

Vuka! Let's unite towards a TB-free world!

9th SA
TB
Conference
8 - 11 June 2026

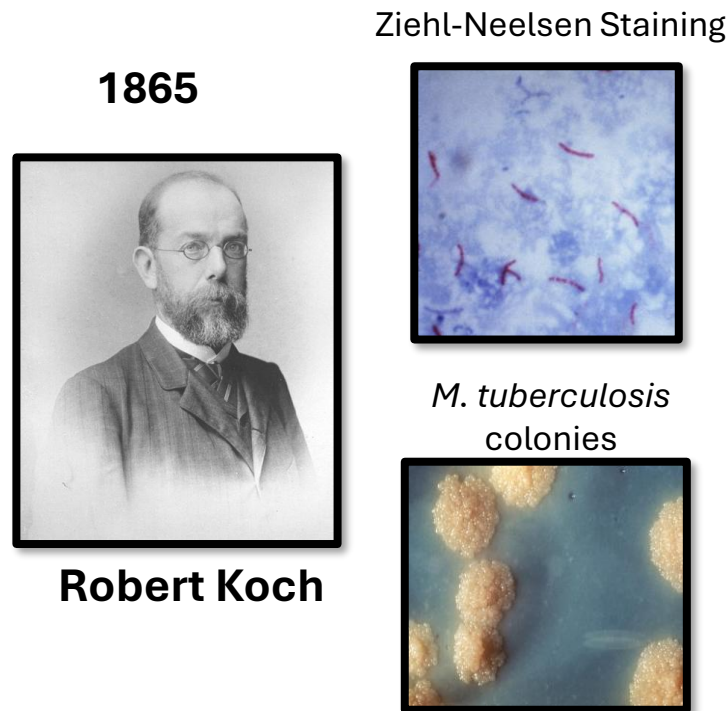


Clinical strains of *Mycobacterium tuberculosis* exhibit distinct propensities to adopt the differentially culturable state

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Heterogeneity of mycobacterial growth in sputum sample

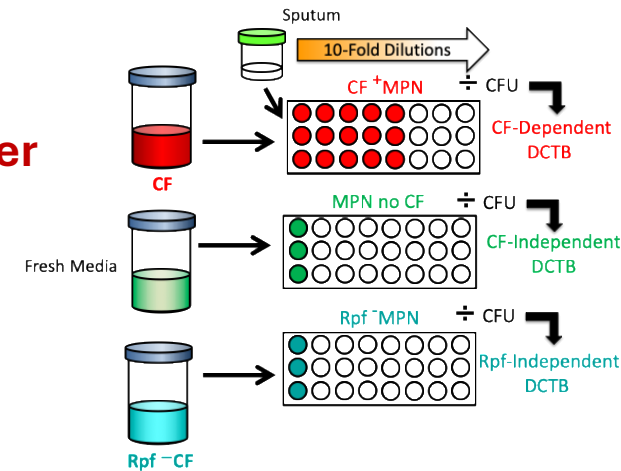


Colonies = Life

151 years later

2016

Liquid based
Most probable Number (MPN) assay



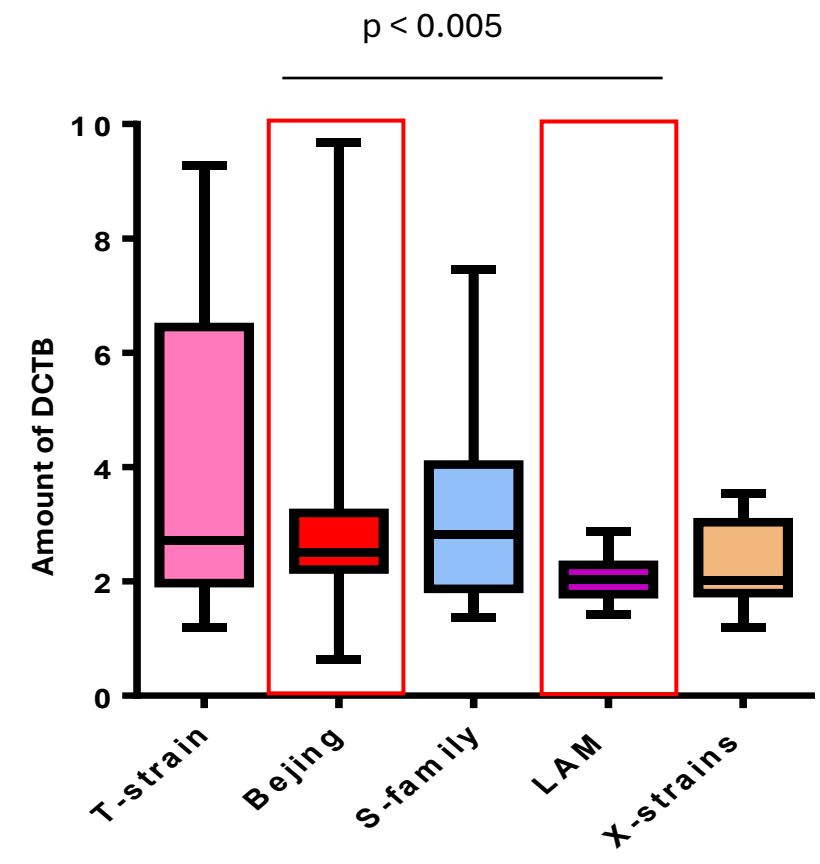
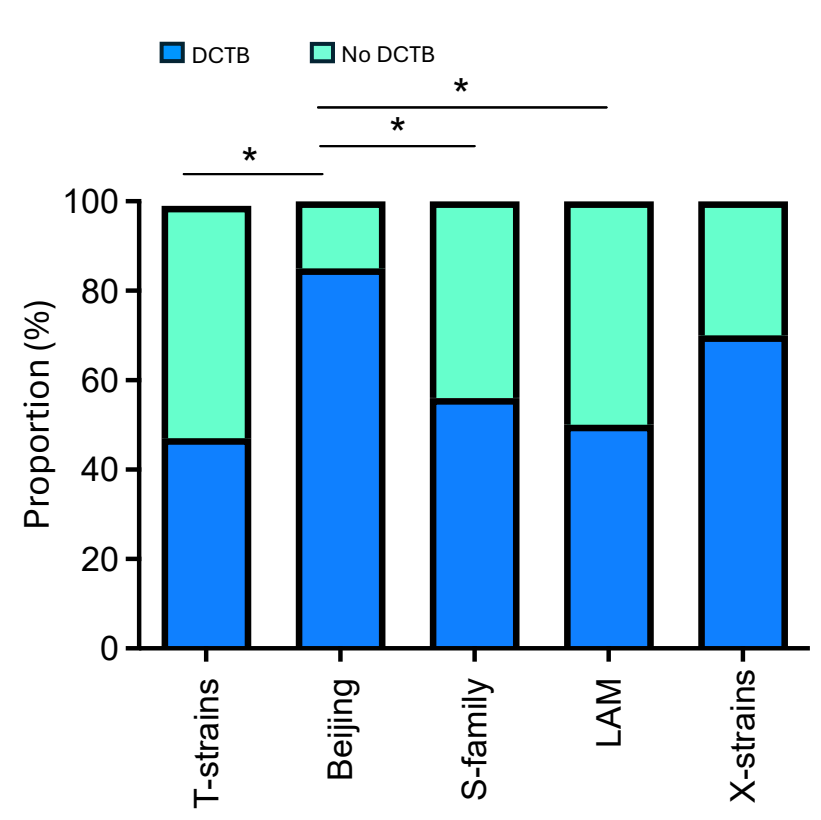
The Hidden World of Non-Culturable TB Bacteria

Differentially Culturable Tubercle Bacteria (DCTB) - fail to grow on solid media - resuscitate in liquid culture in the presence or absence of growth stimulatory factors

Persistent and drug tolerant phenotypes

in vivo cohorts

proportion of individuals with DCTB
in sputum



Strain-specific adaptive mechanisms influence DCTB generation and recovery

Aims

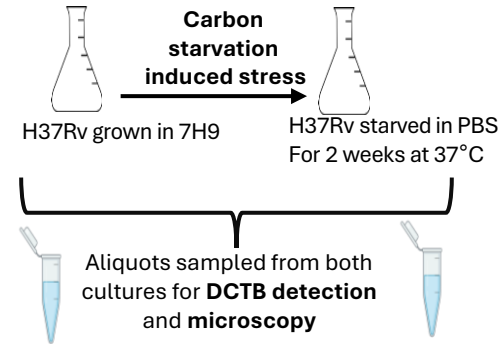


Little known about the capability of different genotype families of *M. tuberculosis* to generate DCTB.

1. Investigated whether Beijing and LAM clinical strains differ in their propensity to generate DCTB and in their resuscitation dynamics following stress.
2. Investigate the modalities and spatial patterns of cell wall expansion during DCTB resuscitation.

Generation and detection of Differentially Culturable Tubercle Bacteria (DCTB)

In vitro - carbon starvation model



MOST PROBABLE NUMBER (MPN) assay

Growth Factor
Culture Filter

$$\sum_{j=1}^k \frac{g_j m_j}{1 - e^{-\lambda m_j}} = \sum_{j=1}^k t_j m_j$$

k denotes the number of dilutions,
 g_j denotes the number of positive (or growth) tubes in the j th dilution,
 m_j denotes the amount of the original sample put in each tube in the j th dilution,
 t_j denotes the number of tubes in the j th dilution.

McCarty, M. H. 1915. *Infect. Dis.* 7:183-211

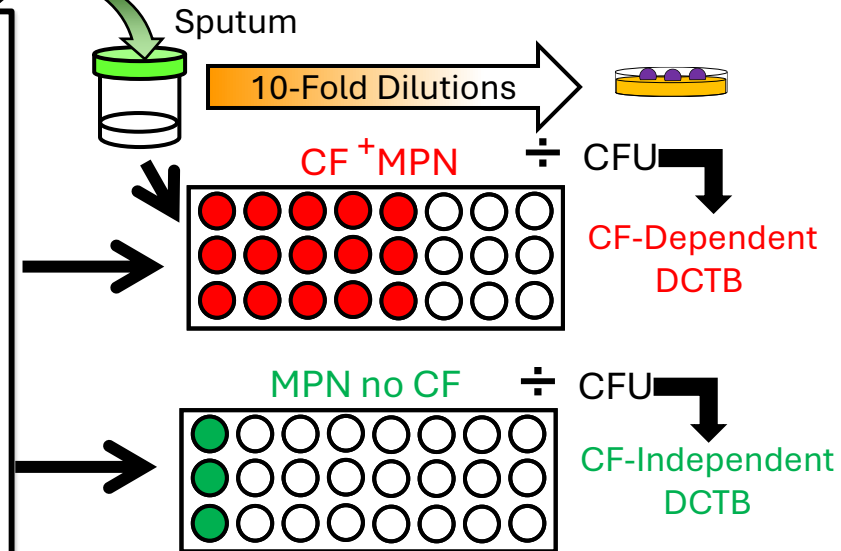
Halvorson, H. O., and N. R. Ziegler. 1933. *Bacteriol.* 25:101-121; 26:331-339; 26:559-567

Haldane, J. B. S. 1939. *Hygiene.* 39:289-293

Cochran, W. G. 1950. *Biometrics* 6:105-116

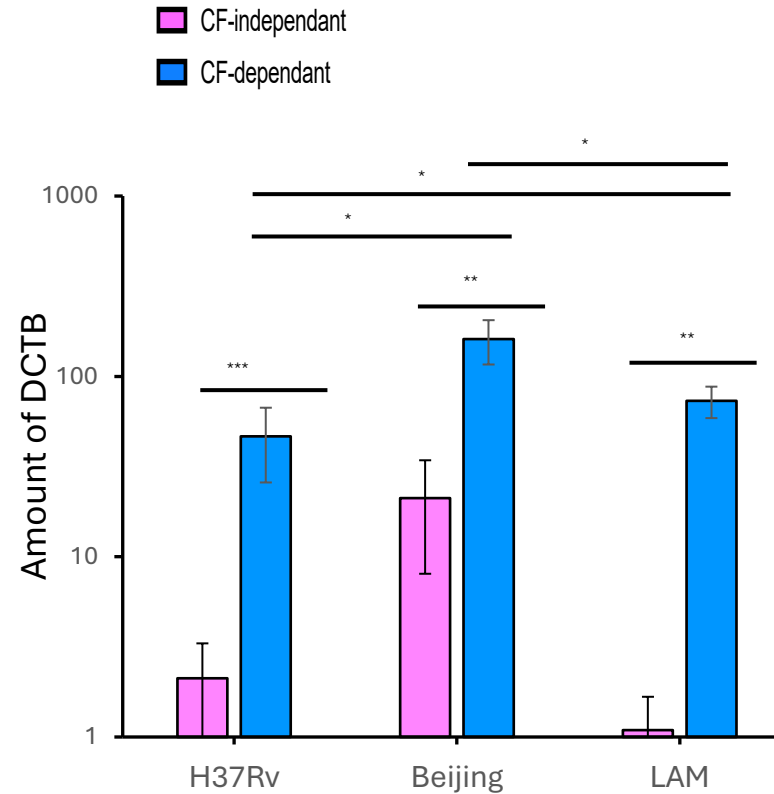
de Man, J. C. 1983. *Eur. J. Appl. Biotechnol.* 17:301-305

Garthright, W. E. and Blodgett, R. J. 2003. *Food Microbiology* 20:439-445



Results

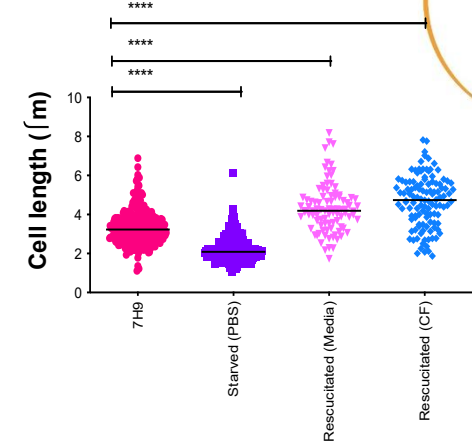
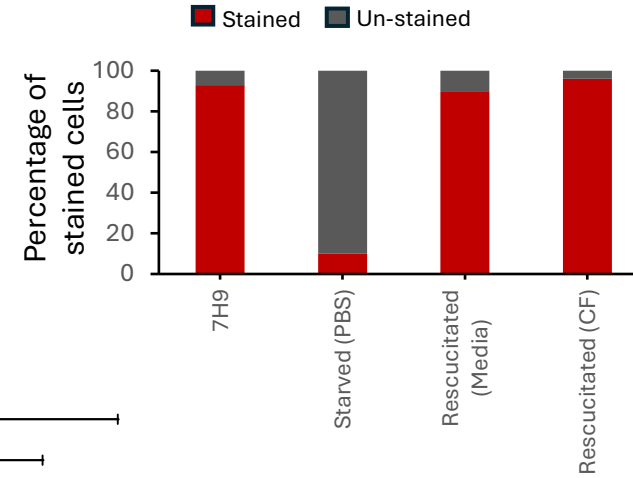
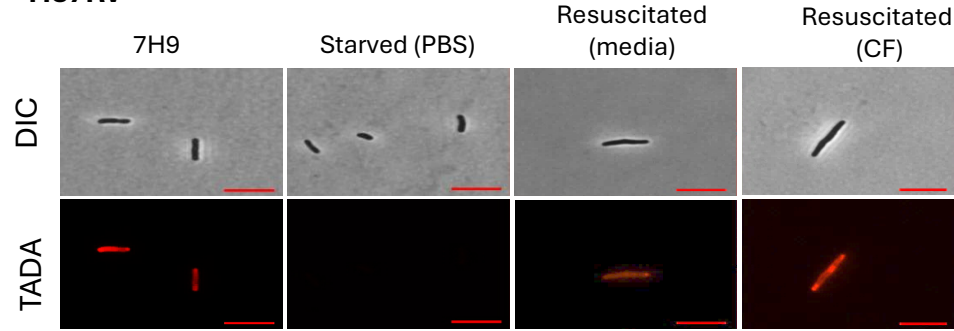
Strain dependent DCTB production



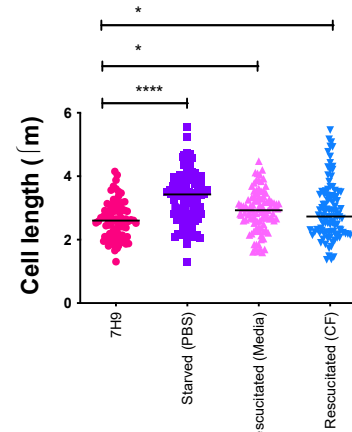
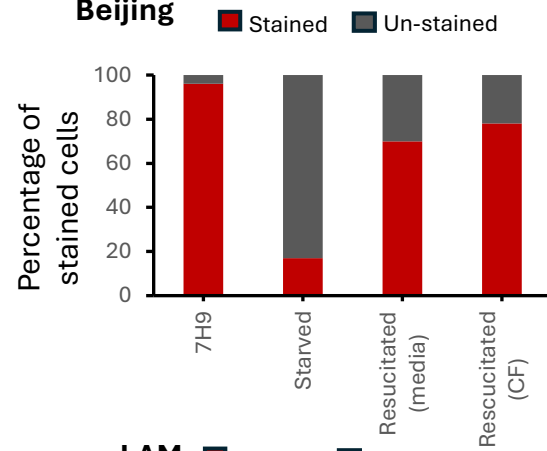
Beijing strains - greater propensity to produce DCTB without CF
LAM strains - dependent on CF for resuscitation.

Microscopy

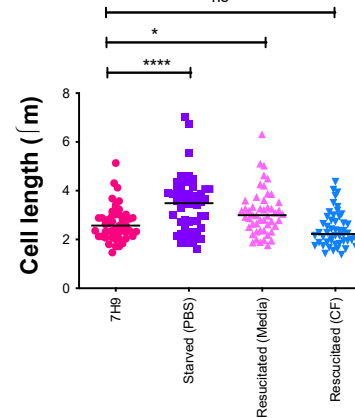
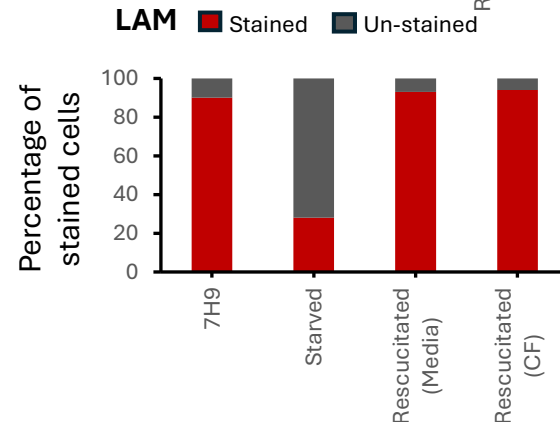
H37Rv



Beijing

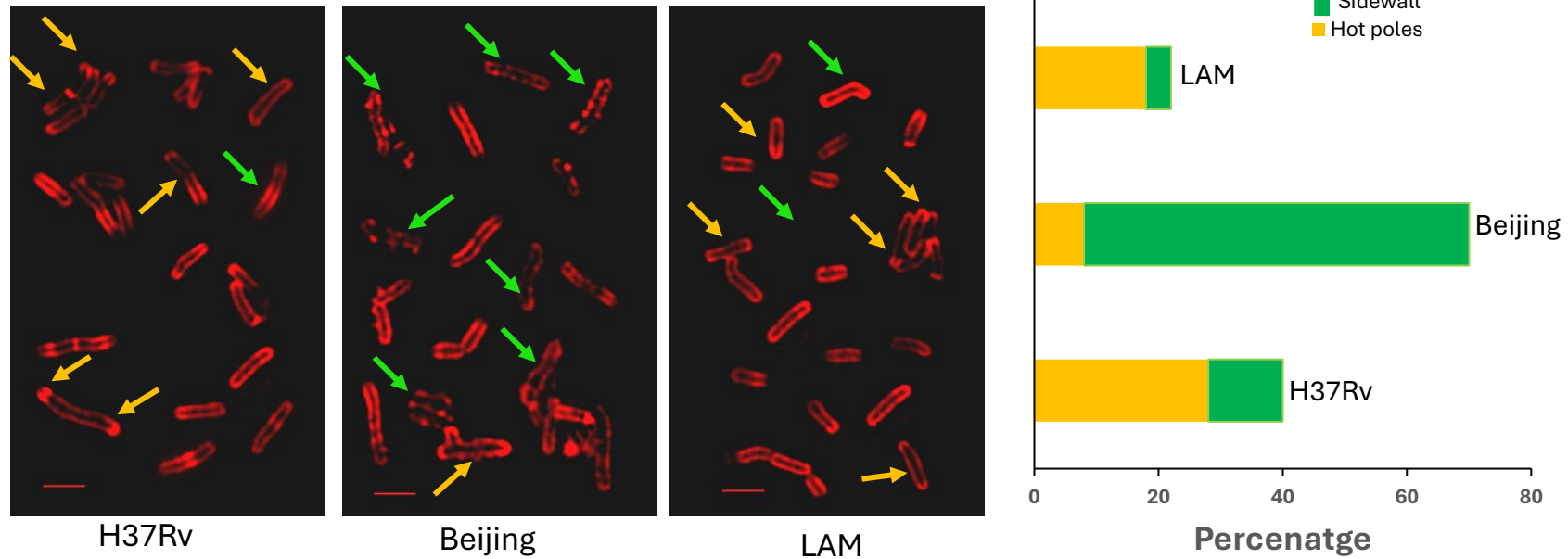


LAM



Starved Beijing and LAM strains show phenotypic differences compared to laboratory strain

Cell wall staining with fluorescent D-amino acids



Strain-specific incorporation patterns, indicating distinct cell surface remodelling during resuscitation between clinical strains

Conclusion

Clinical *M. tuberculosis* strains differ in their capacity to generate DCTB and in their resuscitation characteristics.

Lineage-specific differences may influence bacterial persistence and could have implications for diagnostics, treatment response, TB control strategies and TB treatment success

Acknowledgments



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Kiyasha
Padarath



Astika
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Amanda
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Julian
Peters



Serene
Keenan



Dhanishta
Patel



Limakatso
Lebina



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