A Proteomic Signature of Dormancy in an Actinobacterium: *Micrococcus luteus*

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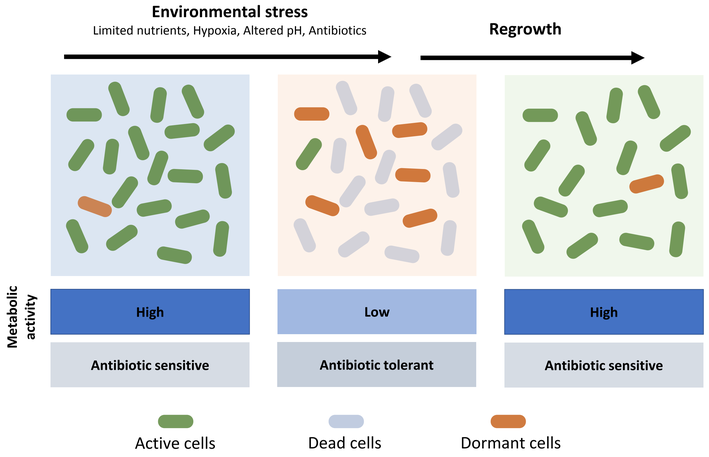
Introduction

- Dormancy is a protective state where diverse bacteria curtail metabolic activity to survive severe external stresses
- Viable but non-culturable (VBNC) dormant bacterial state is important for antibiotic tolerance, latent infection, and reemergence of active infections
- Proteins critical for dormancy are important drug targets to treat dormant bacterial infections such as latent tuberculosis



Micrococcus luteus is a model bacteria

- Non-pathogenic, rapid growth rate
- Small genome
- Belongs to Actinobacteria
- Undergoes VBNC dormant state upon nutrient deprivation
- *M. luteus* resuscitation promoting factor (Rpf) can resuscitate dormant *M. luteus* (C) and *M. tuberculosis* (D)

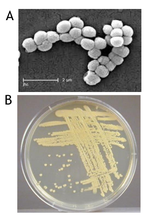
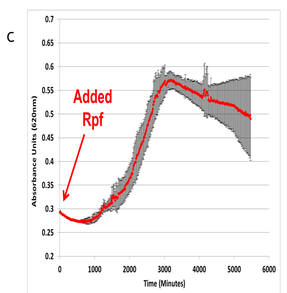
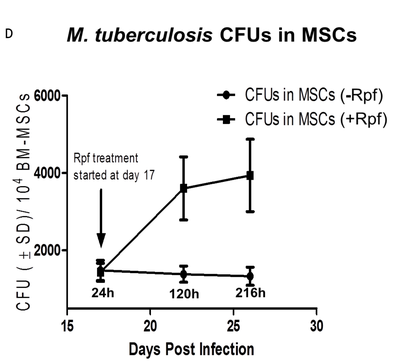


Fig: (A) Scanning electron micrograph and (B) colony of *M. luteus*





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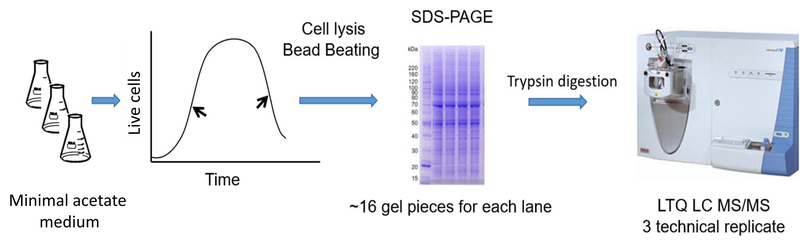
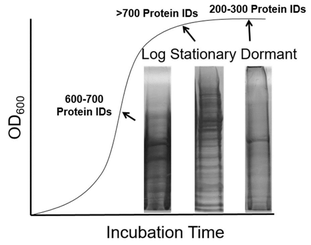
Methods

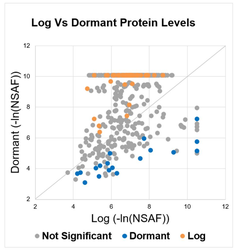
- Workflow of the sample preparation for proteomic study



Results

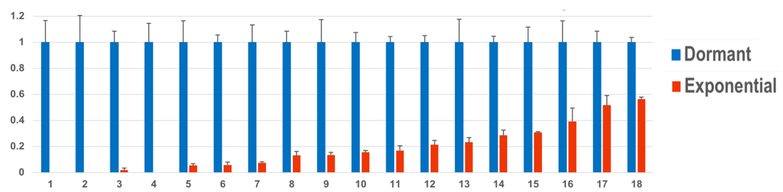
- VBNC state *M. luteus* exhibits a global loss of protein diversity compared to logarithmic (log) or stationary growth phase





- **Proteomic signature of viable but non-culturable (VBNC) *M. luteus***
  Bar graph (A) and Heat map (B) representation of eighteen proteins upregulated in dormant phase compared to log phase

A



B

	PROTEIN IDENTIFICATION		REPLICATE 1			REPLICATE 2			REPLICATE 3		
			Log	Dormant	P-value	Log	Dormant	P-value	Log	Dormant	P-value
1	Isocitrate lyase	Mlut_02080	-10.53	-5.12	***	-8.33	-3.77	***	-9.73	-4.84	*
2	Hypothetical protein Mlut_03760	Mlut_03760	-10.51	-5.72	***	-8.01	-6.14	*	-7.56	-5.05	***
3	O-acetylhomoserine sulfhydrolase	Mlut_06040	-9.264	-5.07	*	-10.80	-6.31	***	-9.50	-5.15	*
4	ABC transporter substrate-binding protein	Mlut_01730	-10.51	-7.21	***	-6.31	-5.76	*	-9.26	-6.55	*
5	Acetyl-CoA synthetase	Mlut_18230	-8.06	-5.16	***	-6.59	-5.32	*	-8.64	-6.50	**
6	Ribosomal biogenesis protein	Mlut_14710	-7.52	-4.64	**	-7.45	-5.61	*	-5.87	-4.92	*
7	Universal stress protein UspA	Mlut_01830	-6.28	-3.69	***	-4.84	-3.89	***	-4.86	-3.54	***
8	Aldolase	Mlut_00870	-6.08	-4.03	**	-8.86	-4.50	*	-9.61	-5.04	*
9	Integration host factor	Mlut_19160	-5.86	-3.87	***	-4.87	-3.23	***	-4.96	-4.39	*
10	Aldehyde dehydrogenase	Mlut_21440	-5.35	-3.50	***	-4.71	-3.43	***	-6.36	-3.36	***
11	Amidohydrolase	Mlut_17580	-7.70	-5.92	**	-6.52	-5.77	***	-7.45	-6.40	*
12	Hypothetical protein Mlut_10450	Mlut_10450	-4.62	-3.07	***	-3.85	-2.90	***	-4.39	-3.47	***
13	Cysteine synthase	Mlut_09450	-5.79	-4.34	**	-6.47	-5.09	***	-6.40	-4.78	*
14	Alcohol dehydrogenase	Mlut_21430	-5.41	-4.16	***	-4.93	-4.12	*	-6.54	-4.38	***
15	Ketol-acid reductoisomerase	Mlut_08550	-5.17	-4.00	***	-5.51	-4.46	**	-5.72	-4.28	*
16	Superoxide dismutase	Mlut_11340	-5.92	-4.97	*	-5.12	-4.41	*	-5.20	-3.90	**
17	ATP synthase subunit beta	Mlut_08180	-4.39	-3.72	***	-4.37	-3.79	**	-4.68	-4.04	***
18	Molecular chaperone GroEL	Mlut_16540	-4.23	-3.65	***	-3.93	-3.34	**	-3.84	-3.59	**

Low Abundance — High Abundance
p-Value: * = <0.05 ** = <0.01 *** = <0.005

Discussion

- The VBNC dormancy signature proteins are conserved across actinobacteria
- The proteins upregulated in VBNC implicate the role of glyoxylate shunt, redox and amino acid metabolism, and ribosomal regulatory processes in VBNC transition
- Upregulation of DNA binding proteins suggests bacterial chromosome compaction which correlates with reduced protein synthesis

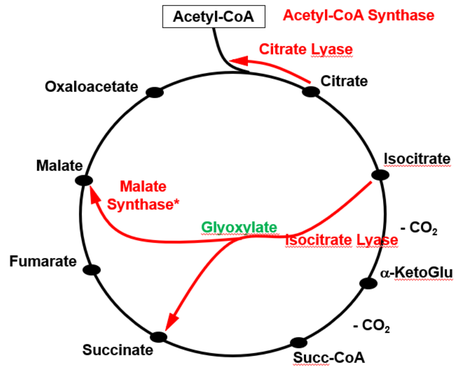
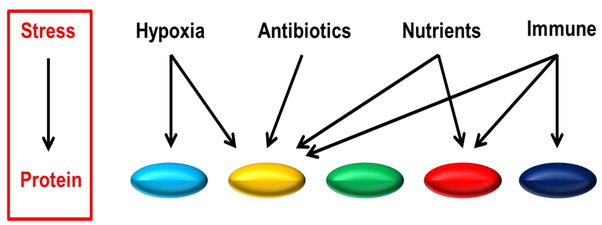


Fig: Glyoxylate Shunt and TCA proteins that were upregulated (red) in the VBNC state of Ml-2665 are mapped onto the TCA Cycle

Future Work

- Identify critical proteins to develop antibiotics against dormant bacteria
- Determine if protein mechanisms are the same across different external stress conditions and different bacteria



References

- Mali *et al.* (2017) A Proteomic Signature of Dormancy in an Actinobacterium: *Micrococcus luteus*. Journal of Bacteriology
- Li *et al.* (2014) The importance of the viable but non-culturable state in human bacterial pathogens. Frontiers in Microbiology
- Zhao *et al.* (2017) Current perspectives on viable but non-culturable state in foodborne pathogens. Frontiers in Microbiology

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