



Characterizing the hydrogen peroxide resistance mechanisms of *Acinetobacter radioresistens* 50v1



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Introduction:

- Isolated on surface of Mars Odyssey spacecraft.
- First gram-negative bacterium isolated from a spacecraft surface.
- Acinetobacter radioresistens* 50v1: as indicated by genomic and microbiological studies.
- H₂O₂ and UV resistance...
- 1-2 log reduction in 5% aqueous H₂O₂.
- This survival is rare for gram-negative bacteria.
- Type strain (43998^T) shows ~6-log reduction.

Methods:

- 16s rDNA analysis of cultivable heterotrophs.
- Survivability in aqueous and vaporous H₂O₂.
- Effects of additional stress: desiccation and UV.
- Proteomic analysis of 50v1 and type strains.
- Catalase assays of whole cell extracts.
- Initial purification of catalase from 50v1.
- Fatty acid analysis of 50v1 and type strains.

Results:

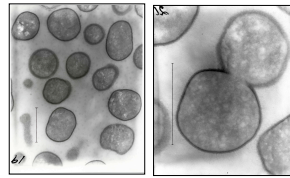
- Identity: *Acinetobacter radioresistens* 50v1
- Remarkable survival under multiple stress.
- Significant proteome difference between strains.
- 50v1 has a ~ 1.3-fold excess of catalase.
- Relative catalase contents for 50v1 increase as power of ultrasonication increases.
- In gel FeCl₃/K₃Fe(CN)₆ stain of partially purified catalase indicates MW of ~ 240kD.
- Anion exchange provides two major bands as shown by native electrophoresis.
- Fatty acid analysis shows slight increase in palmitoleic acid content for 50v1.

Conclusions:

- A. radioresistens* 50v1 may possess:
 - a modified cell wall and membrane...
 - an increased biosynthesis of proteins...
 - an increased biosynthesis of fatty acids...
 - an decreased catabolism of unsat. fatty acids...
 - an increase in enzymatic peroxide degradation...
- A. rad.* 50v1 may be a good target for medical and astrobiological studies towards bacterial resistance and sterilization.

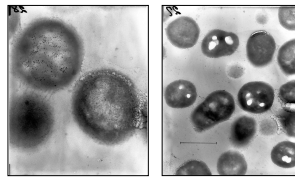
• survivability : H₂O₂ with desiccation and UV exposure

A. radioresistens 50v1

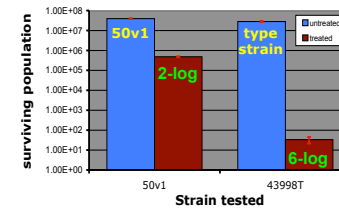


ESEM

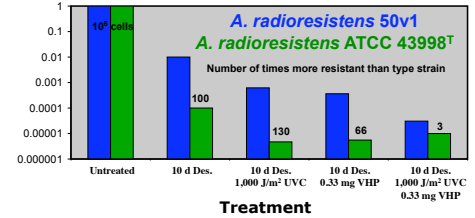
after exposure to H₂O₂



survivability (5% H₂O₂)

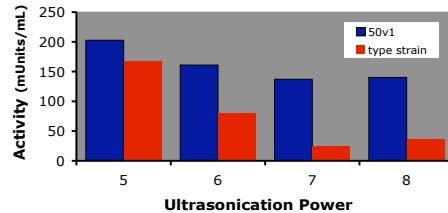


effects of multiple stress (desiccation/UV-C/vaporous H₂O₂)

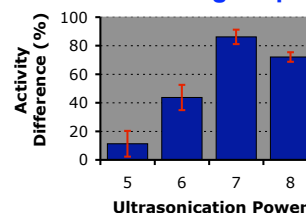


• catalase content : extract analysis and purification

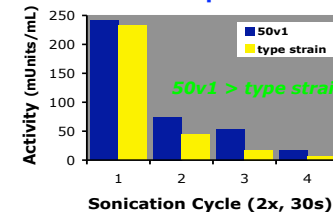
effect of ultrasonication power on the release of catalase



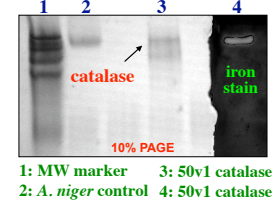
50v1 extracts retain more catalase at higher powers



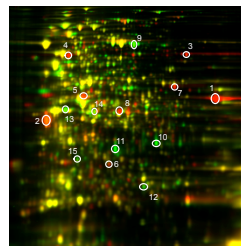
release of catalase at low power



partially purified catalase (2 anion exchange columns)



• proteome and fatty acid analysis : 50v1 versus type strain



spot protein

2	OmpA-like protein precursor	+8
5	EF-Tu, protein elongation factor	+27
6	NADH-dependent enoyl-ACP reductase	+4.2
8	(Zn)-Alcohol Dehydrogenase	+35
10	putative ring oxidizing protein	-29
11	enoyl-CoA isomerase	-74
14	succinylornithine transaminase	-3.7
15	dihydrodipicolinate reductase	-3.7

ratio biochemical role

+8	outer membrane protein; emulsifying reagent
+27	ribosome; protein translation
+4.2	membrane fatty acid biosynthesis (type II)
+35	fermentation
-29	benzoate catabolism
-74	catabolism of unsaturated fatty acids
-3.7	arginine catabolism
-3.7	lysine biosynthesis

(+) : 50v1 > type strain (-) : type strain > 50v1 [1,3,4,7,9,12,13: No Hits...possible new proteins]

fatty acid content

- 50v1 has a ~ 8.5% higher abundance of 16:1 w7c/15 iso 20H
- Abundance of other fatty acids are similar (< 3% diff.)



palmitoleic acid: 16:1(Δ⁹) [16:1 (ω7c)] 2-hydroxy-13-methylmyristic acid [15:0 iso 20H]

• results : 50v1

- Significant survival under stress
- 1.3-fold more catalase
- A stronger cell wall
- ~ 8.5% more 16:1(Δ⁹)

- Upregulated emulsifying reagent
- Upreg. protein translation
- Upreg. membrane FA synthesis
- Downreg. unsat. FA catabolism