Bacterial cell death

Passive and unregulated or
highly regulated?

antimicrobials

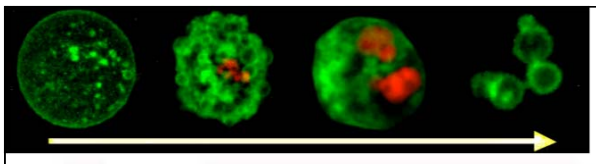
- Wide array of compounds directed against other bacteria:

- Classical antibiotics, typically broad killing spectrum

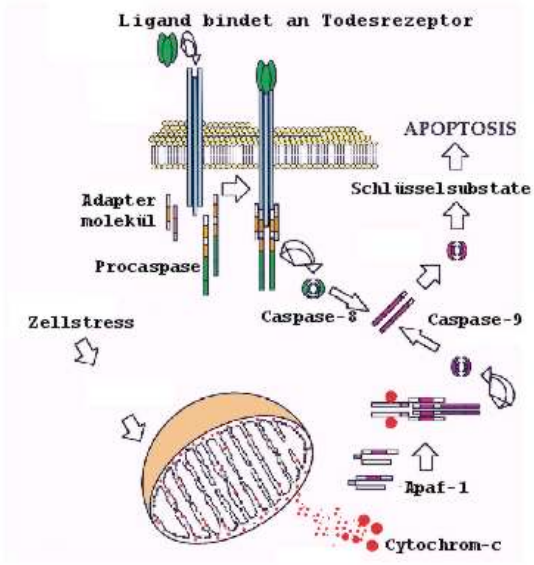
- Bacteriocins, protein exotoxins and murein hydrolases: only affect bacteria that are related to the producer strain.

Bakterieller Zelltod

- Konsequenz von nicht-balanciertem Wachstum am Ende des Lebenszyklus?
- Passiert, nachdem alles wirklich interessante physiologische in der Zelle abgelaufen ist?



Apoptosis



Changing paradigm..

Cell death in bacteria

- Highly regulated
- Role in
  - Developmental processes (competence and biofilm development, sporulation)
  - Elimination of damaged cells

- Analogous to PCD (programmed cell death, i.e. apoptosis) in eukaryotes

Literatur

Molecular control of bacterial death and lysis.Rice KC, Bayles KW.Microbiol Mol Biol Rev. 2008 Mar;72(1):85-109, table of contents. Review

Cannibalism and fratricide: mechanisms and raisons d'être.Claverys JP, Håvarstein LS.Nat Rev Microbiol. 2007 Mar;5(3):219-29. Review.

Autolyse

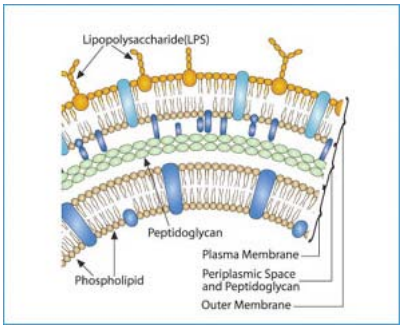
- Ist Temperatur-empfindlich
- Wird induziert durch

  Sauerstoff,

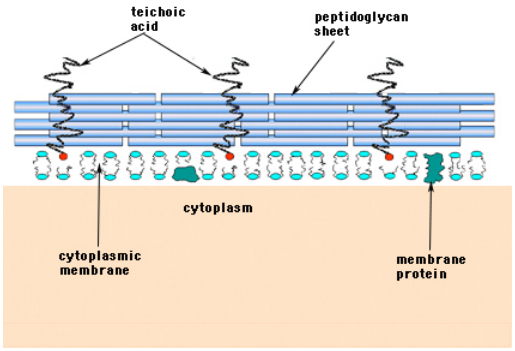
  Glukoseüberschuss,

  Stickstoffmangel
- Weitere Faktoren, die Autolyse beeinflussen: NaCl, pH, Wachstumsphase, Proteasen, Teichonsäuren

Bakterielle Zellwände

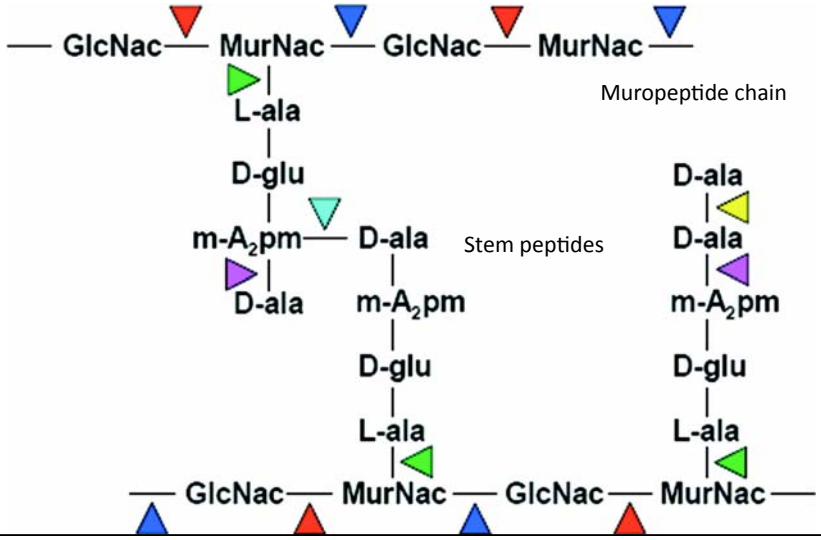


Gram -

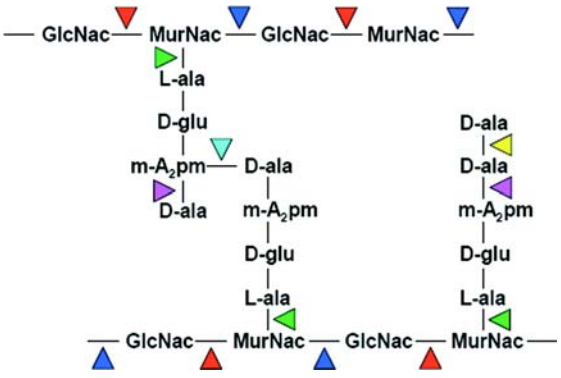


Gram +

Murein Hydrolasen



Murein Hydrolasen



β-N-acetylglucosaminidase (red),
lytic transglycosylase (blue),
N-acetylmuramyl-L-alanine-amidase (green),
D,D-carboxypeptidase (yellow)
L,D-carboxypeptidase (pink)

Funktion von Mureinhydrolasen ist assoziiert mit:

- Zellwandbildung (mit PBPs)
- Peptidoglycan Recycling und turnover
- Trennung der Tochterzellen

- Pathogenität von Bakterien
- Antibiotika-sensitivität

Mureinhydrolasen

...deren Aktivität zur Zerstörung der Zellwand und damit zur Lyse der Zelle führen:

heißen **Autolysine**

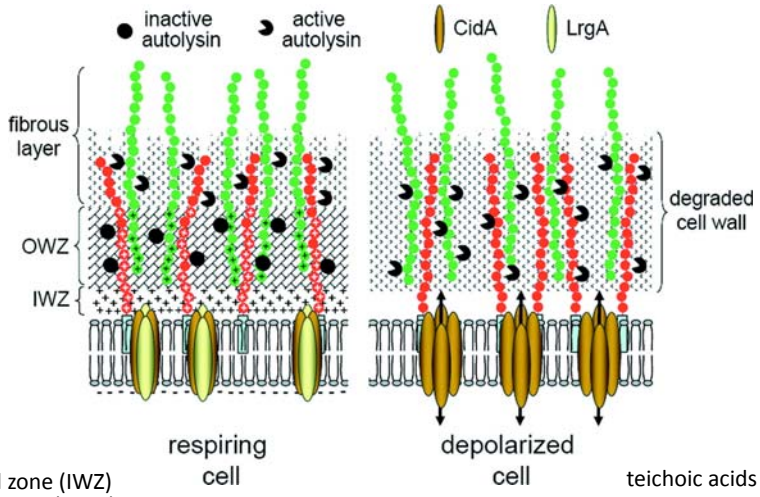
Murein Hydrolasen

Regulation in gram- Bakterien:

Posttranslationale regulatorische Mechanismen
Sequestierung in Lipidmembran
Kontrollierter Transport über die Zytoplasmamembran
Topografische Kontrolle im Peptidoglycan

Details unverstanden

Model for the control of murein hydrolase activity in gram+



inner wall zone (IWZ)
outer wall zone (OWZ)

teichoic acids (green)
lipoteichoic acids (red)

Regulation in gram+

- respiring cell produces a proton gradient outside of the cytoplasmic membrane
- causes a localized reduction in the pH within the cell wall
- acidic pH suppresses the activity of murein hydrolases associated with the teichoic acids (green) and lipoteichoic acids (red) by promoting the protonation of the D-Ala ester linkages (+ signs in figure).
- Upon dissipation of the membrane potential, the pH in the cell wall increases and destabilizes or deprotonates the D-Ala ester linkages, thus derepressing the murein hydrolases.

REGULATED DEATH AND LYSIS

- Best studied system:

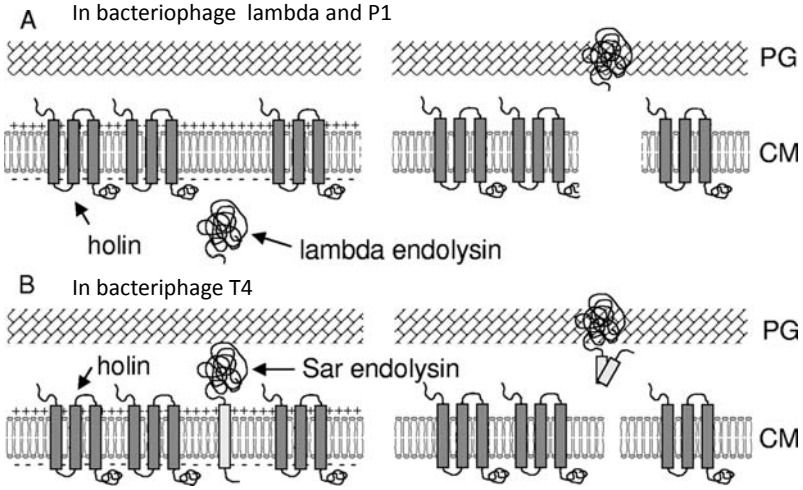
Lysis by bacteriophages: bacteriophage holins

Involves holin and endolysin =murein hydrolase

Precisely timed and rapid cell lysis

Holin controls activity of endolysin

2 Models of holin function



2 different Lysis mechanisms

Mechanism 1:

- Transport across the membrane:
- Mureinhydrolases accumulate in cytoplasm, oligomerization in the membrane, membrane permeabilization, passive cross

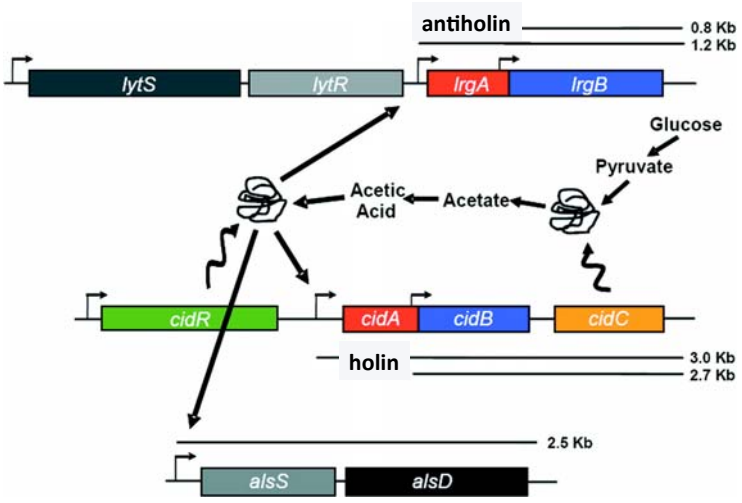
Mechanism 2:

- Murein hydrolases encoded by bacteriophage
- Have signal-arrest-release (SAR) domains
- SAR travers protein to SEC machinery
- Protein anchored to the outside of the membrane, until holin releases it

The holin clock

- Holin
- antiholin (with +charged N-terminus to the inner side)
- antiholin becomes inactive when it is flipped to the outside due to change in membrane potential

CidR-mediated regulation in S. aureus



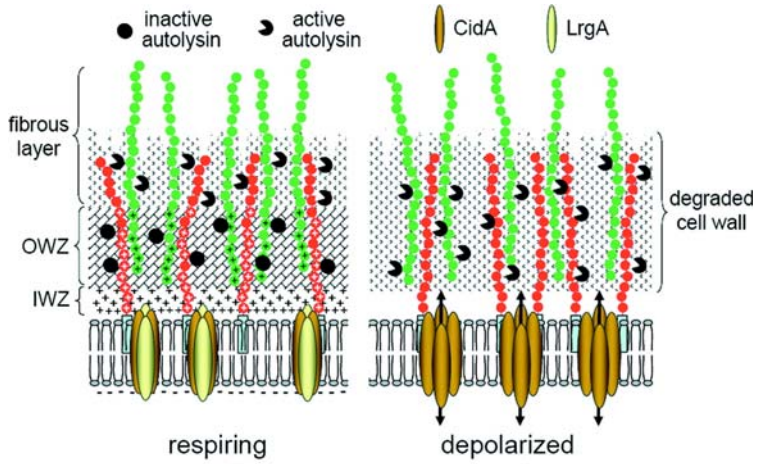
Cid/Lrg Regulatory System S. aureus

- cidA and lrgA genes encode homologous hydrophobic proteins

= holin and an antiholin,

Function of cidB and lrgB genes is unknown

Model for the control of murein hydrolase activity in gram+



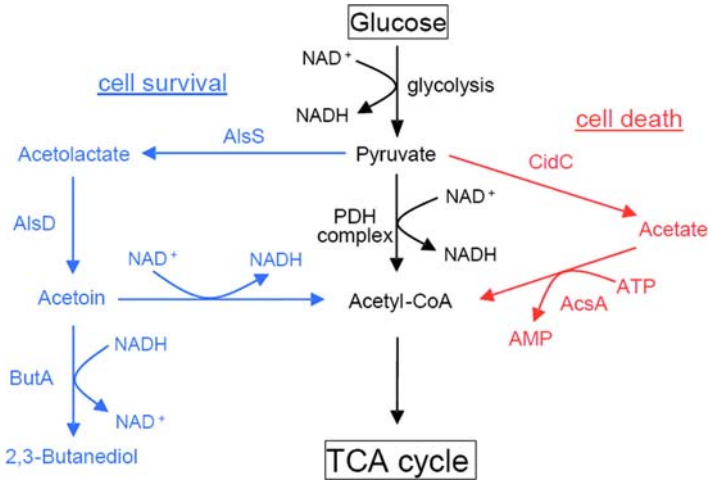
inner wall zone (IWZ)
outer wall zone (OWZ)

teichoic acids (green)
lipoteichoic acids (red)

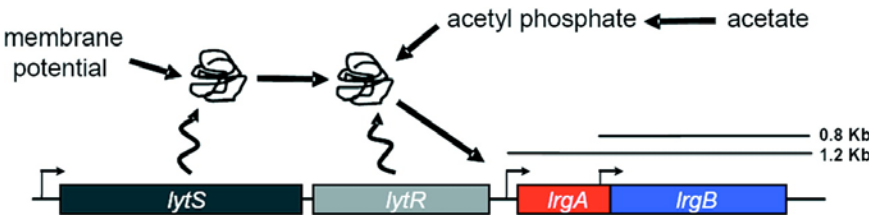
cid – mediated cell lysis plays a significant role during biofilm development

- DNA released of a subset of the bacterial population is an important cohesive component of the biofilm structure.
- cidA mutation caused reduced biofilm formation in animal models of biofilm development
- Altered antibiotic sensitivity
- clinical relevance

Conversion of pyruvate to acetyl-CoA in *S. aureus*



LytSR-mediated regulation



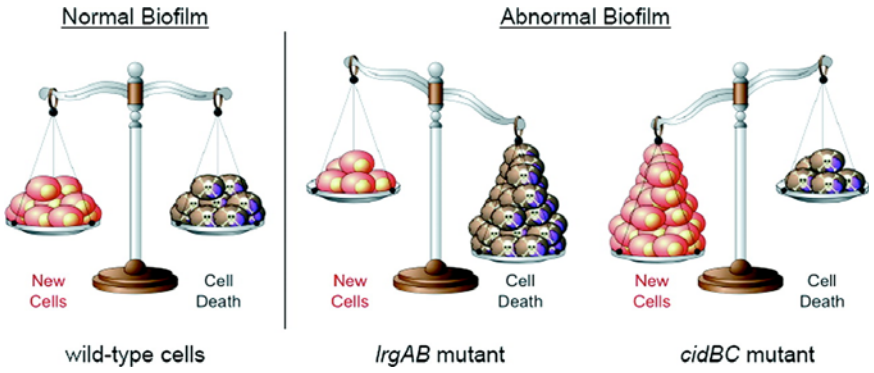
Cid/Lrg regulatory system and eukaryotic apoptosis

- Cid/Lrg: widely conserved in bacteria
- Bax/Bcl-2 widely conserved in eukaryotes
- Bcl-2 (antiholin)-like protein, Bax: holin
- Like holins, Bax can cause membrane permeabilization in a process requiring protein oligomerization
- Release of cytochrome C, but also depolarization of mitochondrial membrane?
- Some bacteria have caspases

Consequences of cell death/delayed cell death

- Antibiotic treatment activates lrgAB, causes upregulation of antiholin,
- Delayed death: gives time to accumulate mutations? (for resistance or survival)
- Similar to tumor cells, that accumulate mutations (c-myc etc)
- defense against virus infection (as in eukaryotes), prevent infection of neighbours
- lytS/R senses infection (via membrane potential)
- Role of carbohydrates

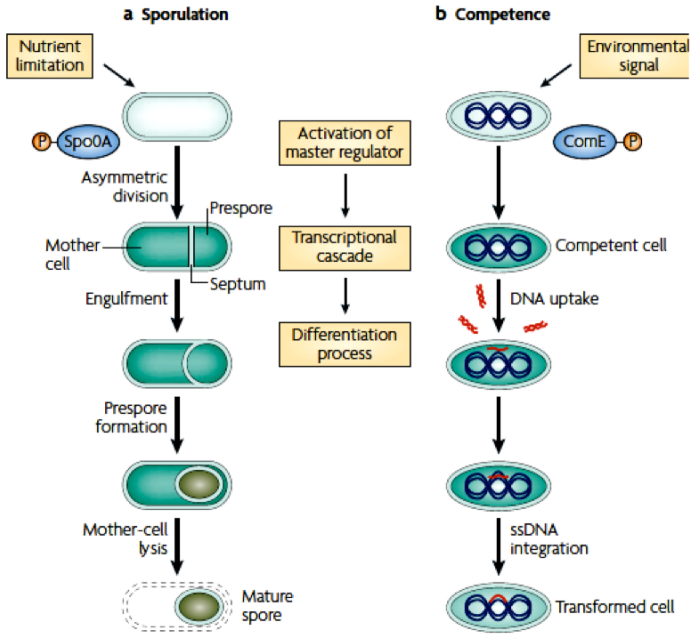
Balance between life and death within a biofilm



- Altruistic cell suicide (biofilms)
- Fratricide: certain cells kill other sibling cells

  During competence development in S. pneumoniae
- Cannibalism

  B. subtilis during sporulation



Why fratricide?

- Streptococcus pneumoniae:
- Induced in rich media, early logarithmic growth phase
- Nasopharynx normally nutrient-limited
- Signal Host-bacterium interactions perturbed, host defense might start
- Aerobic metabolism induces reactive oxygen species.

= stress response

Competence and Fratricide

- S. pneumoniae cells
- Competence regulated by ComDE two-componenent regulatory system.
- ComD hiskinase senses the accumulation of a peptide pheromone called competence-stimulating peptide (CSP) (encoded by comC)
- When accumulated to a treshold level, CSP triggers autophosphorylation of ComD
- ComD phosphorylates ComE
- ComE upregulates early com genes, including sigma factor X
- Factor x induces late com genes, for DNA binding and uptake and recombination.
- Where does the free DNA come from?
- Fratricide (=heterolysis/allolysis) cells that are killed by others (intraspecies killing)

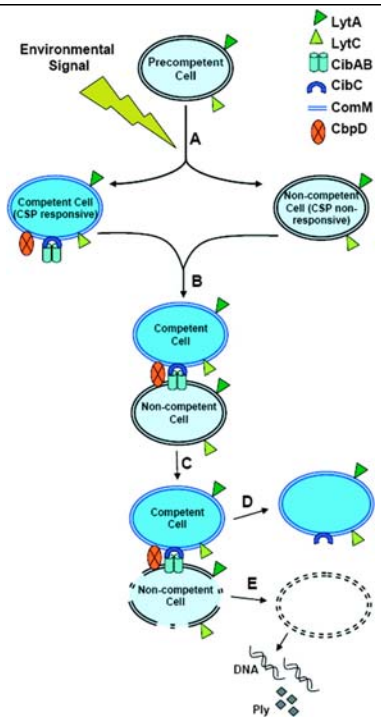
4 killing factors
lytA: cell wall associated murein hydrolase (comDE regulated)

lytC: murein hydrolase

CbpD: novel type of murein hydrolase (choline binding protein)

cipAB: two-peptide bacteriocin (kills first the cell), then lysis

Immunity:
cibC: protector protein: immunity to CibAB in competent cells
comM: early immunity



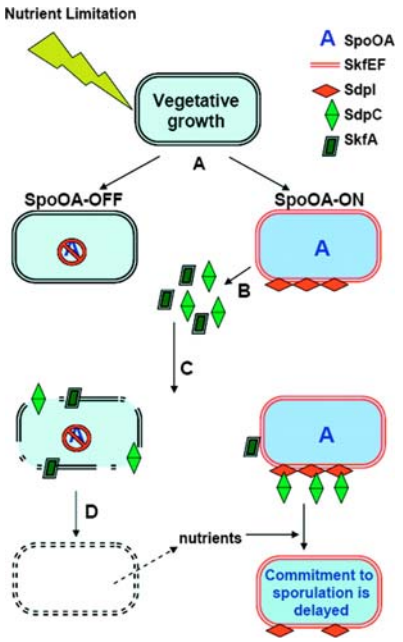
Which cells become competent and which cells are killed?

- Unclear,
- However likely a bistable regulatory switch as in comK (Bacillus)

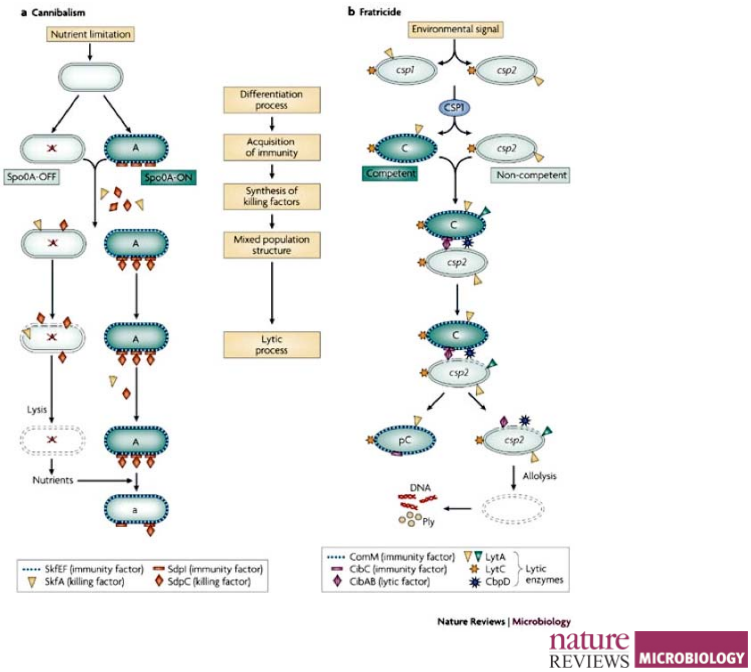
Cannibalism

- B. subtilis sporulation
- Last-resort response to nutritional stress
- Also triggered by cell density and cellular redox state.
- Master regulator SpoOA
- Only few cells initiate sporulation
- Phosphorylated state of SpoOA : bistable switch

Cannibalism during *B. subtilis sporulation*



killing factors SkfA and SdpC
cognate immunity proteins SkfEF and SdpI.



Claverys and Håvarstein *Nature Reviews Microbiology* **5**, 219–229 (March 2007) | doi:10.1038 / nrmicro1613

Comparing fratricide and cannibalism

Similar :

- differentiating cultures
- Induce of system as part of stress-response
- require mixed population
- Two sets of killing factors and immunity devices

Different:

Intrinsic killing and immunity mechanisms

Raisond'etre: provide nutrients versus provide genetic diversity (?)

Comparing fratricide and cannibalism

- Cannibalism: provides the „last supper“ before sporulation
- S. pneumoniae: needs DNA? Contribution to virulence?