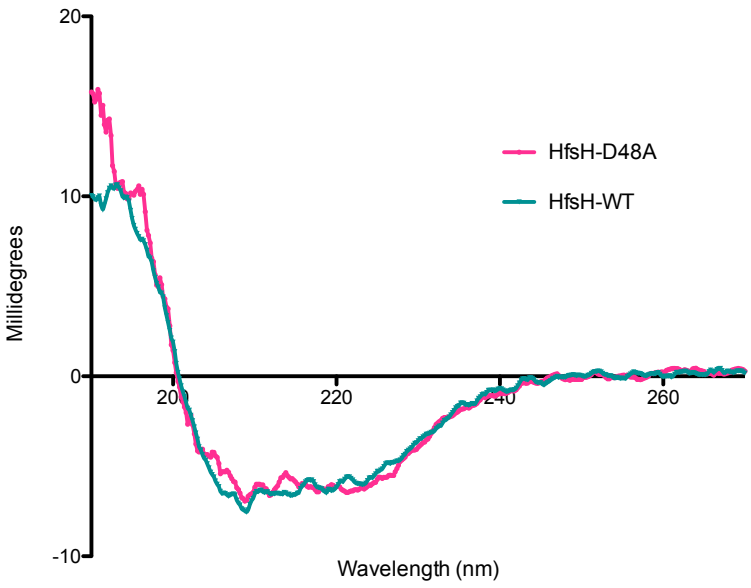
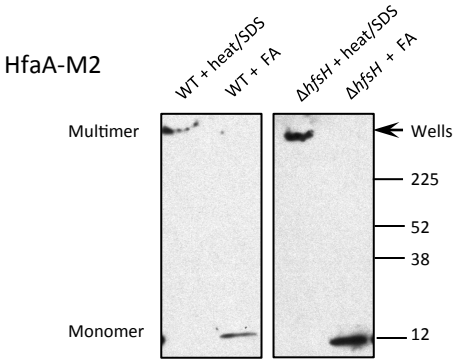
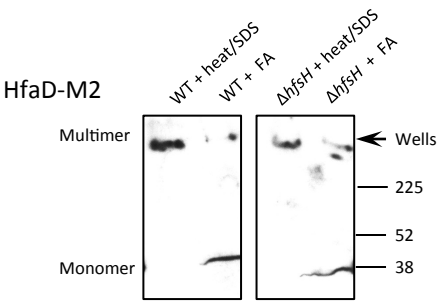

20
10
0
-10
Millidegrees
200
220
240
260
Wavelength (nm)
HfsH-D48A
HfsH-WT

Fig. S3

A.


HfaA-M2
WT + heat/SDS
WT + FA
ΔhfsH + heat/SDS
ΔhfsH + FA
Multimer
Monomer
Wells
225
52
38
12

B.


HfaD-M2
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WT + FA
ΔhfsH + heat/SDS
ΔhfsH + FA
Multimer
Monomer
Wells
225
52
38

C.

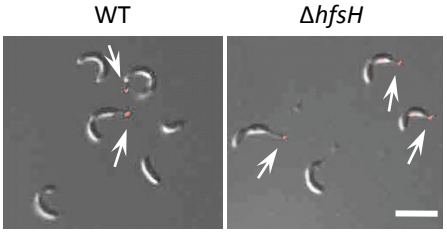
WT
ΔhfsH

Fig. S4