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Characterizing *Acinetobacter radioresistens* 50v1, an extremophile isolated from the Mars Odyssey spacecraft

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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.
- Multiple stress resistance: dessication, H_2O_2, and UV exposure…
- ~ 2 log reduction in 5% aqueous H_2O_2.
- 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 H_2O_2.
- Effects of additional stress: desiccation and UV.
- Catalase assays of whole cell extracts using Lysozyme and Ultrasonication methods…
- Initial purification of catalase from 50v1.
- Proteomic analysis of 50v1 and type strains.
- Fatty acid analysis of 50v1 and type strains.

Results:

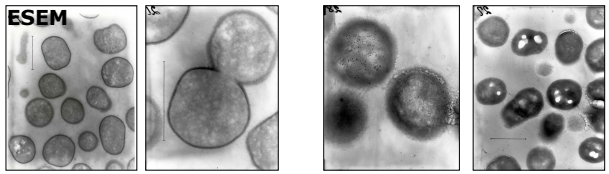
- Identity: *Acinetobacter radioresistens* 50v1
- Remarkable survival under multiple stress.
- Significant proteome difference between strains.
- 50v1 has 6.5-fold excess catalase over *E. coli*.
- Lysozyme is the preferred milder method.
- Ratios are dependent upon ultrasonication power.
- In gel $FeCl_3/K_3Fe(CN)_6$ 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:

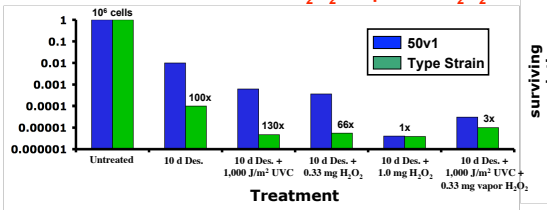
- *A. radioresistens* 50v1 may possess:
  - *(A) an increase in enzymatic peroxide degradation…*
  - *(B) a modified cell wall and membrane…*
  - *(C) an increased biosynthesis of proteins…*
  - *(D) an increased biosynthesis of fatty acids…*
  - *(E) an decreased catabolism of unsat. fatty acids…*
-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

• survivability/growth : H_2O_2, desiccation, and UV exposure

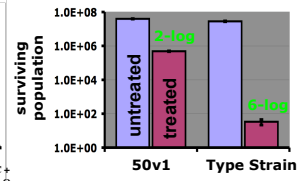
A. radioresistens 50v1 …after exposure to H_2O_2



survivability under multiple stress – desiccation / UV-C / H_2O_2 / vaporous H_2O_2

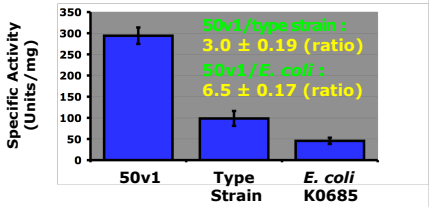


survival in H_2O_2 *(aq)* – 5% aqueous H_2O_2

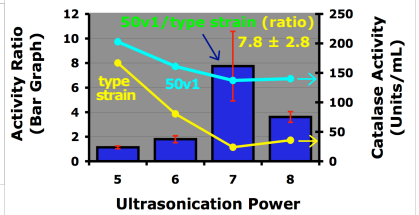


• catalase content : lysozyme vs. ultrasonication, purification

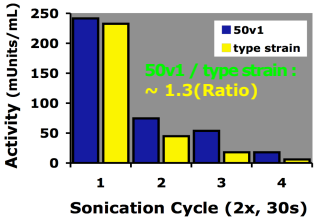
effect of lysozyme degradation on the release of catalase



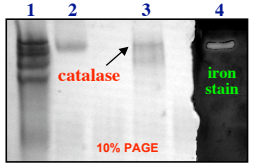
ultrasonication power effects the relative release/activity of catalase



release of catalase at low power (p = 5)

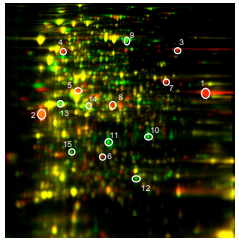


partially purified catalase (2 anion exchange columns)



1: MW marker 3: 50v1 catalase
2: *A. niger* control 4: 50v1 catalase

• proteome and fatty acid analysis : 50v1 vs. type strain



spot	protein	ratio	biochemical role
2	OmpA-like protein precursor	+8	outer membrane protein; emulsifying reagent
5	EF-Tu, protein elongation factor	+27	ribosome; protein translation
6	NADH-dependent enoyl-ACP reductase	+4.2	membrane fatty acid biosynthesis (type II)
8	(Zn)-Alcohol Dehydrogenase	+35	fermentation
10	putative ring oxidizing protein	-29	benzoate catabolism
11	enoyl-CoA isomerase	-74	catabolism of unsaturated fatty acids
14	succinylornithine transaminase	-3.7	arginine catabolism
15	dihydrodipicolinate reductase	-3.7	lysine biosynthesis

(+) : 50v1 > type strain (−) : type stain > 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 2OH*
- Abundance of other fatty acids are similar (< 3% diff.)

palmitoleic acid: 16:1(Δ^9) [16:1 (ω7c)] 2-hydroxy-13-methylmyristic acid [15:0 iso 2OH]

• results : 50v1

- Remarkable survival under stress
- ~7-fold more catalase over *E. coli*
- Sonication suggests stronger cell wall
- ~ 8.5% more 16:1(Δ^9)
- Increased emulsifying reagent
- Increased protein translation
- Increased membrane FA synthesis
- Decreased unsat. FA catabolism