

Small colony variants of *Staphylococcus aureus*:  
A challenge for the researcher,  
the clinical microbiologist,  
and the clinician

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# *S. aureus* colonization and infections

- ❖ healthy nasal carriers
- ❖ community and nosocomial acquired infections

## Skin- and soft tissue infections



furuncle



carbuncle



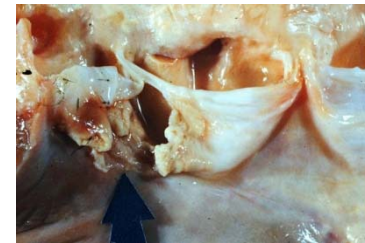
bursitis

## Life-threatening infections

osteomyelitis



endocarditis



pneumonia

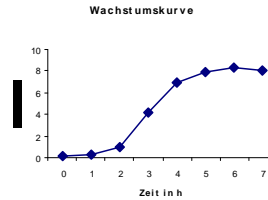


sepsis

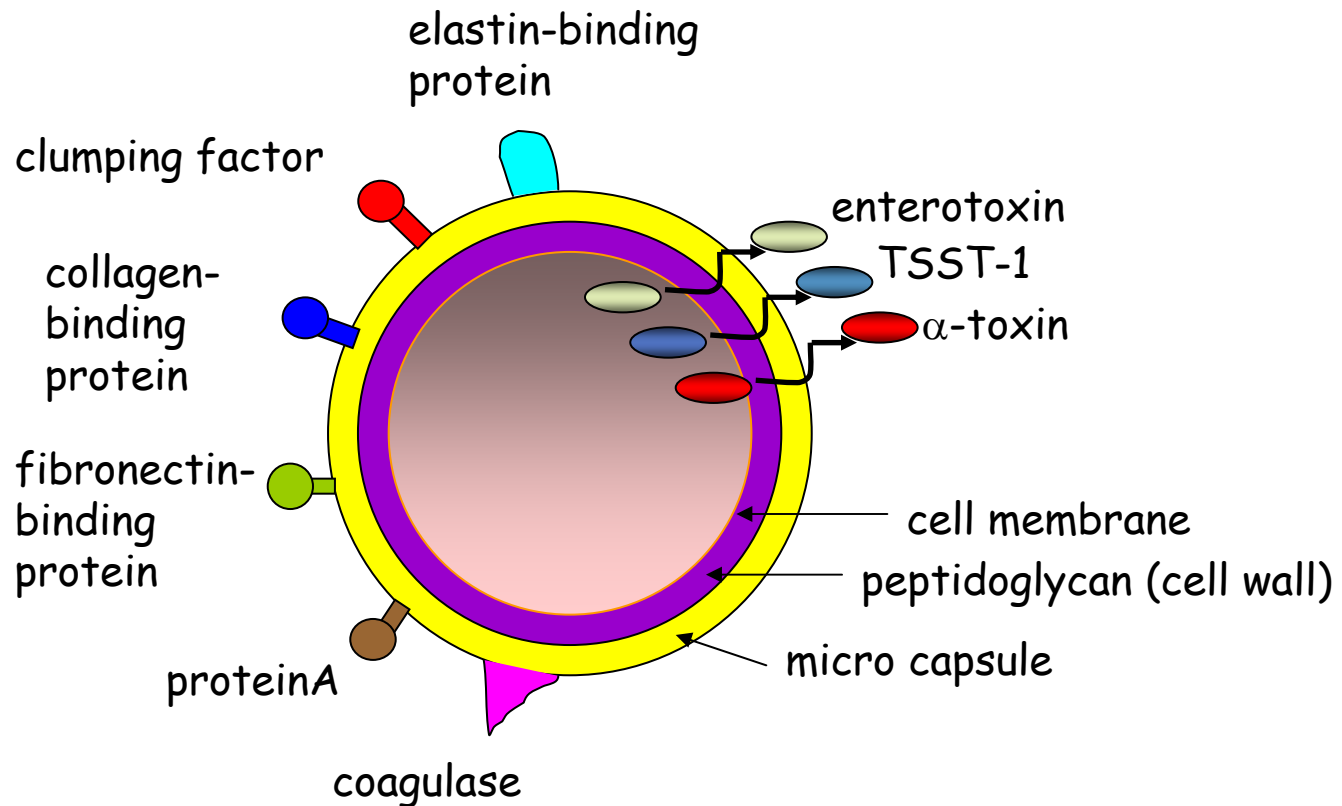


# Virulence factors of *S. aureus*

adhesins  
early log phase

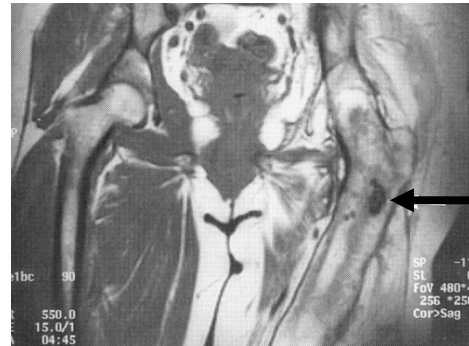
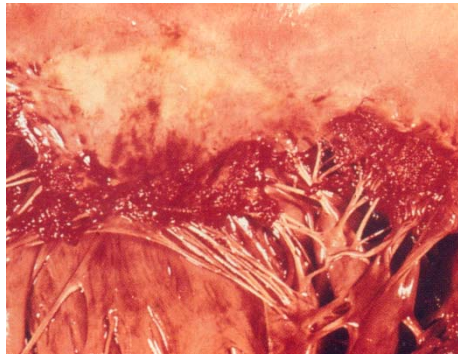


secreted proteins  
late log/stationary phase



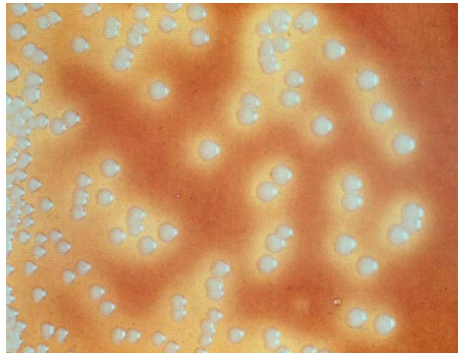
# Acute versus chronic disease

endocarditis



femoral abscess

normal *S. aureus*



small colony  
variant (SCV)  
*S. aureus*

# Small colony variants (SCVs)

- subpopulation of *S. aureus*
- emerge after longterm antibiotic therapy
- associated with persistent, recurrent infections, difficult treatable infections
  - osteomyelitis, device-related infections, cystic fibrosis
- more resistant to antibiotics (aminoglykosides, TMP/SMX,  $\beta$ -lactams)
- persist intracellularly in *in vitro* studies

SCV

normal *S. aureus*

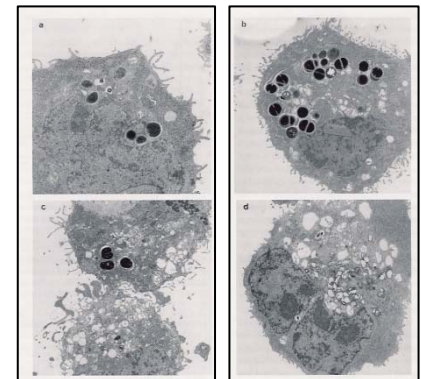


30 min

48 h

*S. aureus*  
SCV

normal  
*S. aureus*



# Various and undetected mechanisms for SCVs occurrence

## mechanisms:

- **hemin- or menadione-dependent** (Proctor, von Eiff, Becker, McNamara, Peters, Lannergard AAC2008; Malouin J Bacteriol, 2006)



# Various and undetected mechanisms for SCVs occurrence

## mechanisms:

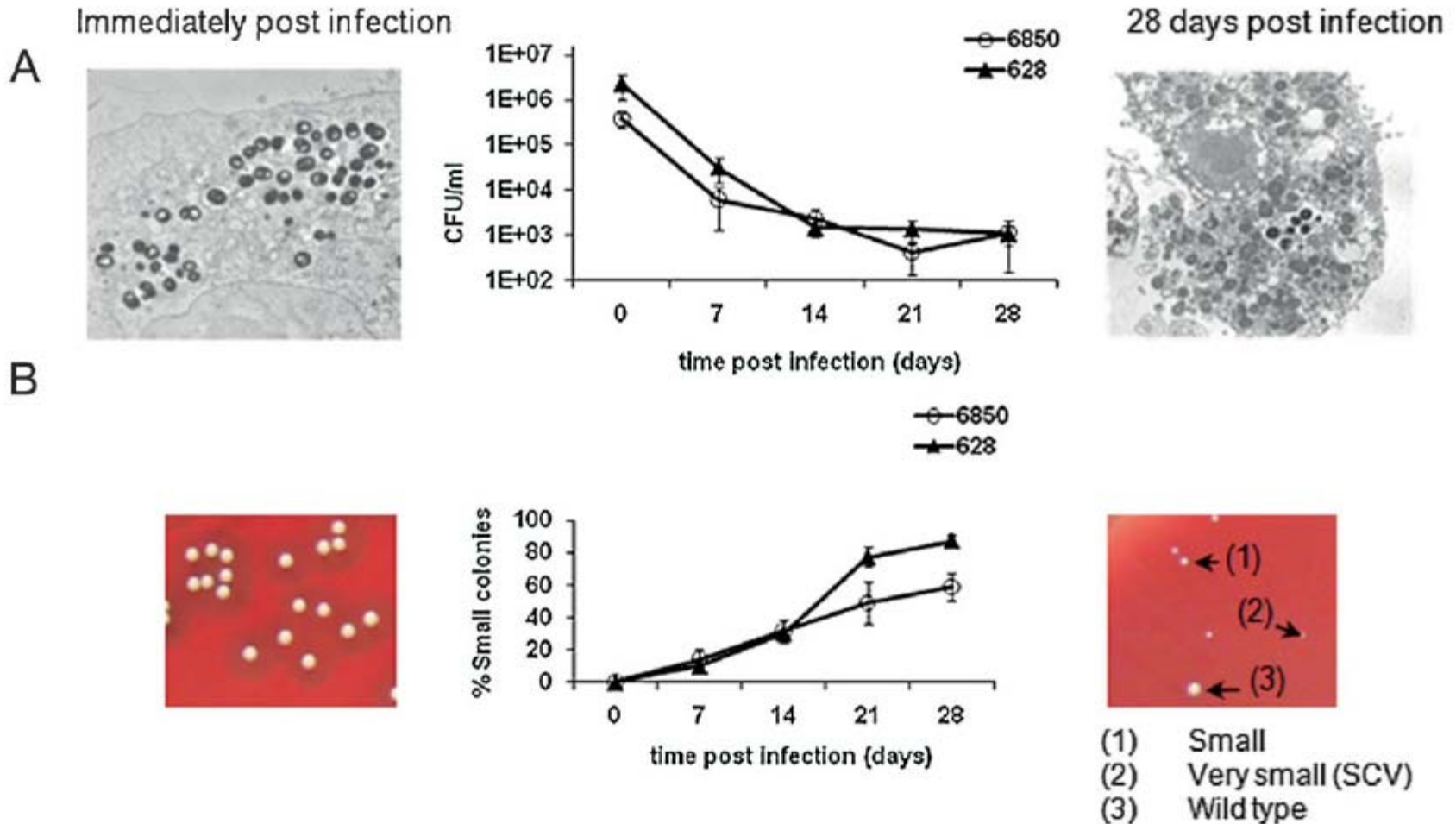
- **hemin- or menadione-dependent** (Proctor, von Eiff, Becker, McNamara, Peters, Lannergard AAC2008; Malouin J Bacteriol, 2006)
- **CO<sub>2</sub>-dependent** (Gomez-Gonzalez, J Clin Microbiol 2010)
- **mutations in stringent stress response genes** (Gao et al. Plos Pathogen 2010)
- **thymidine-dependent** (Gilligan JCM1987, Besier I&I2008, Kahl, JID1998)
- many SCVs with so far unknown underlying mechanism

## SCVs:

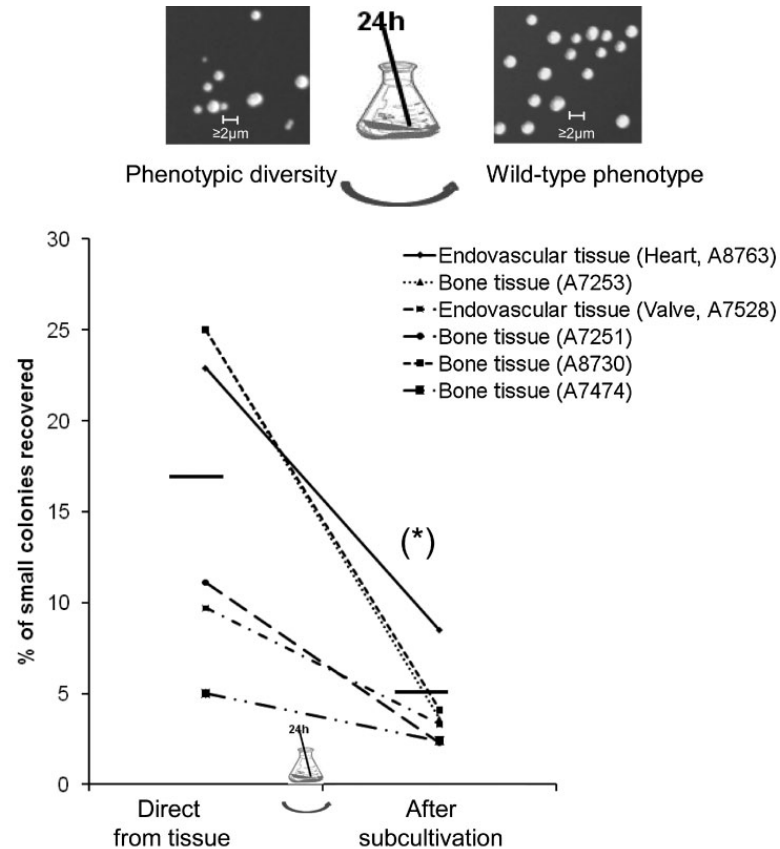
- can **revert** to the normal phenotype within short periods



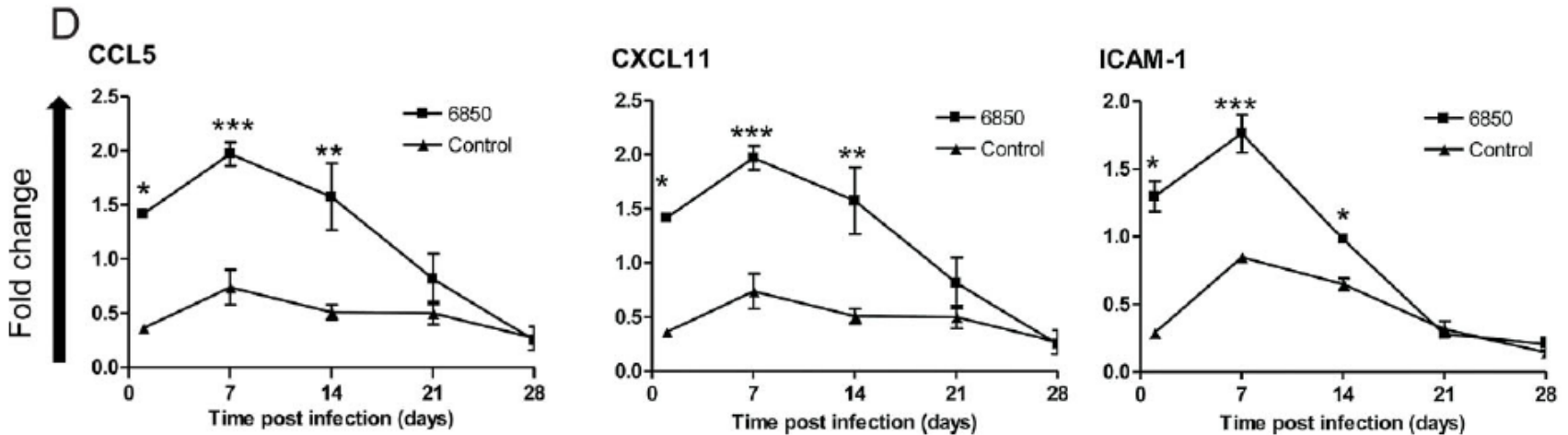
# Intra/extracellular phenotypic switching



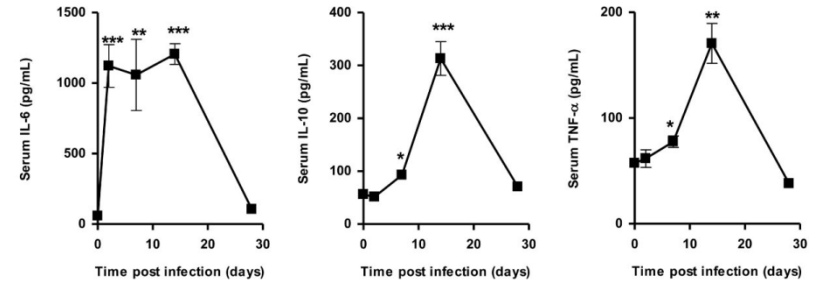
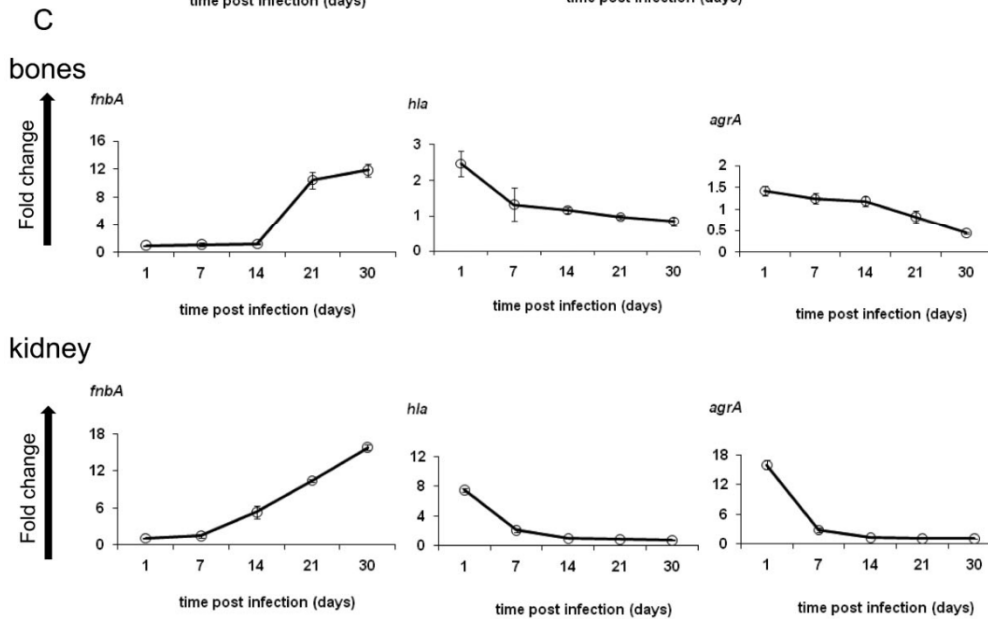
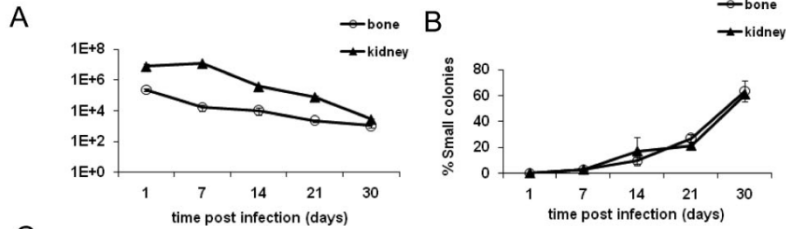
# Primary cultures from clinical specimens



# Altered bacterial gene expression and host cell response



# Chronic infection in mice



**D**

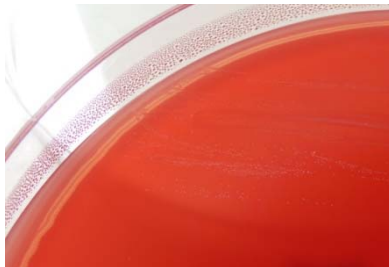
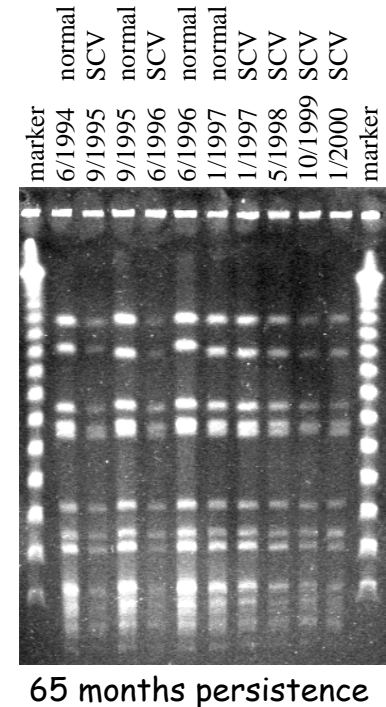
# First conclusions

- Bacterial phenotype switching is an integral part of the infection process, which enable the bacteria to hide inside the host thereby providing a reservoir for chronic infection.

# Thymidine-dependent (TD) SCVs

- ❖ emerge in vivo after treatment with trimethoprim/sulfamethoxazole (TMP/SMX)
- ❖ rely on extracellular thymidine (no growth on Mueller-Hinton Agar)
- ❖ are TMP/SMX resistant
- ❖ survive only in the presence of thymidine
- ❖ in many patients present even when no normal *S. aureus* was cultured
- ❖ persisted after TMP/SMX therapy was stopped (>4 years)
- ❖ induction of TD-SCVs of *S. aureus* Newman after in vitro culture in BHI after TMP/SMX challenge

## Longterm persistence of SCVs



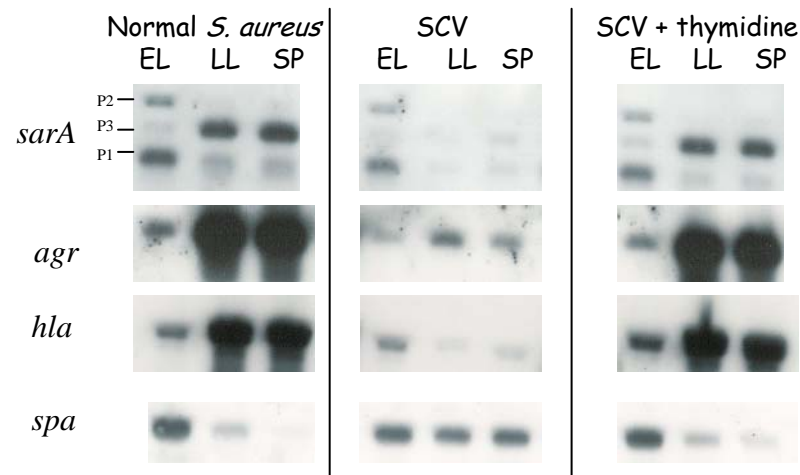
Columbia  
blood agar



Schaedler  
agar  
CO<sub>2</sub>

From Kahl B. C. et al. J. Clin. Microbiol. 2003, 41:410-3;  
Kahl B. C. et al. J. Clin. Microbiol. 2003, 41:4424-7; and unpublished data

# Decreased transcription of *agr* and *hla* in clinical thymidine-dependent (TD) SCVs



low thymidine    low thymidine    high thymidine

⇒ less virulent phenotype  
specialized for persistence

# TD-SCVs occur not only in CF, but also in other infections and in other species

- ❖ 11% SCVs of 3972 isolates from a CF multicenter study (193 patients from 17 centers)  
40% TD-SCVs
- ❖ are reported in other CF-centers in Belgium, US, Germany, Turkey, Czech Republic
- ❖ in other chronic infections:
  - soft tissue infection
  - recurrent abscesses
  - chronic bronchitis
  - septicaemia
  - tympanitis

(Besier S J Clin Microbiol 2008; 46:3829; Seifert H, Emerg Infect Dis 1999; 5:450)
- ❖ not only in humans but also in chronic bovine mastitis (Atalla H VetMicro09)
- ❖ can complicate correct diagnosis of MRSA (Cleeve VJ, Hosp Infect 2006)
- ❖ also reported in other species: *Salmonella*, *Escherichia*



# When to expect TD-SCVs?

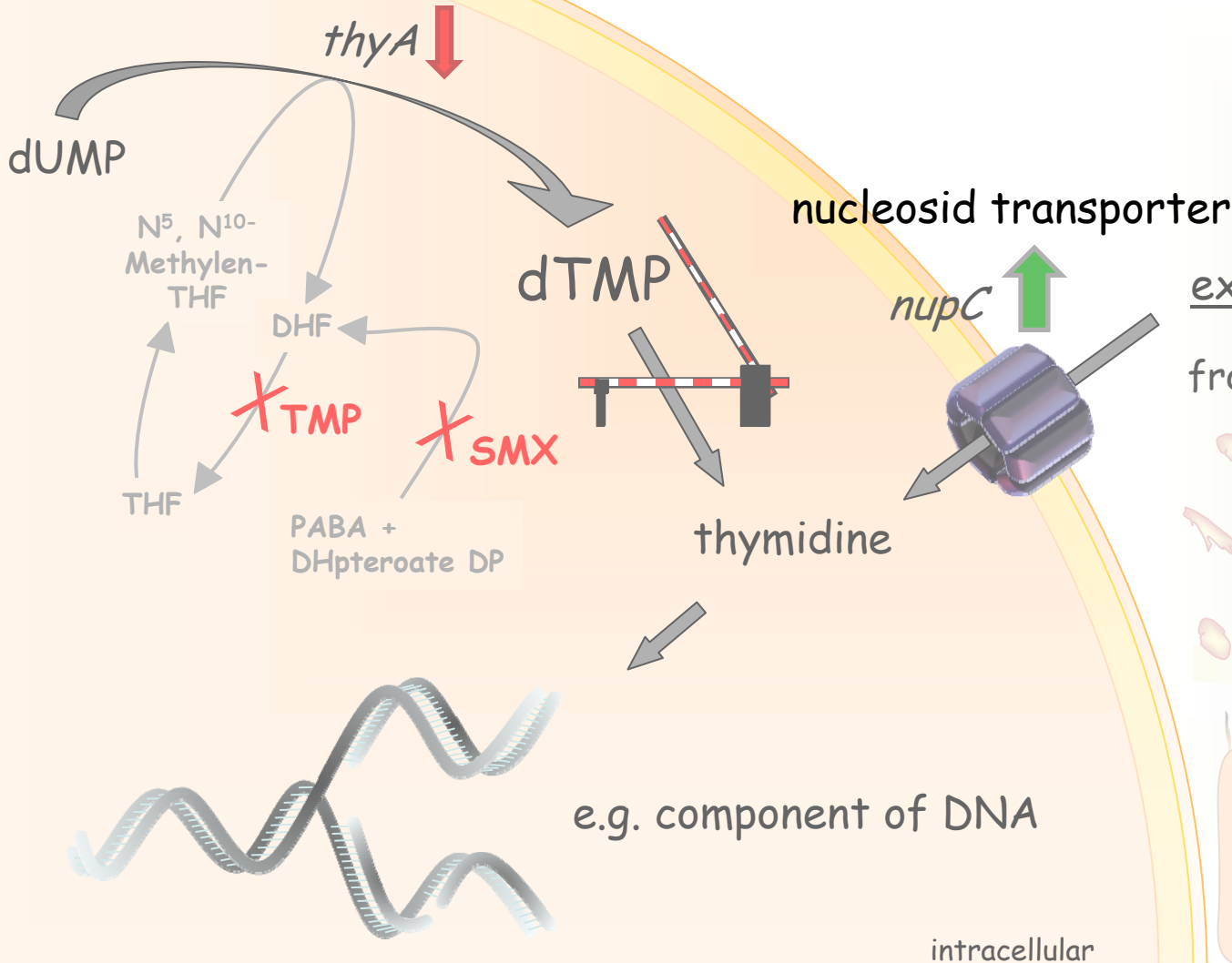
1. *S. aureus* in high density
2. extracellular thymidine
3. treatment with TMP/SMX
4. Due to the rise of CA- and HA-MRSA  
recommendations of the IDSA to treat with TMP/SMX  
critical response (Proctor RA, Clin Infect Dis 2008; 46:584)

## Concentration of thymidine or dTMP in various human specimens\*

CF sputum	346 $\mu\text{g/l}$	34,8 $\mu\text{g/l}$
Pus	nd	18,19 $\mu\text{g/l}$
Urine	540 $\mu\text{g/l}$	1,818 $\mu\text{g/l}$
Liquor	nd	375 $\mu\text{g/l}$

# Model for thymidine-dependency of *S. aureus* SCVs

thymidylate synthase



extracellular thymidine

from respiratory secretions  
in CF lung  
with  
destroyed cells  
and pus

intracellular

extracellular

# Thymidine-dependent SCV expressing *thy* exhibits normal phenotype

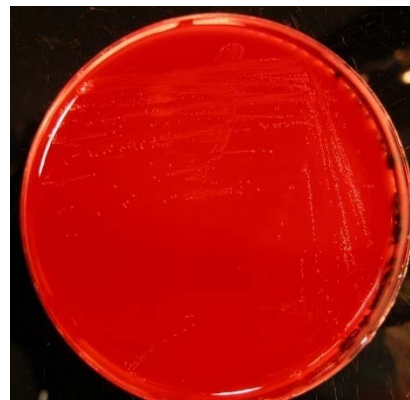
Normal *S. aureus*



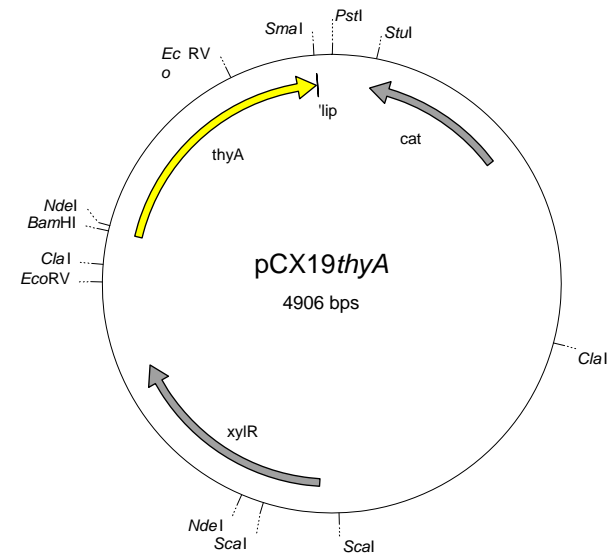
Thymidine-dependent SCV



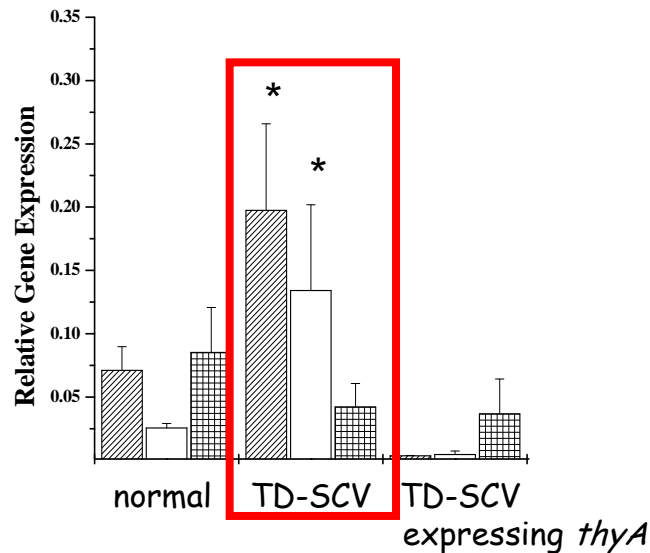
SCV expressing *thy*



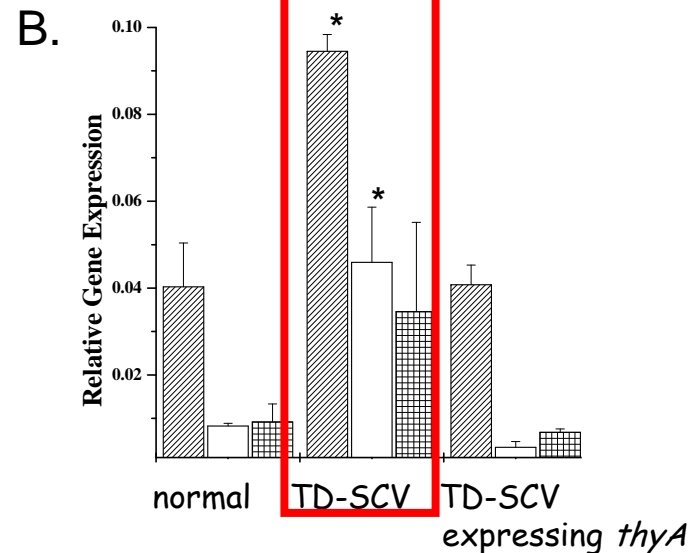
Cured mutant



# Increased transcription of *thyA* and *nupC* in TD-SCVs



*thyA*



*nupC*

- unexpected: increased transcription of *thyA*
- expected: increased transcription of *nupC*

# Conclusions

- For the clinical microbiology laboratory: important to know when TD-SCVs are to be expected and how they look like
- patho-adaptive mechanism lead to a loss of function of thymidylate synthase - an essential protein
- clinical and in vitro data provide evidence that TD-SCVs are optimized for survival in the hostile environment of the lung
- TD-SCVs are attenuated in their virulence
- Intracellular location of bacteria difficult to treat
  - Therefore, the work of defining the cellular pharmacokinetics and -dynamics of antibiotics against these bacteria are of importance.

## Münster

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# Greetings from Münster

