Exosporium Hairs Affect Spore Adhesion on Surfaces

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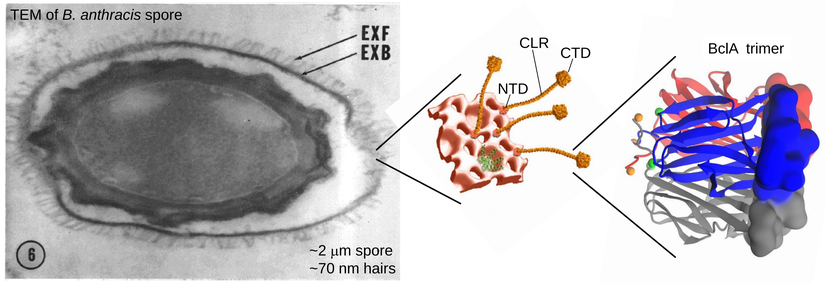
Introduction

Found in:
Bacillus anthracis/thuringiensis/cereus/mycoides
Clostridium botulinum

Plays critical role in spore adhesion in environment and *in vivo* [Turnbull 2008; Faille 2007]
and in dissemination, targeting, and germination [Kailas 2011]

Exosporium: hairs + basal layer

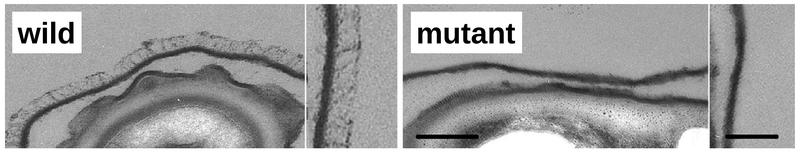


Moberly, J. Bacteriol. 92: 220–228 (1966).
Kailas, PNAS 108:16014-16019 (2011).

EXF = Exosporium Filaments (hairs)
EXB = Exosporium Basal Layer
(cotE glues basal layer to coat)

Threaded from BclA-*Btk* sequence using SwissModel

Lequette, Appl. Environ. Microbiol. 77: 4905–4911 (2011).

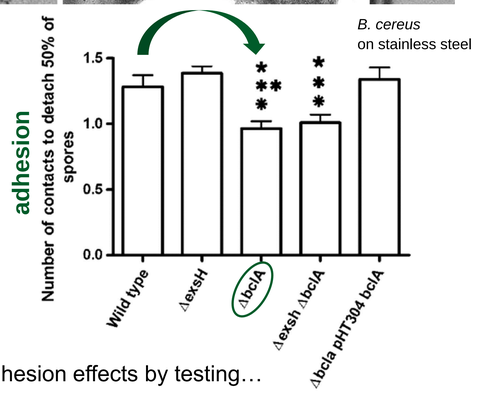


Mutation removed hairs

↓ adhesion

Flexibility of exosporium helps increase surface contact area, promoting adhesion, but in-depth study lacking

↑ hydrophobicity



B. cereus
on stainless steel

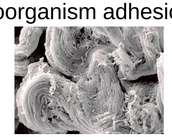
Objectives

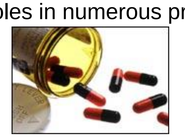
Provide insight into exosporium adhesion effects by testing…

Hypothesis: Orientation of exosporium hairs affects spore adhesion to surfaces
- Hairs (not basal layer) primarily contribute to observed adhesion enhancement
- Changes in environment (hydrophilic vs. phobic) affect hair patterning
- Specific interactions (electrostatic forces and hydrogen-bonding) affect surface area and adhesive force

Guide development of novel coatings/materials for detection, protection, decon

Why? Microorganism adhesion plays important roles in numerous processes





Courtesy of Web

Approved for Public Release

Methods

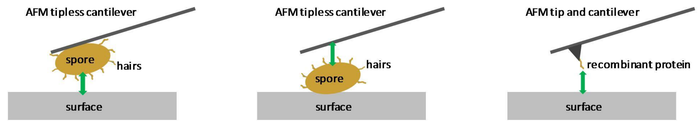
Controls also substrate and RH

hair + basal | basal | no exo

Hairy (*B. thuringiensis*/ΔSterne) spores

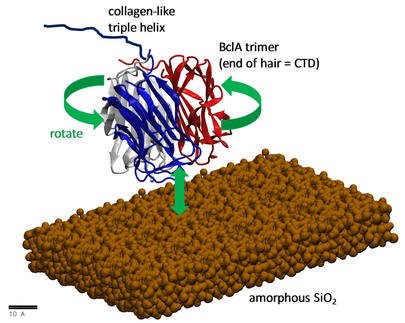
No (*B. globigii*; ΔcotE/ΔbclA mutants; PSL beads)

Orthogonal approaches to explore trends

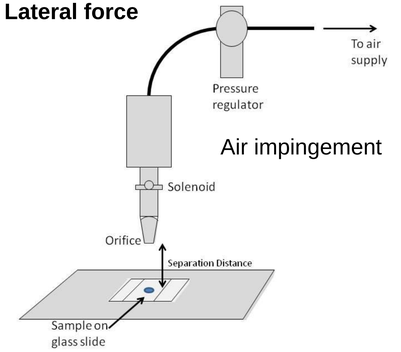


Normal force

Single hair **Modeling**

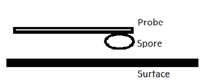


Lateral force

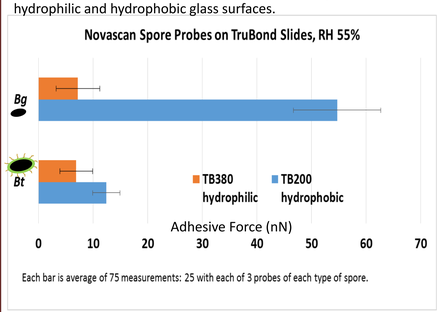


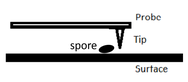
Results

AFM

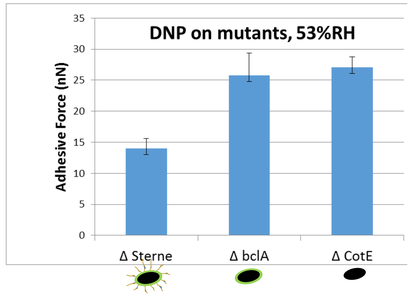


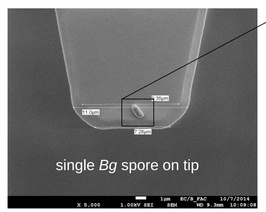
Comparison of ECBC *Bg* and *Bt* spores on Novascan probes on hydrophilic and hydrophobic glass surfaces.

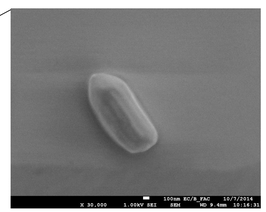




Comparison of *ΔSterne* and mutants' adhesion with standard cantilever probe (DNP D silicon nitride - hydrophilic)





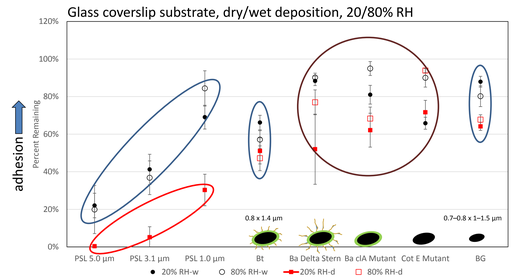


Nonhairy *Bg* spores and mutants adhere more to certain substrates than do hairy *Bt*/*ΔSterne*. Possible contradiction to Lequette's work (*B.cereus* on stainless steel) may be due to specie/substrate differences.

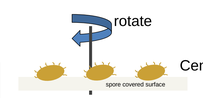
Novascan Technologies spore-probe; nominal spring constant = 0.01 nN/nm (SEM Photomicrographs, JEOL FLV 7001, 1kEV)

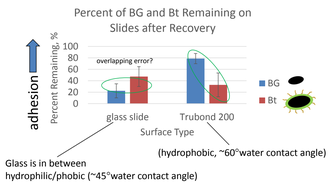
Hairs and basal layer affect stickiness

Aerosol



Vortex





- Nonhairy *Bg* reaerosolizes less (adheres more) than hairy *Bt* (in agreement w/ EPA report) but possible contradiction to Lequette's work may be due to smaller *Bg* spore, short *Bt* hairs, or aerodynamic lift/drag
- Higher humidity (more capillary forces) may increase adhesion of smaller PSL particles
- PSL size vs. adhesion effect (small -> high adhesion due to surface area)

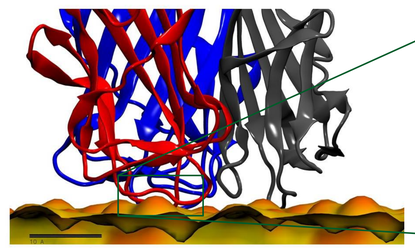
Hairs respond to substrate

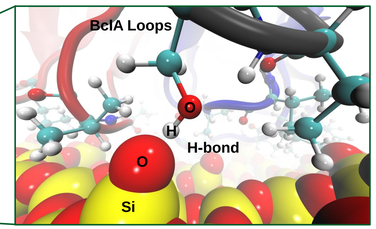
Tip/surface	p < 0.05	F_1, max force, nN	(a) to (b) stretching distance, nm	F_2, last jump force, nN
BclA on Quartz CAW < 10° n = 40	Mean	0.77	32.08	0.60
	St dev	0.12	3.26	0.10
BclA on TB200, CAW = 62.7° ± 2.70° n = 11	Mean	0.91	29.69	0.88
	St dev	0.05	2.92	0.04
BclA on TB380, CAW=33.5° ± 0.77° n = 20	Mean	1.33	30.33	1.16
	St dev	0.31	4.94	0.36

- Curve between (a) and (b) indicates stretching/unfolding of protein/linker couple as protein is pulled from surface.
- Circular dichroism secondary structure study in progress.

Modeling single hair on SiO_2

Loops interact with surface contour





scale bar = 1 nm

Conclusions

- Nonhairy *Bg* has stronger adhesion than hairy *Bt* on hydrophobic surfaces (confirmed by AFM, vortex, and aerosol experiments), in agreement w/ EPA SPORE report. This may refute part of our hypothesis (hairs enhance adhesion), but specie differences (size, hydrophobicity, and short hair issues) may further complicate comparison.
- Surface type (hydrophobic/philic) affects adhesion of all spore types (confirmed by AFM and vortex experiments). This may support part of our hypothesis (hairs respond to different environments and moderate adhesion).
- *ΔSterne* spore and *ΔbclA*/*ΔcotE* mutant adhesion comparison in aerosol data (on glass coverslips of intermediate hydrophobicity) may suggest basal layer effects on adhesion, in possible contradiction to part of our hypothesis (hair dominates adhesion).
- Humidity (capillary forces) appears to play a minor role in adhesion of *Bg* and *Bt* and a stronger role in that of PSL beads, *ΔSterne* spores, and *ΔbclA*/*ΔcotE* mutants.
- MD simulations have observed H-bonding between BclA protein (hair) and SiO2 glass substrate. This may explain how hairs respond to different environments, in support of hypothesis and our previous spore peel-off AFM work.

Acknowledgements

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