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Observation of motility machinery by quick-freeze deep-etch and replica electron microscopy

急速凍結レプリカ電子顕微鏡法が明らかにする運動マシナリー

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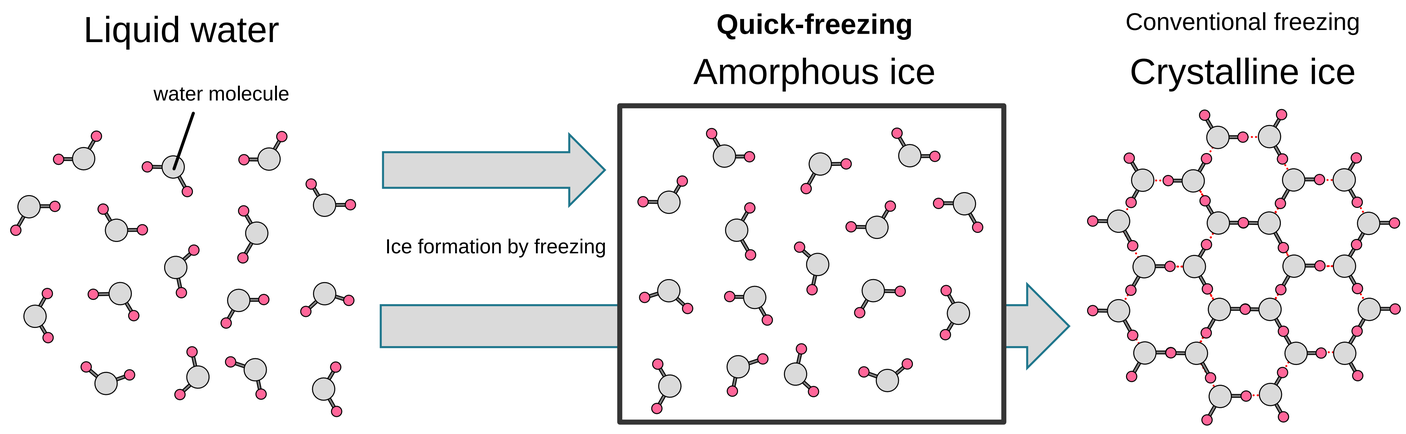
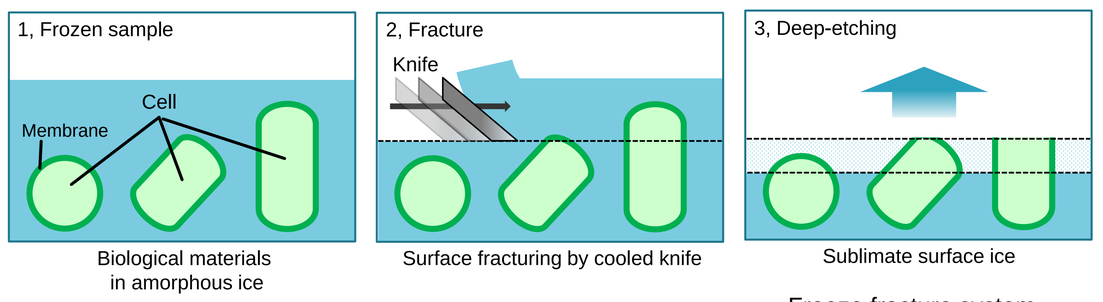
QFDE (quick-freeze deep-etch and replica)

Quick-Freezing of water



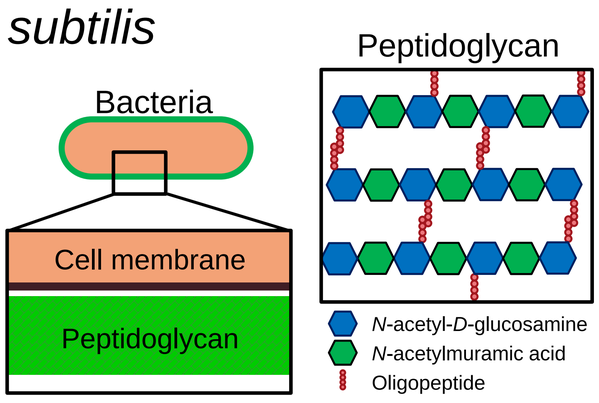
Deep-etch replication
side view

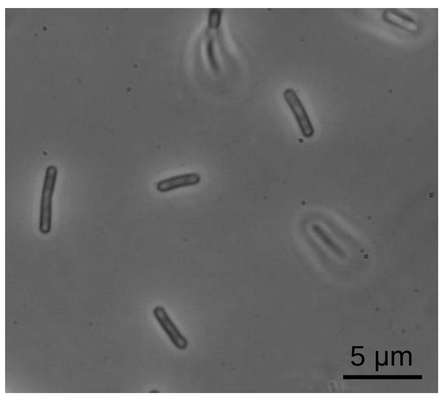


Freeze fracture system

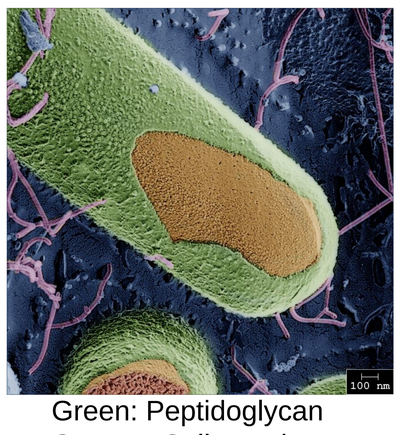
Visualizing PG layer of *Bacillus subtilis*

Peptidoglycan (PG) is the cell wall of bacteria, in which linear glycan strands composed of linked amino-sugar disaccharides are cross-linked by short elastic peptide stems. PG is essential for the cell shape maintainance and resistance to turgor pressure, and therefore its synthesis is tightly coupled with the cell reproduction cycle. Many antibiotics target to this PG synthetic process. The PG structure has been shown as filamentous network on cell surface by scanning electron microscopy (SEM), but high resolution images have not been provided.

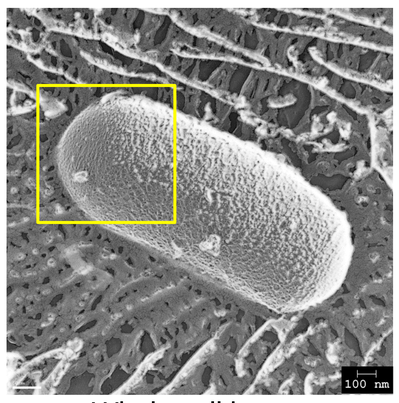




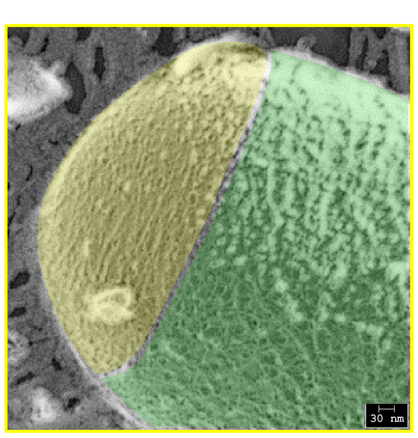
168CA strain (WT)
Grow in LB media



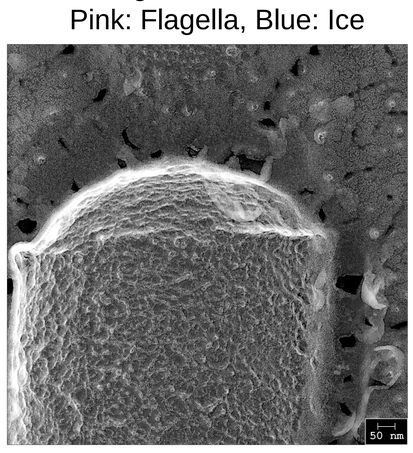
Green: Peptidoglycan
Orange: Cell membrane
Pink: Flagella, Blue: Ice



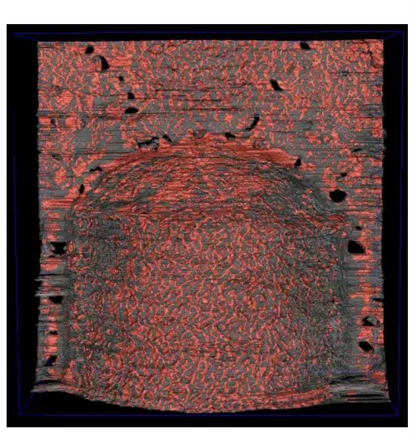
Whole cell image



Magnified image
Yellow: concentric circle pattern
Green: meshwork pattern

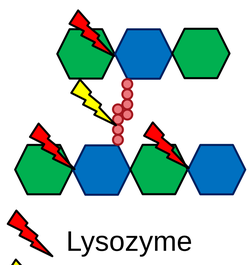


3D reconstruction

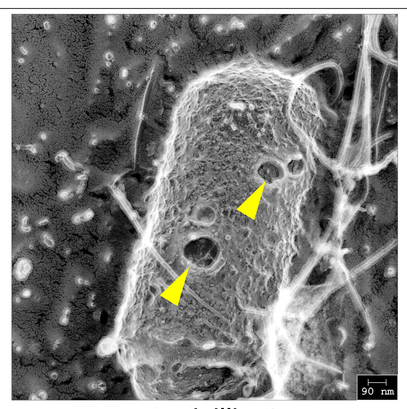


3-D Image of Quick-Freeze Replica Specimen
Prepared by Our New Algorithm
(Baba & Katayama, 1996)
[Topography-Based-Reconstruction;Hitachi High-Tech]

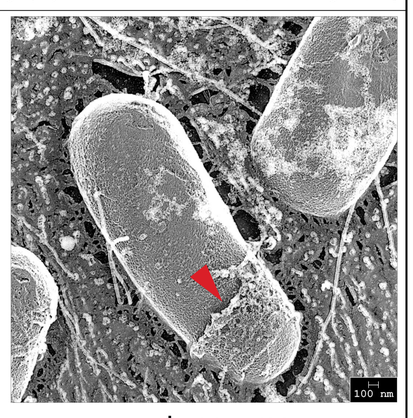
Peptidoglycan disruption



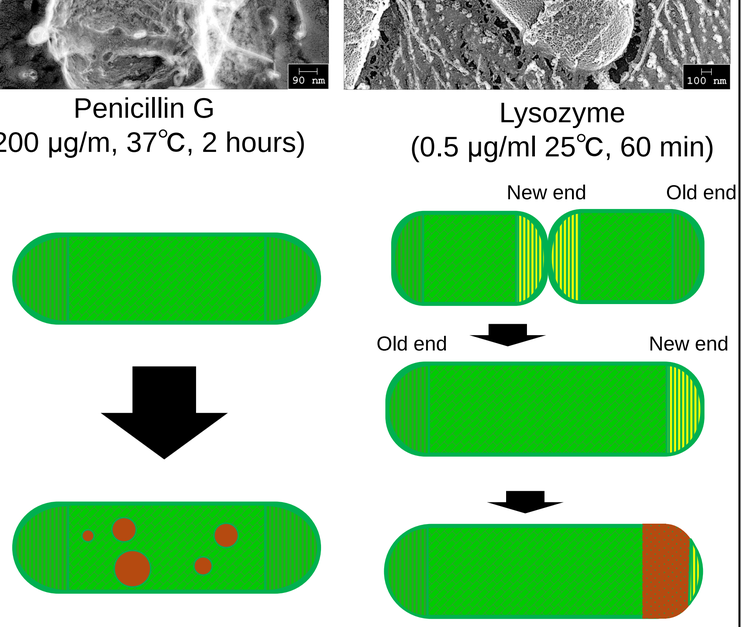
- Lysozyme hydrolyze glycoside bond in peptidoglycan.
- Penicillin G inhibits crosslinking of oligopeptide in PG.



Penicillin G
(200 μg/m, 37℃, 2 hours)



Lysozyme
(0.5 μg/ml 25℃, 60 min)



Conclusions

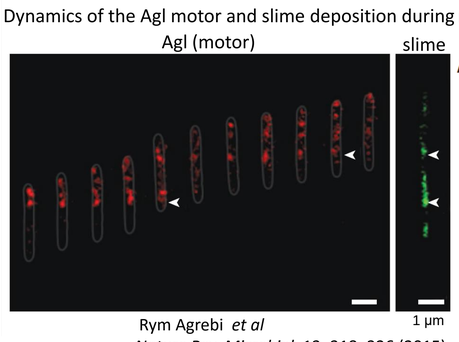
1. Site of cell division had concentric circle pattern and body part had meshwork pattern.
2. Penicillin G made hole on body part.
3. Lysozyme digested from new end.

Visualizing focal adhesion of *Myxococcus xanthus* by sapphire-disc freezing

Motility of *M. xanthus*

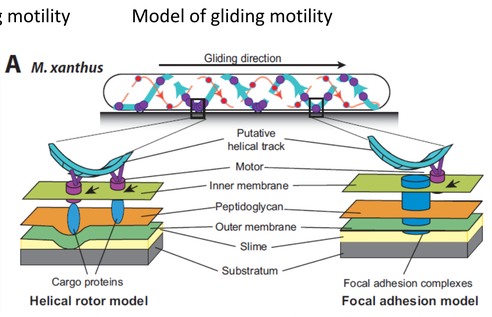
S motility: pili
A motility: gliding motility
crucial motility proteins accumulate at periodic sites along the cell body, forming 'focal adhesion complexes' at the interface between the cell body and the underlying surface.

Dynamics of the Agl motor and slime deposition during motility

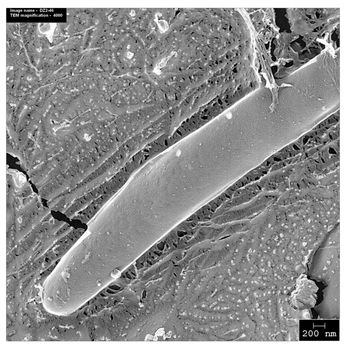


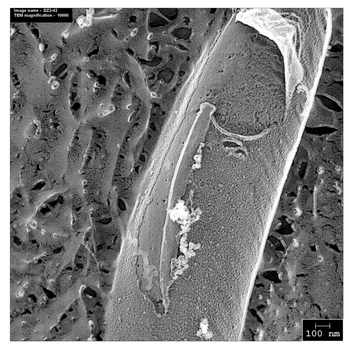
Rym Agrebi *et al*
Nature Rev. Microbiol. 13, 318–326 (2015)

Model of gliding motility



Nan B, Zusman DR.
Mol Microbiol. 30 (2016)

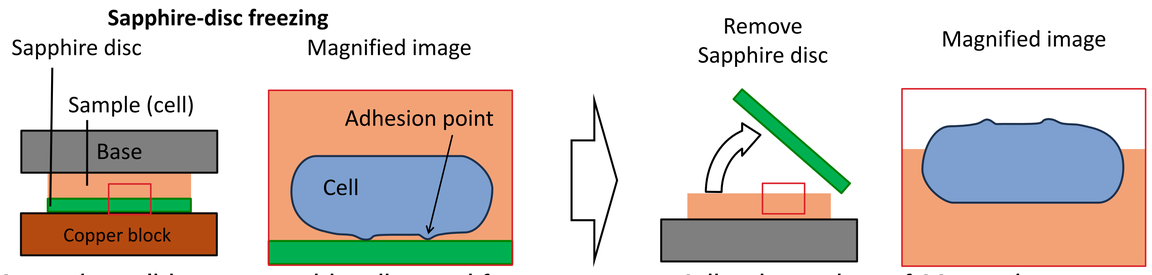




Knife-fractured sample

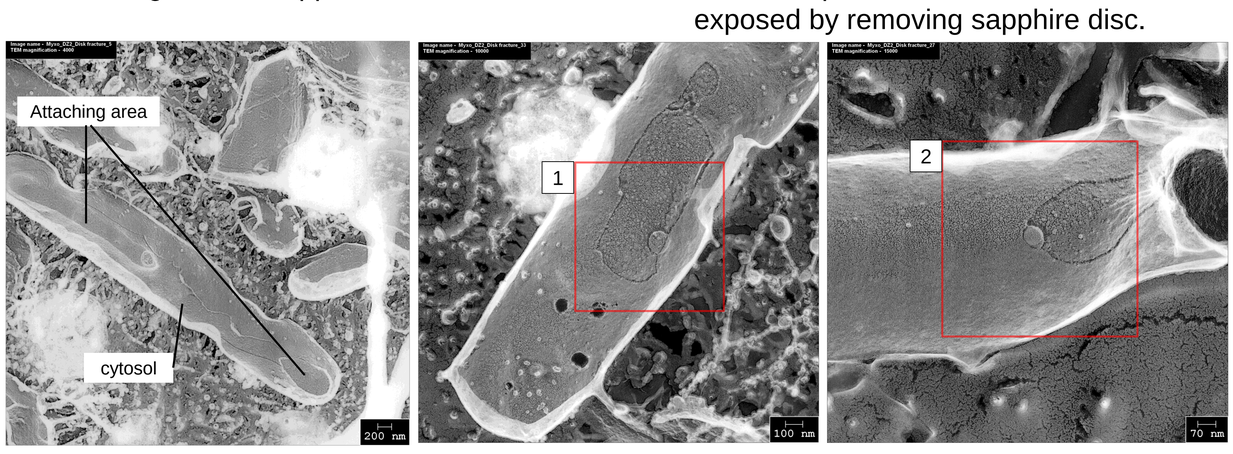
- No structure on sample surface and under the outer membrane
- Adhesion complex aggregate solid surface, so observe from "backside view"
- We apply "sapphire-disc freezing"

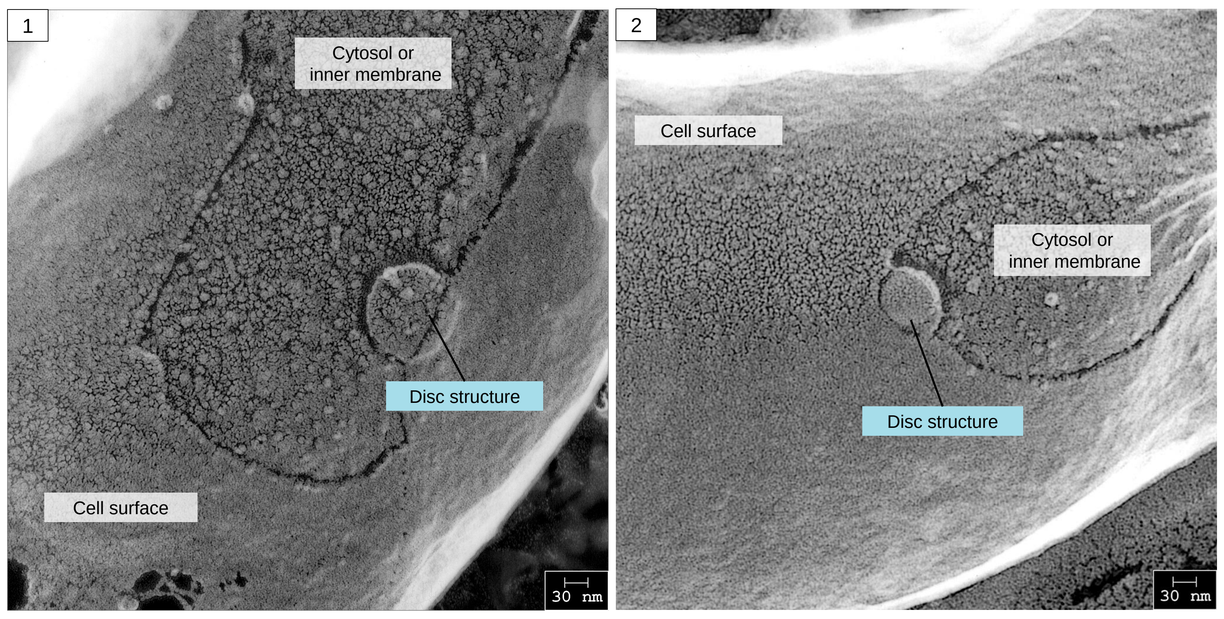
Sapphire-disc freezing



M. xanthus glides on sapphire disc and frozen.

Adhesion points of *M. xanthus* were exposed by removing sapphire disc.





- *M. xanthus* has disc structure under membrane.

大阪市立大学での実験について

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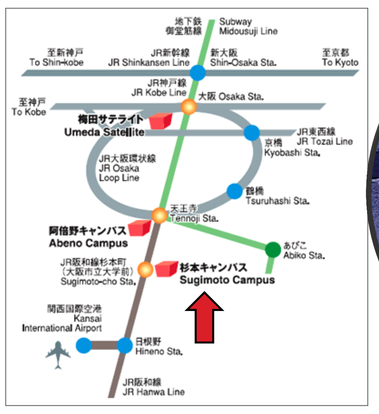

アクセス

Smile of *M. xanthus*