Catalase activity in *Staphylococcus aureus* is associated with increased susceptibility to hydrogen peroxide killing and to decreased intracellular growth

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Content of the presentation

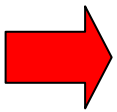
- What is the "accepted" role of catalase in *S. aureus*
- What about low or catalase-negative natural mutants ?
- What did we find ?
- What could we not do ?
- What are our current hypotheses ?

as you can see, many " ? "

The accepted role of catalase in S. aureus…

- *Staphylococcus aureus* invades eukaryotic cells, which shelters it from immune defenses and reduces its susceptibility to most antibiotics…
  - ➢ Intracellular survival may contribute to the persistent and relapsing character of many staphylococcal infections
- In eucaryotic cells, however, *S. aureus* becomes exposed to reactive oxygen species (ROS) generated by the respiratory burst…
  - ➢ Thus, it is generally assumed that the expression of catalase by *S. aureus* will protect it and favour its intracellular survival

This concept is essentially based on original observations that high-catalase producing strains are killed relatively poorly by PMNs…



Catalase, Superoxide Dismutase, and Virulence of Staphylococcus Aureus

IN VITRO AND IN VIVO STUDIES WITH EMPHASIS ON STAPHYLOCOCCAL—LEUKOCYTE INTERACTION

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However, absence or low activity of catalase is not always detrimental …

- A double catalase- and β-toxin negative mutant survives more readily in murine macrophages *in vitro* than its wild-type counterparts [1].
- *S. aureus* with a low catalase activity resist the bactericidal activity of leucocytes [2].
- A SCV (menadione-dependent phenotype) that survives intracellularly has a significantly diminished *katA* expression compared to its isogenic parental strain [3].
- *S. aureus* decreases its expression of *katA* immediately after invasion and during early survival in lung epithelial cells [4].

1. Martinez-Pulgarin S, Dominguez-Bernal G, Orden JA, de la FR (2009) Simultaneous lack of catalase and beta-toxin in Staphylococcus aureus leads to increased intracellular survival in macrophages and epithelial cells and to attenuated virulence in murine and ovine models. Microbiology 155: 1505-1515.
2. Nishihara S, Seki K, Masuda S (1985) Resistance of a mutant with an extremely low catalase production from Staphylococcus aureus Cowan-I strain to the bactericidal activity of human leukocytes. Microbiol Immunol 29: 151-155.
3. Kriegeskorte A, Konig S, Sander G, Pirkl A, Mahabir E, Proctor RA, von Eiff C, Peters G, Becker K (2011) Small colony variants of Staphylococcus aureus reveal distinct protein profiles. Proteomics 11: 2476-2490.
4. Garzoni C, Francois P, Huyghe A, Couzinet S, Tapparel C, Charbonnier Y, Renzoni A, Lucchini S, Lew DP, Vaudaux P, Kelley WL, Schrenzel J (2007) global view of Staphylococcus aureus whole genome expression upon internalization in human epithelial cells. BMC Genomics 8: 171

Strains used in the study

Parameter	strain no.				
	ATCC 25923	**2008-S080**	**2008-S419**	**UCN61**	**SH1000**
Catalase activity (U/mg protein)	**1.28 ± 0.15**	0.38 ± 0.13 *	0.11 ± 0.06 *	**0.0015 ± 0.003** *	0.67 ± 0.25 *
Susceptibility to cumene hydroperoxide (mm) [a]	**20.2 ± 0.4**	15.5 ± 0.5 *	16.3 ± 1.2 *	**19.7 ± 0.6**	13.3 ± 0.1 *
Susceptibility to paraquat (mm) [a]	**29.8 ± 1.9**	ND	ND	**28.3 ± 1.6**	9.7 ± 0.5 *
Staphyloxanthin production [b]	+	+	+	+	+++

[a] diameter of growth inhibition zone around disks impregnated with 100 mM cumene hydroperoxide or 500 mM paraquat.
* significantly different from the corresponding value of strain ATCC 25923 (one way ANOVA with Dunnett multiple comparisons test; P< 0.01)
[b] visual inspection
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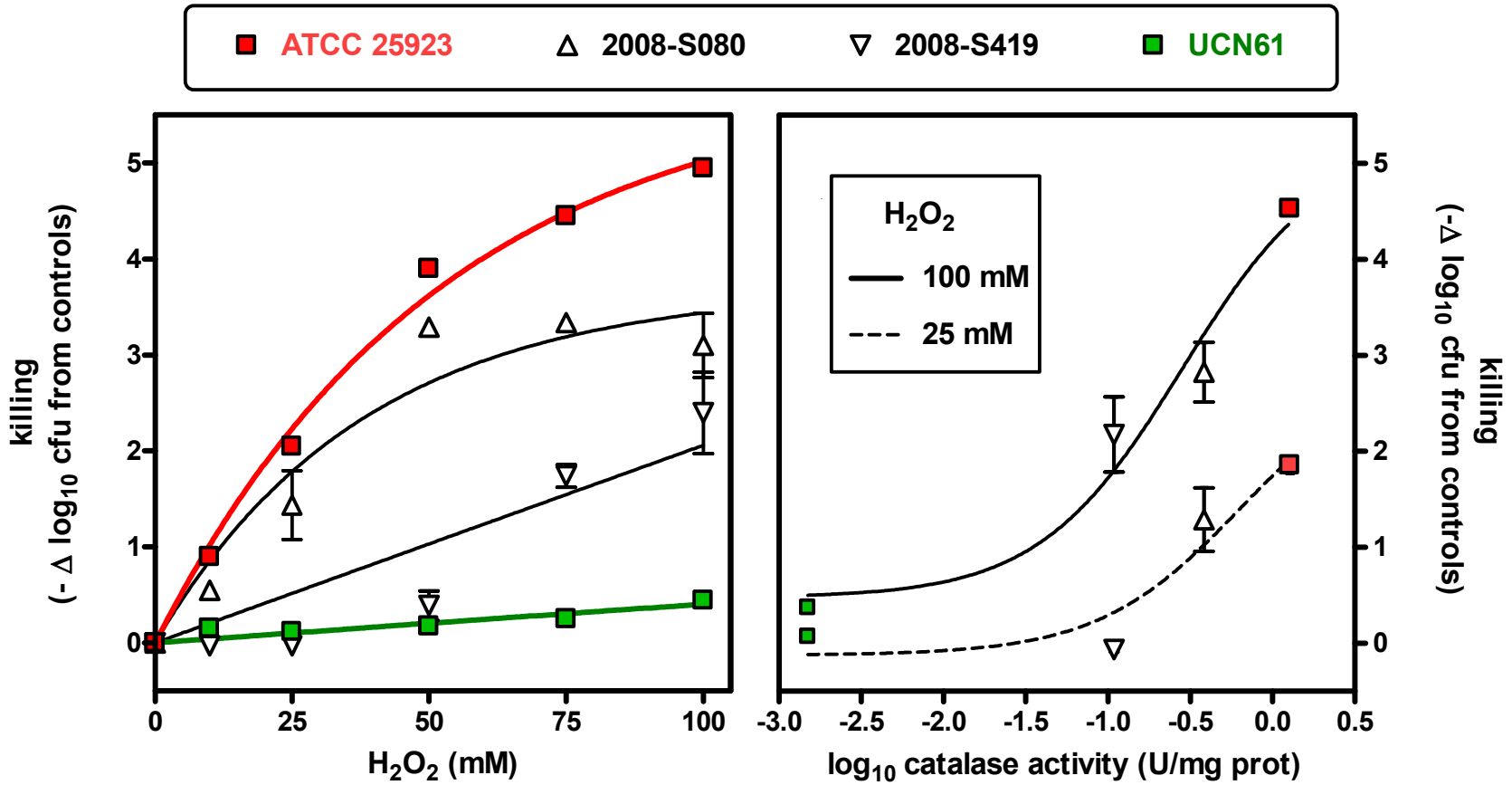
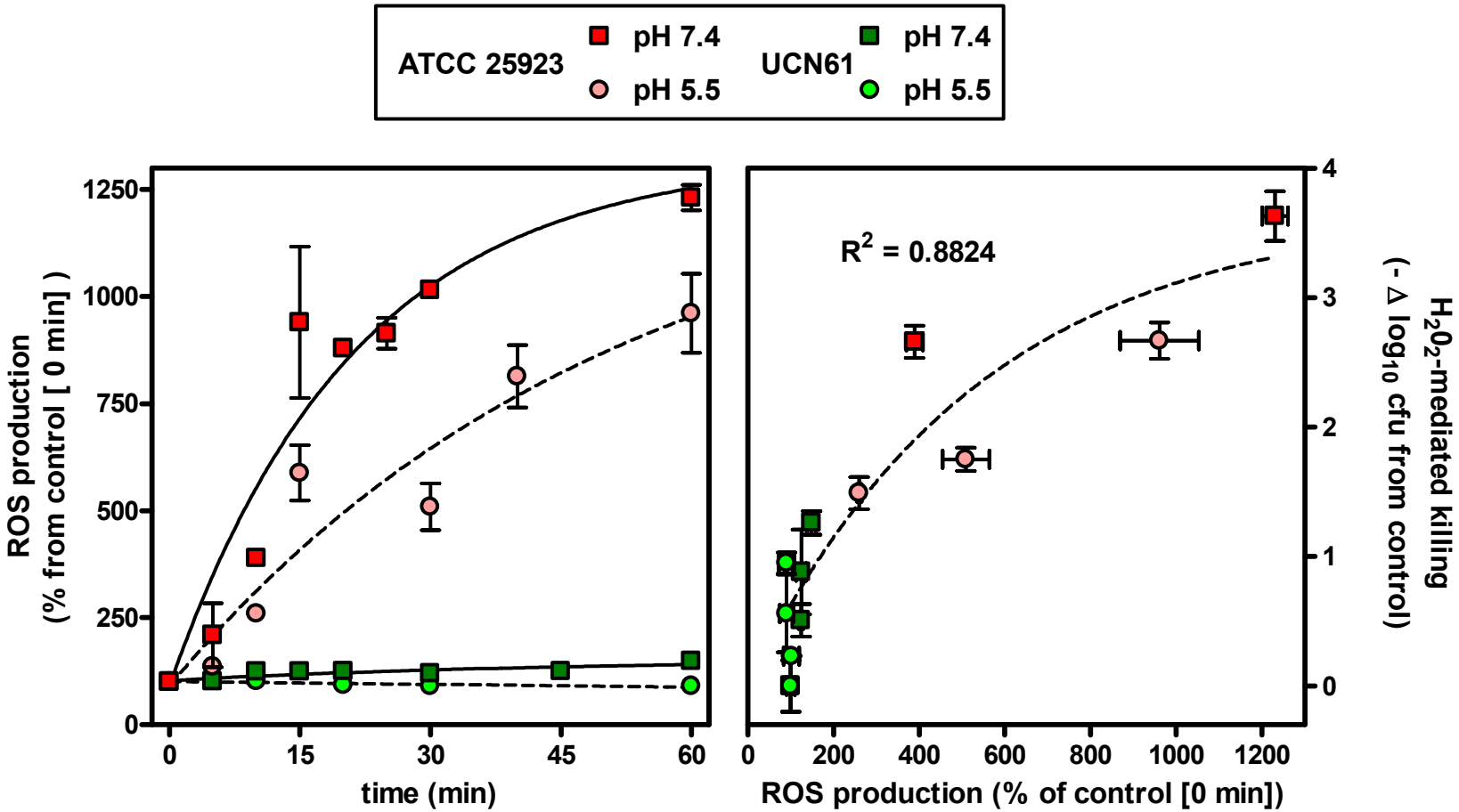
This strain produces a lot of staphyloxanthin

1. Killing of *S. aureus* by exposure to H_2O_2



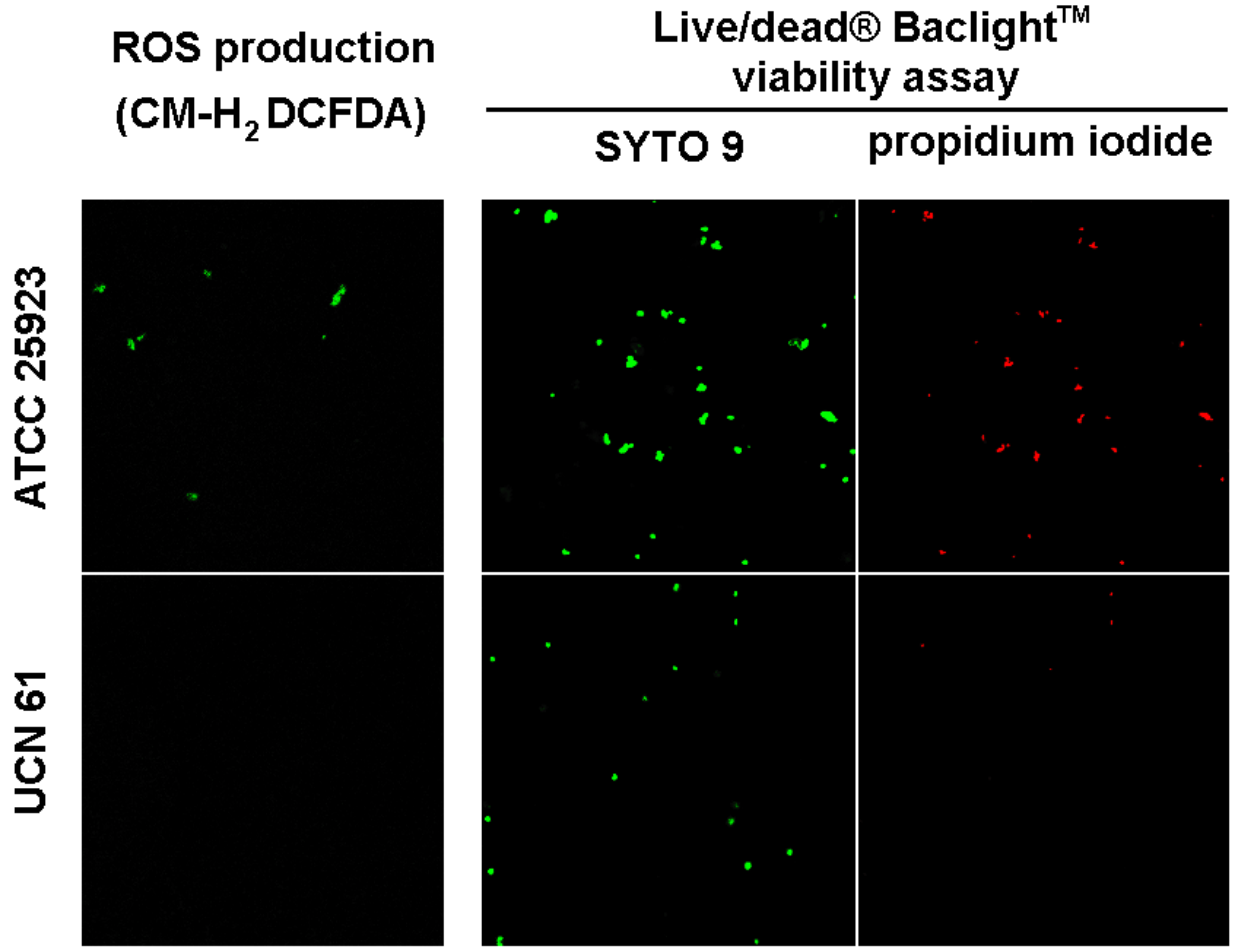
- **Left: Strains with increasing catalase activity (UCN61 < 208-S419 < 2008-S080 < ATCC 25923) exposed to increasing concentrations of H_2O_2 for 45 min, after which surviving bacteria were enumerated by colony counting**
- **Right: same results for two concentrations of H_2O_2 (25 and 100 mM) expressed as a function of the catalase activity of each strain.**

2. Production of ROS by *S. aureus* upon exposure to H_2O_2 and correlation with H_2O_2-induced killing



- Left: strains loaded with CM-H2DCFDA, exposed to 250 mM H_2O_2 at pH 7.4 or 5.5, and monitored for ROS production
- Right: correlation between bacterial killing and ROS production for the same strains and same pH conditions.

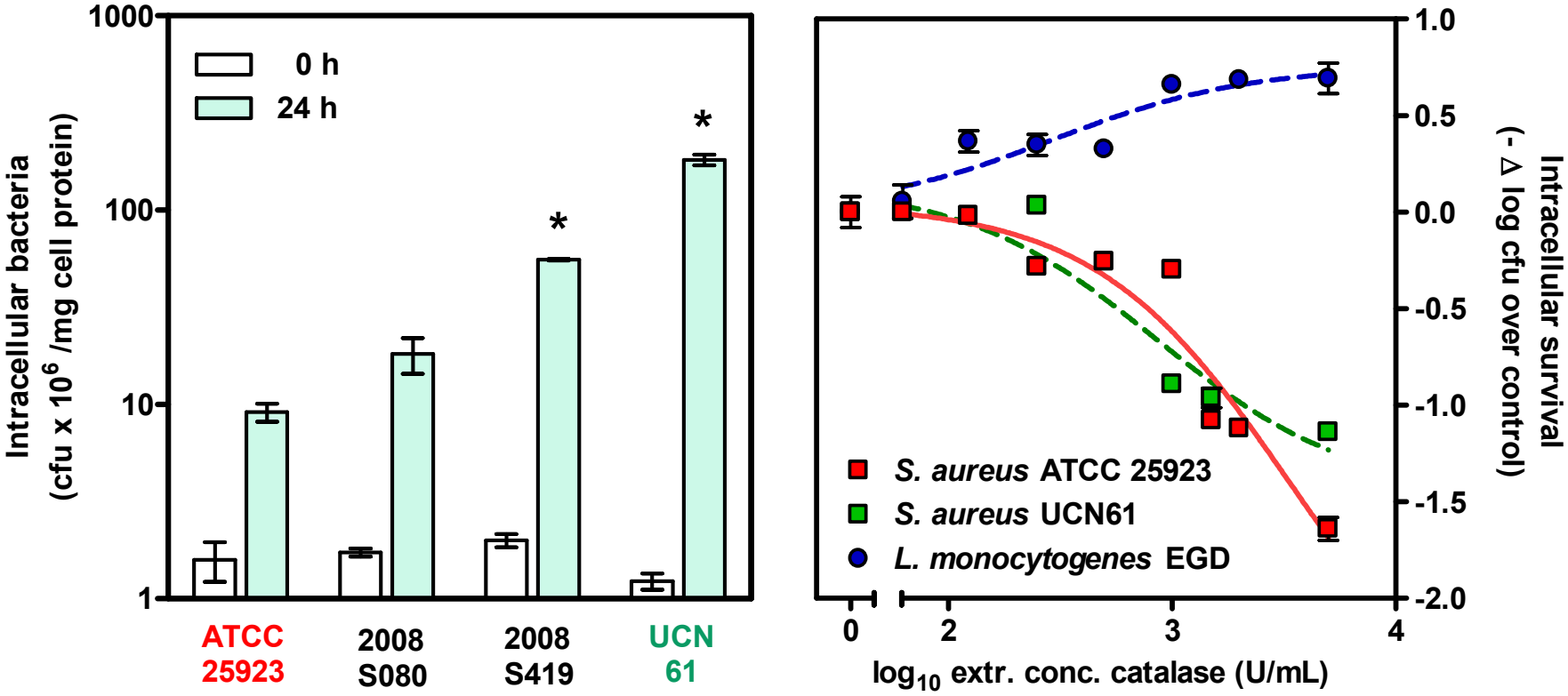
3. Visual evidence of ROS production and bacterial killing of *S. aureus* upon exposure to H_2O_2



Confocal microscopy images of bacteria exposed for 30 min at 37°C to 250 mM H_2O_2 at pH 7.4.

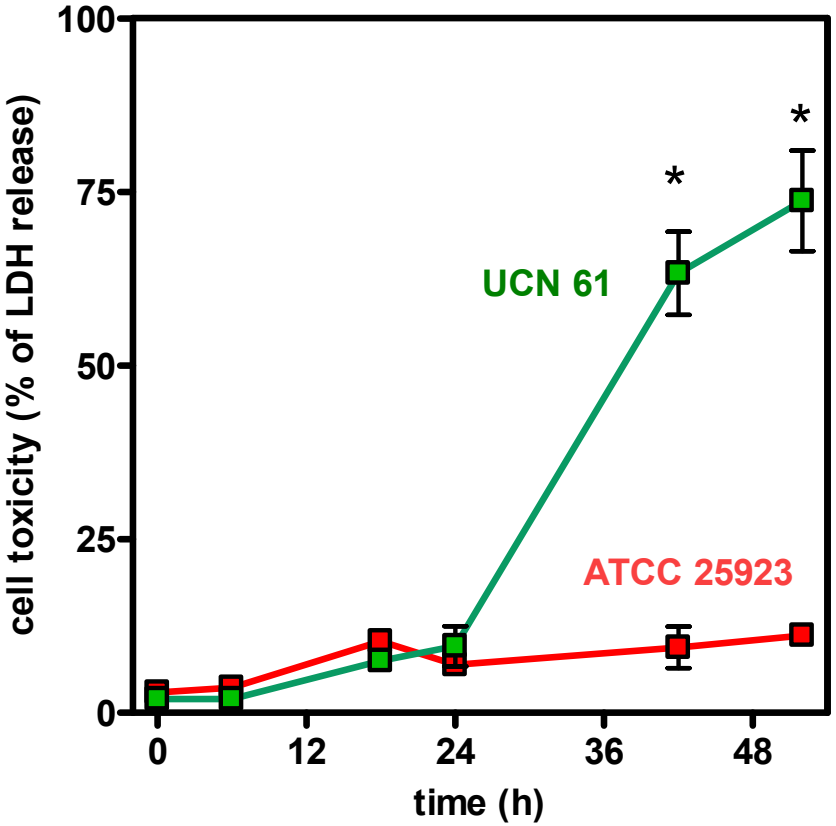
- **Left panels: ROS detection (green signal);**
- **Middle panels: all bacteria (living or dead: green signal);**
- **Right panel: dead bacteria (red signal).**

4. Intracellular growth of *S. aureus* and influence of added catalase (compared to *L. monocytogenes*)



- Left: Intracellular growth of S. aureus strains over 24 post-phagocytosis
- Right: Effect of added catalase on the intracellular survival of *S. aureus* and *L. monocyogenes*

5. Cytotoxicity of phagocytized *S. aureus* (THP-1 monocytes) upon long term intracellular growth



Loss of viability of the monocytes examined by the releas of the cytosolic enzyme lactate dehydogenase (LDH) indicating cell membrane permeabilization.

In a nutshell…

- *S. aureus* strains with a **high catalase activity** are **more readily killed** by exposure to H_2O_2 than those with low activity;
- Strains with **high catalase activity** produce **large amounts ROS**;
- Strains with **high catalase-producing strains** are **less capable of multiplying** in THP 1 monocytes
- Intracellular survival of *S. aureus* is **decreased** by addition of **exogenous catalase.**
- Strain **UCN61** (very **low catalase activity**) readily kills THP-1 monocytes through its **excessive growth** within 24 h whereas ATCC25923 does not.
- Not illustrated: strain **SH1000** (**high** producer of **staphyloxanthin**) is more susceptible to H_2O_2 than strain **UCN61** (low producer of staphyloxanthin) ruling out a major role of this pigment in our findings

What we could not do…

- We failed, in spite of continuous efforts, to construct *katA* negative mutants and the requisite complemented strains in the ATCC 25923 background.
- We also failed to restore catalase activity in the *S. aureus* UCN61 background.

  - *Staphylococcus aureus* is an "untransformable bacterium" unless using specific DNA cytosine methyltransferase mutant (DC10B [1])

  - Catalase may have other critical roles for bacterial survival than its turnover of hydrogen peroxide (maintained or compensated for in the natural strain UCN61)

Note: Isogenic catalase-negative mutants have been described but obtained from the genetically modified SH1000 laboratory strain (restored rsbU activities), which shows an exceptionally large production of staphyloxanthin and is accordingly more resistant than ATCC 25293 to H_2O_2-induced killing [2].

1. Monk IR, Shah IM, Xu M, Tan MW, Foster TJ (2012) Transforming the untransformable: application of direct transformation to manipulate genetically Staphylococcus aureus and Staphylococcus epidermidis. MBio 3. 2012 Mar 20;3(2).
2. Olivier AC, Lemaire S, Van Bambeke F, Tulkens PM, Oldfield E (2009) Role of rsbU and staphyloxanthin in phagocytosis and intracellular growth of Staphylococcus aureus in human macrophages and endothelial cells. J Infect Dis 200: 1367-1370.

Our current hypotheses…

1. Catalase activity might involve an **hydroxyl radical** as an intermediate

$$HOOH \rightarrow 2\ HO^{\bullet} \rightarrow H_2O + \tfrac{1}{2}\ O_2$$

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2. Catalase is known to also act as an oxidase

$$HOOH + H_2R \rightarrow 2\ H_2O + R$$

and this activity is predominant when the concentration of H_2O_2 is kept at low steady-sate concentrations [1]. Actually, catalase is bactericidal when added to broth containing a system generating H_2O_2 [2].

1. Keilin D, Hartree EF (1945) Properties of catalase. Catalysis of coupled oxidation of alcohols. Biochem J 39: 293-301.
2. Klebanoff SJ (1969) Antimicrobial activity of catalase at acid pH. Proc Soc Exp Biol Med 132: 571-574.

Thank you for all suggestions !