

Název: Molecular aspects of *Staphylococcus aureus* resistant strains

Školitel: Dagmar Chodobová

Datum: 5.4.2013

# *Staphylococcus aureus*

- G<sup>+</sup> bacteria of the genus *Staphylococcus* (Ø 1 µm)
- often cause of infections
- pathogenic - production of toxins
- in 1/3 of the population naturally presented on the skin and mucous membranes
- may be presented in salami, pork, potato salad and ice cream

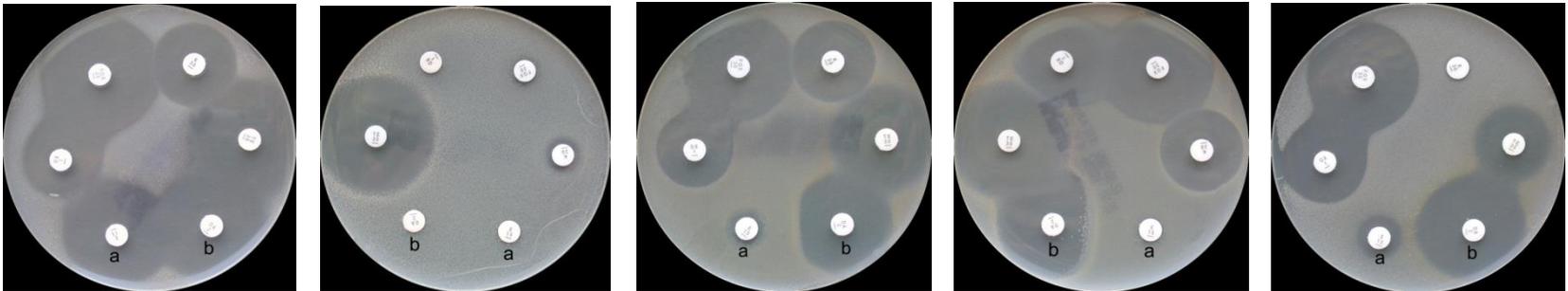
# Treatment and prevention

- localized infections - surgically
- 90 % of staphylococci are resistant to penicillin → use of penicillin, which inhibits beta-lactamase
- macrolides, cephalosporins, aminoglycosides, tetracyclines etc.
- carriage – local antibiotic mupirocin
  
- prevention – vaccines to stimulate specific immunity



# Resistance

- resistance (*from Latin*)
- biological resistance = resistance x environment effects
- resistance genes code genetic information, which can be changed by environmental factors



# Aims of study

- comparison of biochemical markers in resistant (Ag, Cu, Cd, Zn and Pb) and non-resistant *Staphylococcus aureus*
- comparison of DNA changes in resistant (Ag, Cu, Cd, Zn and Pb) and non-resistant *Staphylococcus aureus*

# Preparation of resistant strains of *S. aureus*

- *S.aureus* + 2 mM Ag, Cu, Cd, Zn or Pb
- lowest dose of metal = 50  $\mu$ M
- increase of 50  $\mu$ M of metal to the maximum possible dose for the regeneration of *S.aureus*
- possible to revitalize its resistant strain in medium without the addition of metal

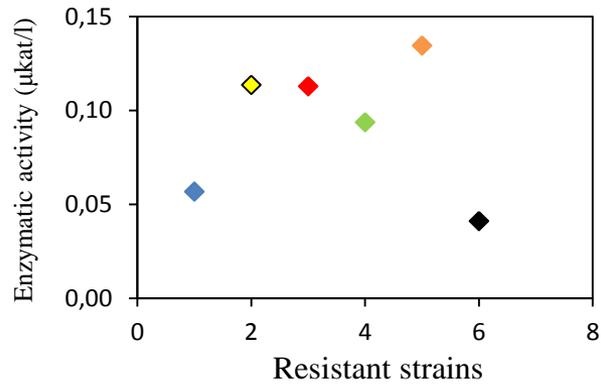
# Measurement

- spectrophotometry – BS 400
- electrochemistry (methalothionein, height of peaks)
- HPLC (GSH, GSSG)
- Multiskan EX (STAPHYtest 24)
- MALDI-TOF
- isolation of DNA (Magna Pure), PCR, gel electrophoresis

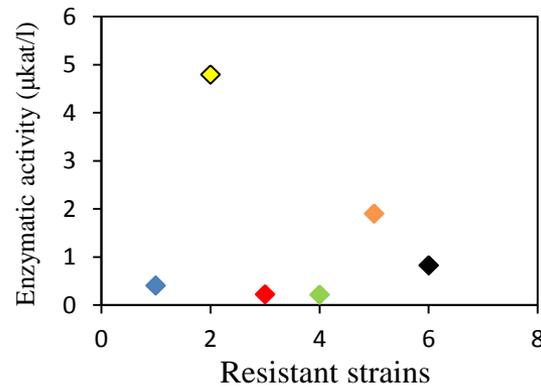
# Spectrophotometry BS 400

◆ S.a. ◆ Ag ◆ Cu ◆ Pb ◆ Zn ◆ Cd

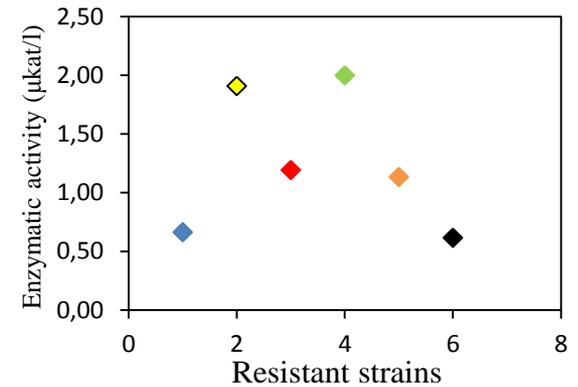
ALT (μkat/l)



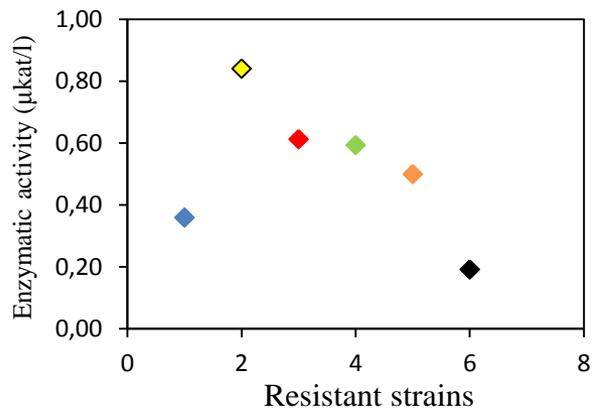
AST (μkat/l)



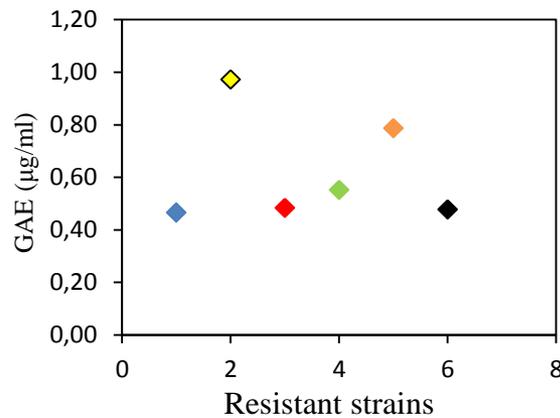
ALP (μkat/l)



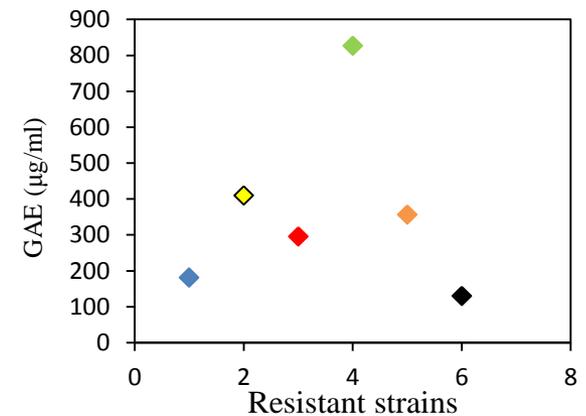
GMT (μkat/l)



FRAP (GAE)

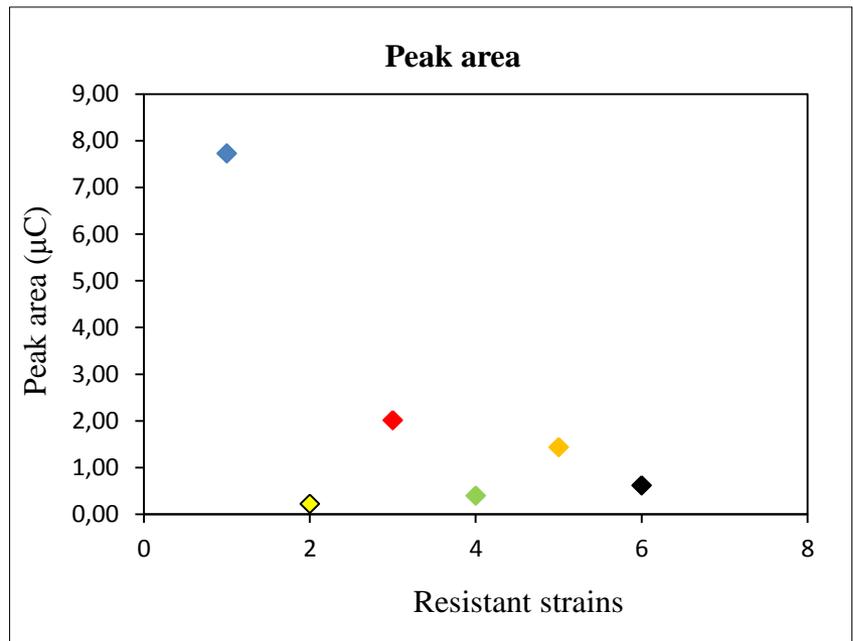
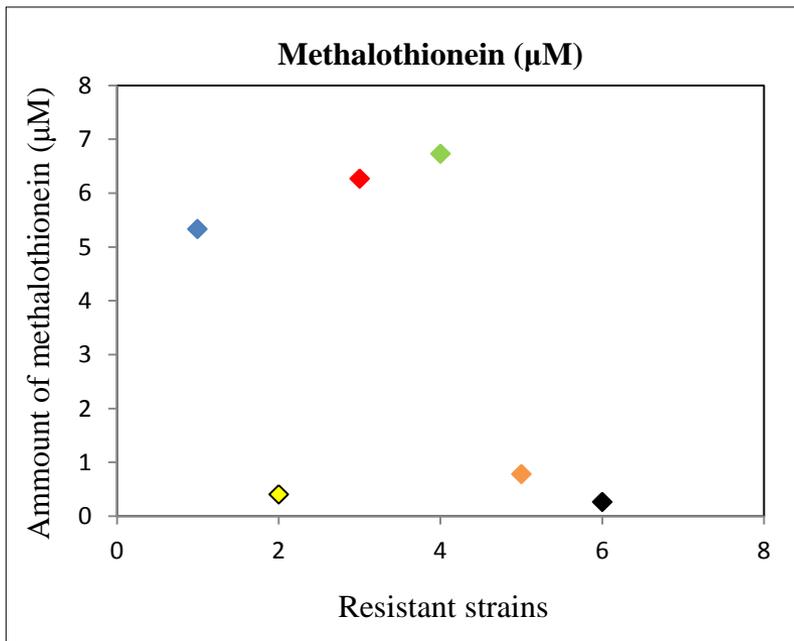


ABTS (GAE)



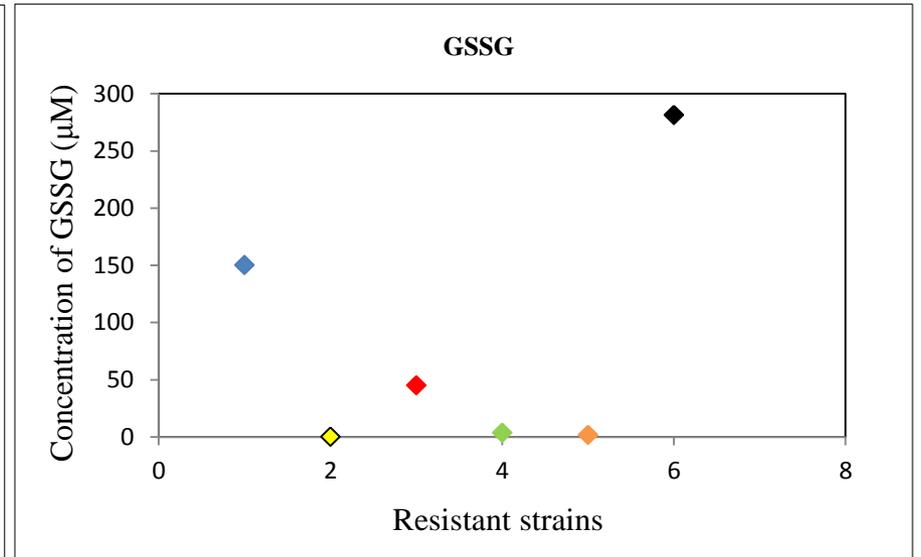
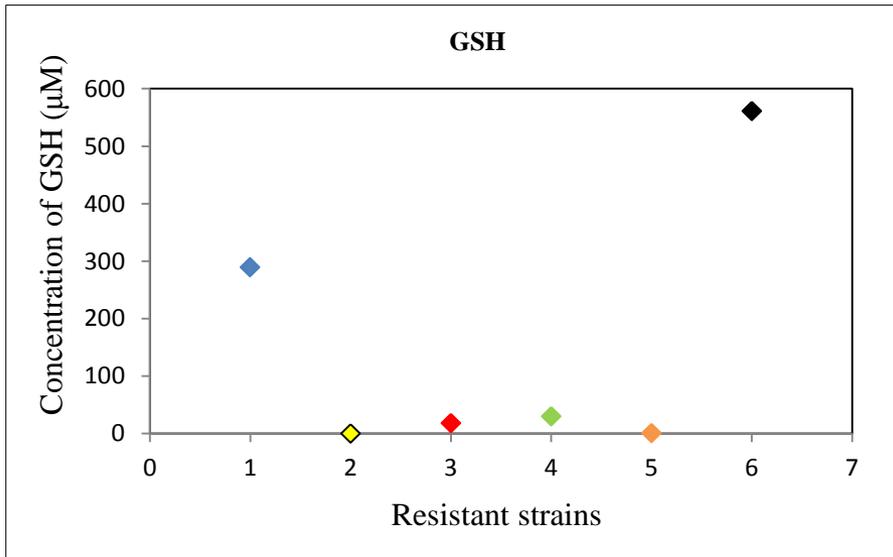
# Electrochemistry

◆ S.a. ◆ Ag ◆ Cu ◆ Pb ◆ Zn ◆ Cd



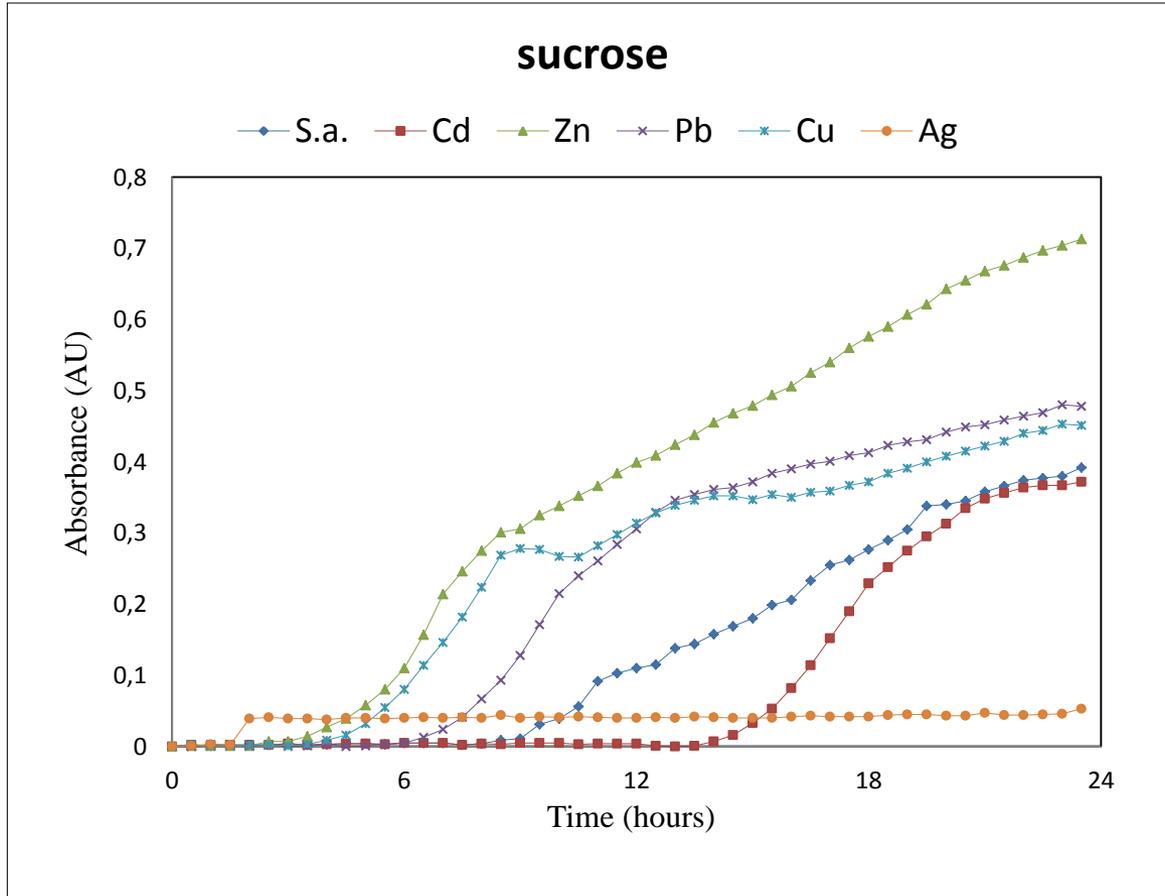
# Glutathione

◆ S.a. ◆ Ag ◆ Cu ◆ Pb ◆ Zn ◆ Cd

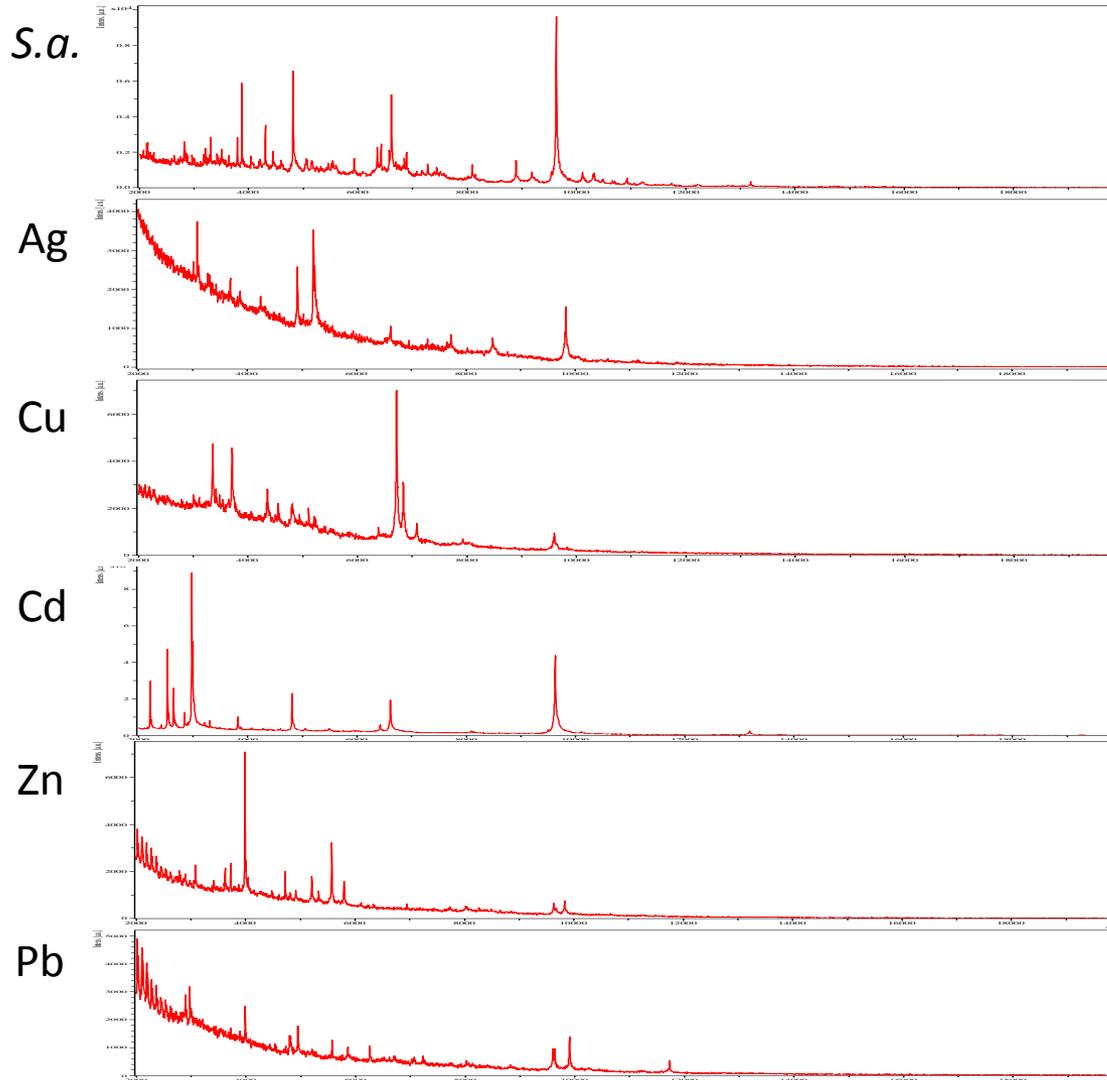


# Multiskan EX (STAPHYtest24)

- 620 nm
- 37 °C
- shaking



# MALDI-TOF



<i>Staphylococcus aureus</i> ssp. <i>aureus</i> DSM 4910	2.158
---	-------

<i>Staphylococcus simulans</i> DSM 20723	1.379
---	-------

<i>Staphylococcus</i> <i>saprophyticus</i> ssp. <i>saprophyticus</i> CCM 2682	1.422
---	-------

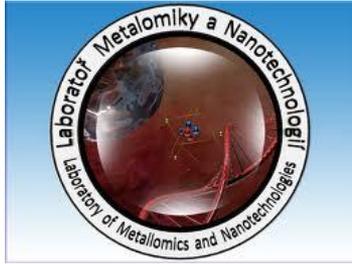
<i>Staphylococcus aureus</i> ssp. <i>aureus</i> DSM 20491	1.798
--	-------

<i>Staphylococcus felis</i> DSM 7377T	1.467
--	-------

<i>Staphylococcus simulans</i> DSM 20324	1.465
---	-------

# Conclusion

- comparison of resistant and non-resistant strains of *S.aureus*
- used metals for resistance production: Ag, Cu, Cd, Zn a Pb
- substantial changes of biochemical properties, DNA structure



# Acknowledgment



- Bc. Monika Kempová
- Ing. Jiří Kudr
- Ing. Kateřina Tmejová, Ph.D.
- Bc. Markéta Komínková
- Bc. Simona Dostálová
- Prof. Ing. René Kizek, Ph.D.

Financial support from:

CEITEC CZ.1.05/1.1.00/02.0068

NanoBioMetalNet CZ.1.07/2.4.00/31.0023



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Thank  
you for  
your  
attention

