Supplementary data

Motility, chemotaxis and aerotaxis contribute to competitiveness during bacterial pellicle biofilm development

Theresa Hölscher[1,#], Benjamin Bartels[1,#], Yu-Cheng Lin[2,#], Ramses Gallegos-Monterrosa[1], Alexa Price-Whelan[2], Roberto Kolter[3], Lars E.P. Dietrich[2], Ákos T. Kovács[1,*]

1 - Terrestrial Biofilms Group, Institute of Microbiology, Friedrich Schiller University Jena, Jena, D-07743, Germany

2 - Department of Biological Sciences, Columbia University, New York, New York, NY 10027, USA

3 - Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA 02115, USA

*Correspondence to Ákos T. Kovács: akos-tibor.kovacs@uni-jena.de

Postal address: Neugasse 23, D-07743 Jena, Germany

Tel: +49 3641 949980

Fax: +49 3641 949292

Fig. S1 Growth curves of *B. subtilis* strains lacking certain genes involved in flagellum synthesis and regulation (A), chemotaxis (B), and aerotaxis (C) compared to the wild-type strain (black line). Growth in LB medium was followed by recording optical density at 595 nm in a microtiter-plate reader. Growth curves represent the average of 6 replicates and error bars indicate standard deviation.

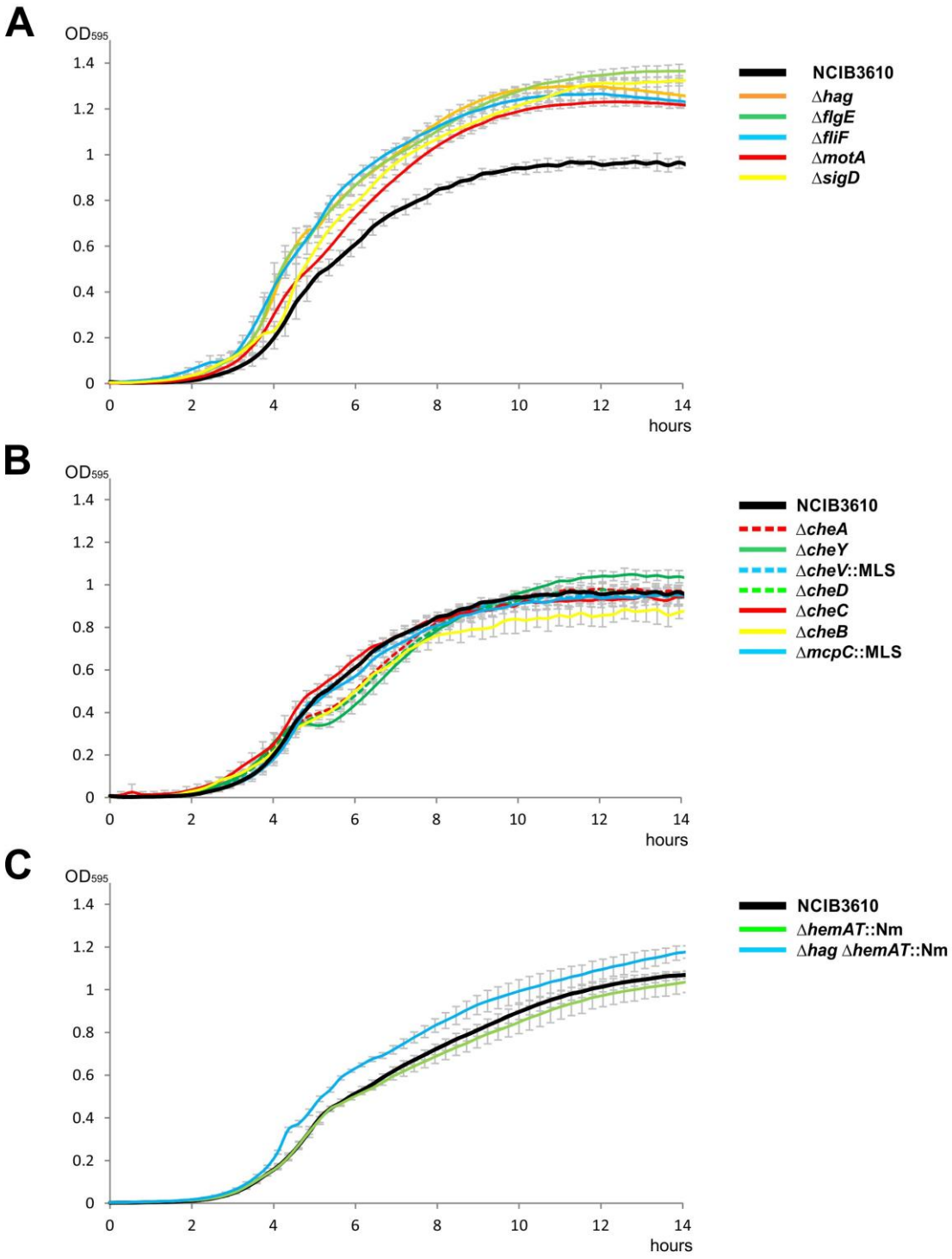


Fig. S2 Competition of GFP or mKATE2 labelled strains of *B. subtilis* strains during pellicle formation. Wells of a 24-well plate containing mixes of cells are shown in the green- or red-fluorescence channels (false coloured in green and red, respectively) together with its bright light images. The images in the right side of the figure show the pellicle pictures of the pure mutant cultures.

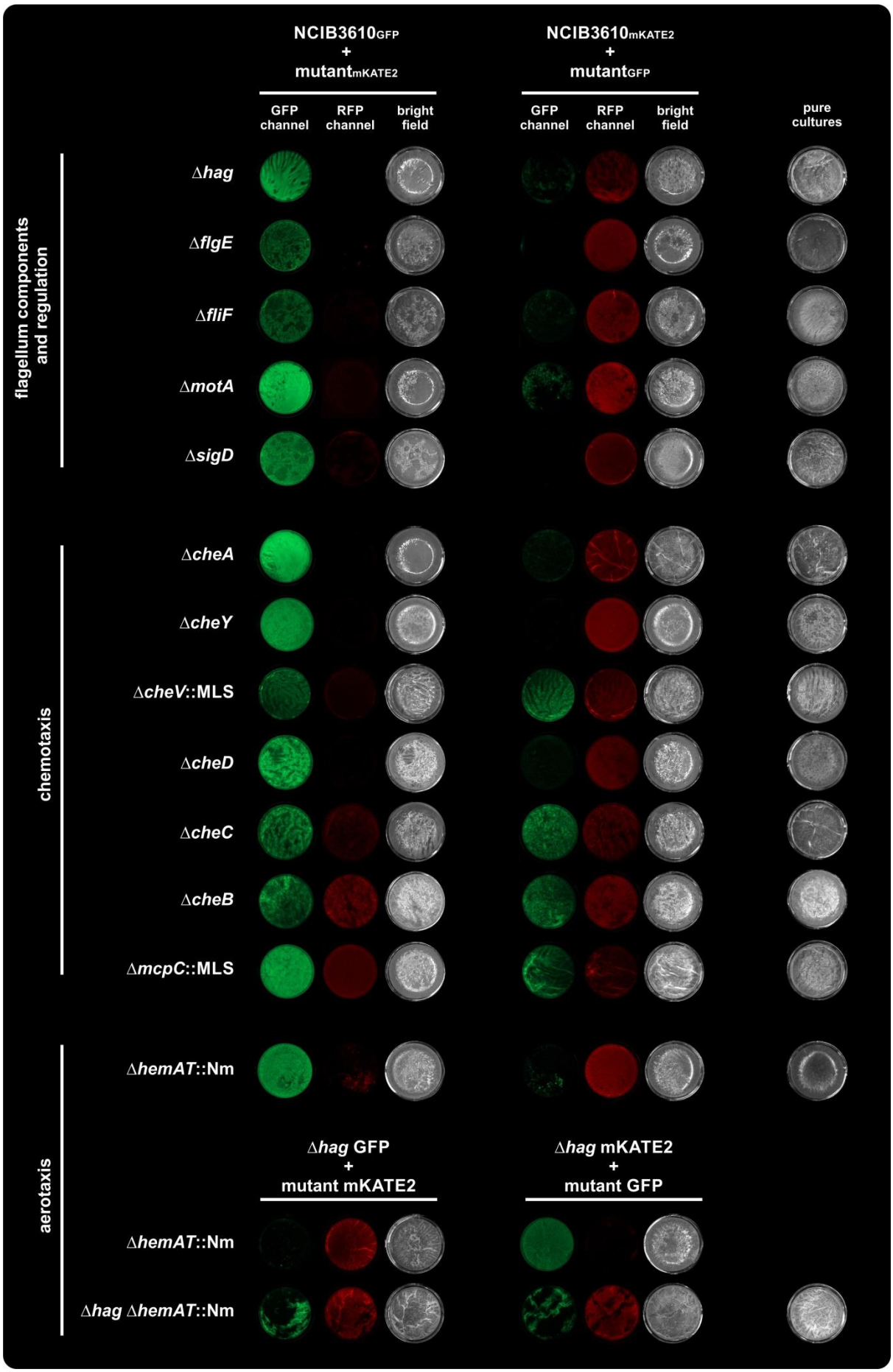
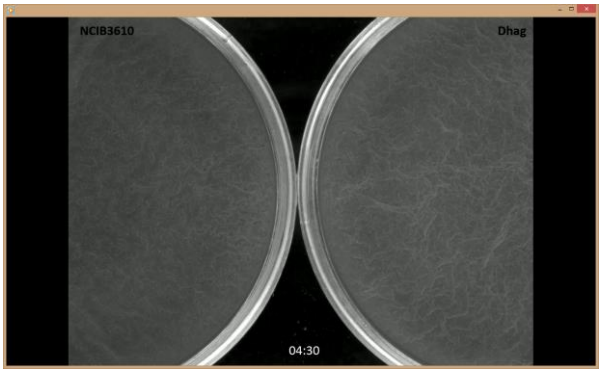

NCIB3610GFP + mutantmKATE2
NCIB3610mKATE2 + mutantGFP
GFP channel
RFP channel
bright field
GFP channel
RFP channel
bright field
pure cultures
flagellum components and regulation
Δhag
ΔflgE
ΔfliF
ΔmotA
ΔsigD
chemotaxis
ΔcheA
ΔcheY
ΔcheV::MLS
ΔcheD
ΔcheC
ΔcheB
ΔmcpC::MLS
aerotaxis
ΔhemAT::Nm
Δhag GFP + mutant mKATE2
Δhag mKATE2 + mutant GFP
ΔhemAT::Nm
Δhag ΔhemAT::Nm

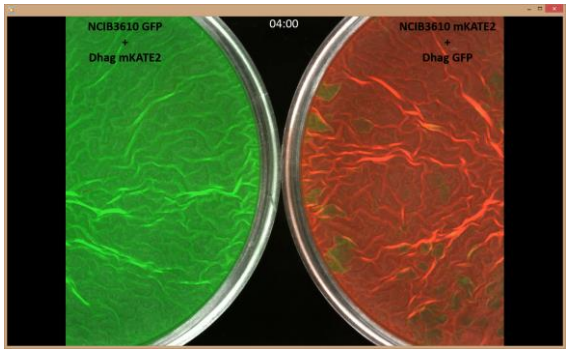
Table S1. Competition experiments of various *B. subtilis* mutant strains lacking motility, chemotaxis or oxygen sensing against wild-type strain (3610) during pellicle formation. Values are arbitrary units as described in the Materials and Methods.

Strains	Signal abundance		Strains	Signal abundance	
	GFP	mKATE2		GFP	mKATE2
flagellum synthesis and function					
3610_{GFP} vs Δhag_{mKATE2}	2.41±0.53	0.03±0.02	3610_{mKATE2} vs Δhag_{GFP}	0.08±0.05	2.51±0.29
3610_{GFP} vs $\Delta flgE_{mKATE2}$	1.67±0.37	0.16±0.08	3610_{mKATE2} vs $\Delta flgE_{GFP}$	0.16±0.11	5.24±2.69
3610_{GFP} vs $\Delta fliF_{mKATE2}$	1.43±0.25	0.11±0.02	3610_{mKATE2} vs $\Delta fliF_{GFP}$	0.59±0.21	2.20±0.35
3610_{GFP} vs $\Delta motA_{mKATE2}$	2.08±0.92	0.21±0.08	3610_{mKATE2} vs $\Delta motA_{GFP}$	0.33±0.17	3.38±0.98
3610_{GFP} vs $\Delta sigD_{mKATE2}$	1.41±0.42	0.04±0.01	3610_{mKATE2} vs $\Delta sigD_{GFP}$	0.38±0.29	2.29±0.24
chemotaxis					
3610_{GFP} vs $\Delta cheA_{mKATE2}$	2.35±0.57	0.03±0.01	3610_{mKATE2} vs $\Delta cheA_{GFP}$	0.26±0.18	3.18±0.56
3610_{GFP} vs $\Delta cheY_{mKATE2}$	1.82±0.25	0.05±0.03	3610_{mKATE2} vs $\Delta cheY_{GFP}$	0.11±0.02	2.32±0.34
3610_{GFP} vs $\Delta cheV_{mKATE2}$	1.31±0.41	0.48±0.15	3610_{mKATE2} vs $\Delta cheV_{GFP}$	0.63±0.12	1.54±0.24
3610_{GFP} vs $\Delta cheD_{mKATE2}$	2.11±0.27	0.09±0.04	3610_{mKATE2} vs $\Delta cheD_{GFP}$	0.09±0.03	2.66±0.44
3610_{GFP} vs $\Delta cheC_{mKATE2}$	0.60±0.18	1.61±0.35	3610_{mKATE2} vs $\Delta cheC_{GFP}$	1.27±0.15	0.76±0.47
3610_{GFP} vs $\Delta cheB_{mKATE2}$	0.75±0.23	1.02±0.28	3610_{mKATE2} vs $\Delta cheB_{GFP}$	1.33±0.46	1.39±0.65
3610_{GFP} vs $\Delta mcpC_{mKATE2}$	0.70±0.14	1.14±0.12	3610_{mKATE2} vs $\Delta mcpC_{GFP}$	1.11±0.11	0.7±0.17
aerotaxis					
3610_{GFP} vs $\Delta hemAT_{mKATE2}$	1.72±0.33	0.10±0.09	3610_{mKATE2} vs $\Delta hemAT_{GFP}$	0.55±0.33	2.20±0.46
Δhag_{GFP} vs $\Delta hemAT_{mKATE2}$	0.59±0.37	1.66±0.24	Δhag_{mKATE2} vs $\Delta hemAT_{GFP}$	1.56±0.26	0.05±0.05
Δhag_{GFP} vs $\Delta hag\ \Delta hemAT_{mKATE2}$	1.57±1.30	1.30±0.40	Δhag_{mKATE2} vs $\Delta hag\ \Delta hemAT_{GFP}$	1.87±0.86	0.69±0.35

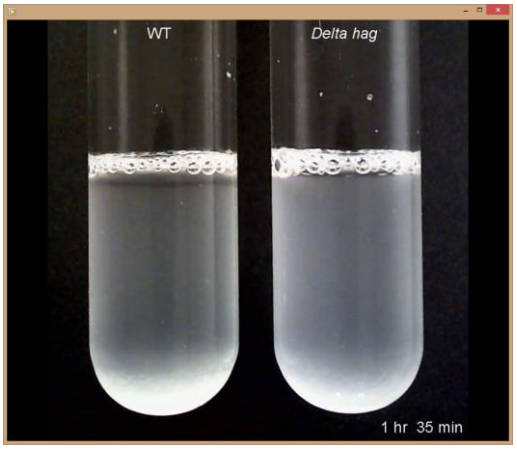
Video S1 Pellicle formation of *B. subtilis* wild-type (left petri-dish) and its Δ*hag* derivative (right petri-dish) is shown in MSgg medium. Cultures were grown in 35 mm diameter Falcon petri dishes at 30 °C and images were recorded every 30 min.



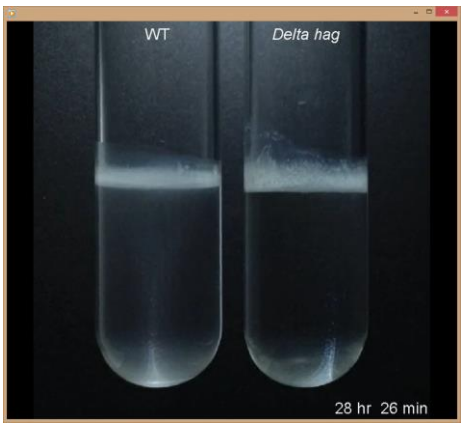
Video S2 Competition of GFP or mKATE2 labelled wild-type and Δ*hag* strains of *B. subtilis* strains during pellicle formation (GFP and mKATE2 signals were false coloured in green and red, respectively). Cultures were grown in 35 mm diameter Falcon petri dishes at 30 °C and images were recorded every 15 min. Left: $NCIB3610_{GFP}$ co-inoculated with Δhag_{mKATE2} strain. Right: $NCIB3610_{mKATE2}$ co-inoculated with Δhag_{GFP} strain.



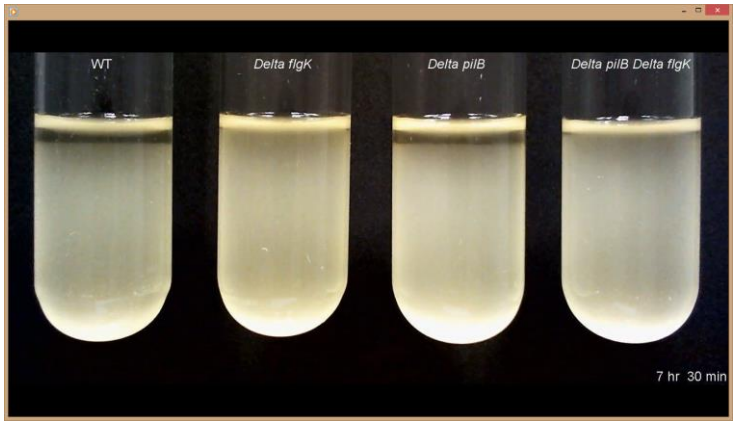
Video S3 Pellicle formation of *B. subtilis* cultures recorded from the side of the glass tubes. Cultures were inoculated in MSgg medium at high cell densities (OD_{500} of 1.2) and incubated at 30 °C.



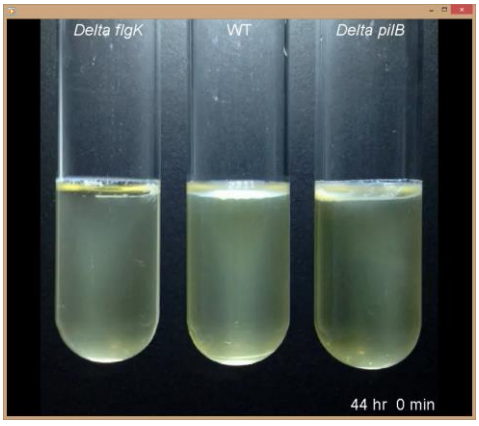
Video S4 Pellicle formation of *B. subtilis* cultures recorded from the side of the glass tubes. Cultures were inoculated in MSgg medium at low cell densities (OD_{500} of 0.005) and incubated at 30 °C.



Video S5 Pellicle formation of *P. aeruginosa* cultures recorded from the side of the glass tubes. Cultures were inoculated in LB medium at high cell densities (OD_{500} of 2.0) and incubated at 37 °C.



Video S6 Pellicle formation of *P. aeruginosa* cultures recorded from the side of the glass tubes. Cultures were inoculated in LB medium at low cell densities (OD_{500} of 0.005) and incubated at 37 °C.



Video S7 Pellicle formation of *B. subtilis* cultures recorded from the side of the glass tubes. Wild type, Δ*hag* and Δ*hemAT* cultures (from left to right) were inoculated in MSgg medium at high cell densities (OD_{500} of 1.0-1.2) and incubated at 30 °C.