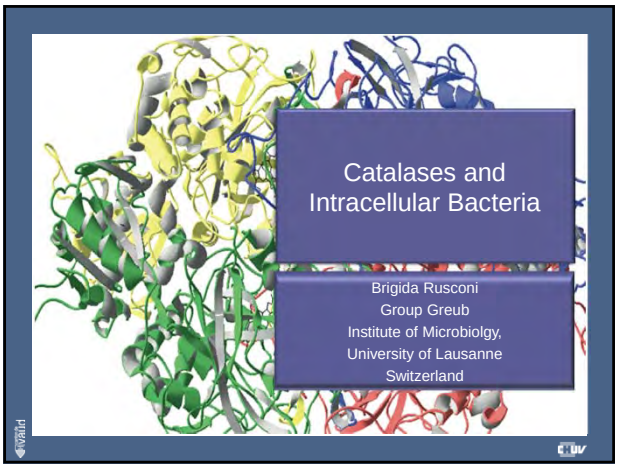


Reactive oxygen species in infection

- Respiratory burst
  - ROS damage of bacteria
- Superoxide produced can be used by myeloperoxidase to produce hypohalous acids
- Decrease in membrane potential
  - Ionic changes affect bacteria
- Important for regulation of cell death in neutrophils
  - Formation of neutrophil extracellular traps (NETs)
- Prevention of hyperinflammation
  - Control of inflammasome
- Cytokine production
  - In CGD several cytokines are over or underexpressed upon infection

Reviewed in Rada *et al.*, *Sem Immunpathol*, 2008

Catalases

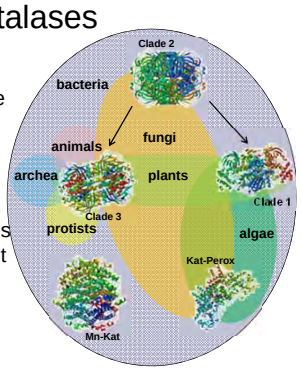
$2H_2O_2 \rightarrow O_2 + 2H_2O$

- Essential for aerobic life
- Control of signaling pathways
- Counteract oxidative stress during infection

Introduction

Catalases

- Found in prokaryotes, eukaroytes, and archeae
- "Monofunctional" catalases, catalases-peroxidases, and manganese catalases
- Monofunctional catalases proteins (already present in eobacteria)



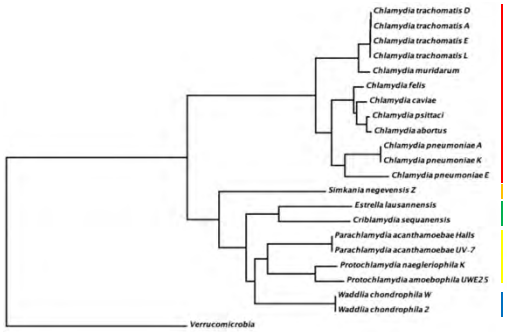
Question: Part I

Catalase was found during genome annotation of *Waddlia chondrophila*

Exception in *Chlamydiales* ?

Introduction

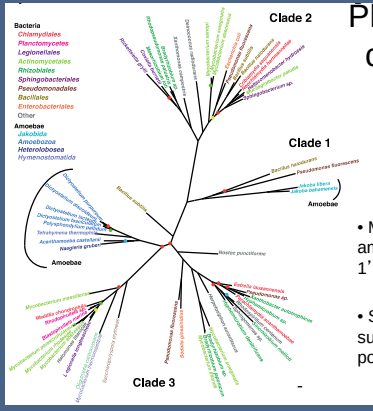
Chlamydiales order



Work by T. Pillonel

Results: Part I

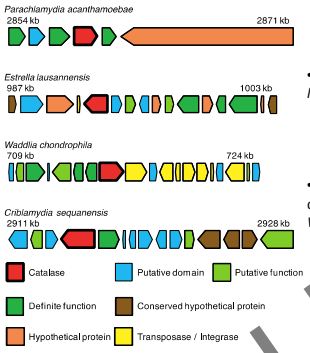
Phylogeny of chlamydial catalases



- Mr. Bayes with mixed amino acid model, 1'000'000 generations
- Separation of clades supported by high posterior probability (1)

Results: Part I

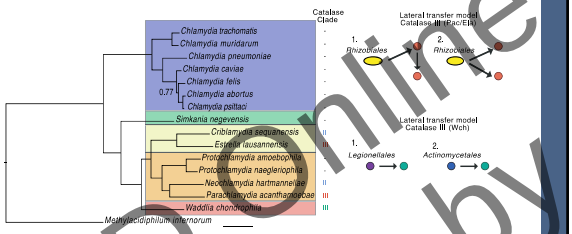
Genetic environment



- Identity *P. acanthamoebae* and *E. lausannensis* 79%
  - -Synteny low among *chlamydia*-related genomes
- •Transposon integrase upstream and downstream (15 genes) of *katA* of *Waddlia chondrophila*

Overview : Part I

Overview catalases phylogeny

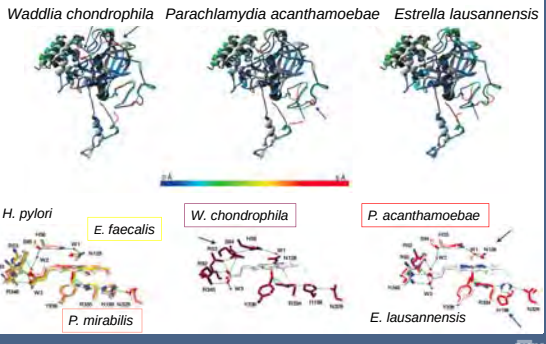


Question: Part II

Are the motifs and structural features of chlamydial catalases conserved ?

Results : Part II

Structural conservation



Overview : Part II

Overview structural conservation

- Motifs are mostly conserved at the sequence level in chlamydial catalases
  - –Distal site motif not recognized by PROSITE for *Parachlamydia acanthamoebae* and *Estrella lausannensis* due to changes F44/45Q and S59/60)
  - –Changes did not affect secondary / tertiary structure
- Tertiary structure of subunit is conserved in chlamydial catalases
- Catalytic site conformation is conserved except for *P. acanthamoebae*
  - –Shift of H198 side chain causes an interruption of the hydrogen bonds linked to the catalytic tyrosine Y338

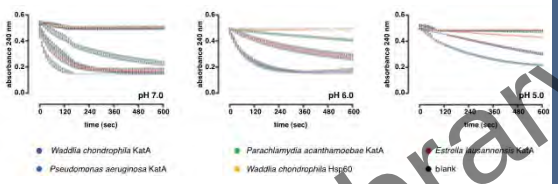
Question: Part III

Are the chlamydial catalases enzymatically active?

Results : Part III

Enzymatic activity

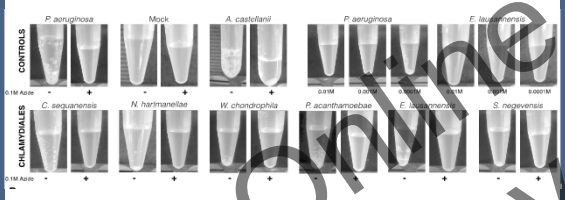
- H_2O_2 absorbs @ 240nm
- Follow degradation of H_2O_2 by catalase activity with microplate reader



W. chondrophila is the most resilient to a drop in pH of the chlamydial small subunit catalases

Results : Part III

Enzymatic activity



Summary

Summary

- Catalases of clade 2 and 3 were found in several species of the *Chlamydia*-related branch of the *Chlamydiales*
- Origin of catalases of clade 3 occured by lateral gene transfer at several occasions
- Structure and motifs of catalases are conserved
  - Catalytic site of *Parachlamydia acanthamoebae* is missing a hydrogen bond
- Chlamydial clade 3 catalases are enzymatically active *in vitro* and in elementary bodies
  - Catalase of *P. acanthamoebae* is significantly less active than others

Enzymatic activity of Catalase-Peroxidase

TABLE 3. Enzymatic activity[a]

Peroxidase cosubstrates	Relative activity (% of catalase activity)[b]	
	KatA	KatB
H_2O_2 + dianisidine	0.26 ± 0.02	0.058 ± 0.0004
H_2O_2 + pyrogallol	0.29 ± 0.05	0.36 ± 0.002
H_2O_2 + NADH	0.022 ± 0.001	0.022 ± 0.004
H_2O_2 + NADPH	0.023 ± 0.005	0.019 ± 0.002

[a] Enzymatic activities of KatA and KatB were measured in periplasm or spheroplast extracts of *E. coli* strain UM283 *katE katG* containing pMMB207*aB*-Km14 *katA* or pMMB207*aB katB* (Table 1). The *E. coli* strains were grown overnight in LB broth with chloramphenicol.

[b] Activities (in micromoles of H_2O_2 or peroxidase cosubstrate converted per minute per microliter of extract) are expressed relative to catalase activity, set at 100%. Where appropriate, corrections were made for absorbance changes in extracts of UM283 with the vector pMMB207*aB*-Km14 alone.

KatA and KatB null mutants have an attenuated growth in macrophages

Bandyopadhyay et al, *J Bact*, 2000

Acknowledgements

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