Disruption of the *mce* operon from *Streptomyces* affects spore resistance and results in precocious germination

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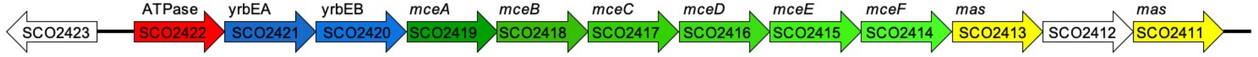

Fig. 1: The mammalian cell entry operon of *Streptomyces coelicolor*

The mammalian cell entry operon (*mce*) of *Streptomyces coelicolor* is composed of 9 core genes, including an ATPase, two integral membrane proteins and six putative secreted proteins. Mce proteins were originally discovered in *Mycobacterium tuberculosis* (*Mtb*) as virulence factors and have since been identified in a wide range of bacteria, being particularly prevalent amongst Actinobacteria, where *mce* operons have a conserved structure and high sequence homology[1].

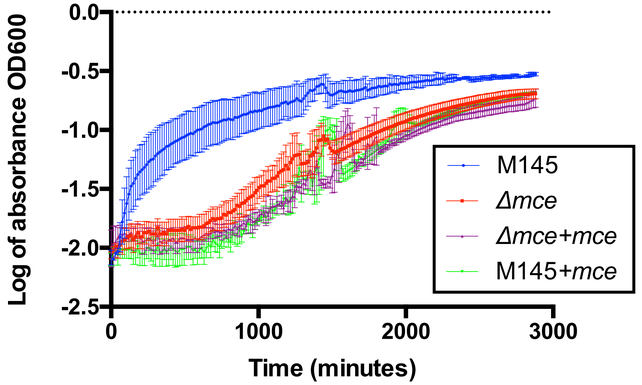

Fig. 1: Strains grown in minimal media supplemented with 0.193% cholesterol.

The *mce* operon of *S. coelicolor* may encode an ABC transporter for cholesterol import

Mce proteins have roles in lipid transport. Mce protein in *E. coli* are involved in transporting lipids across the periplasm[2]. In Actinobacteria, *mce* operons appear to encode ABC transporter assemblies for lipid transport in which the Mce proteins appear to function as the substrate binding proteins of the transporter. It has been shown conclusively that the Mce4 system of Rhodoccocus and *Mtb* is a cholesterol importer, whilst Mce1 in *Mtb* appears to be a fatty acid importer[3].

The function of the single *mce* operon in *S. coelicolor* remains uncertain, although results from this study suggest it may be a cholesterol importer. When grown in minimal media containing 0.193% cholesterol as the sole carbon source, growth of an *mce* knockout (Δ*mce*) was impaired compared to the WT *S. coelicolor* strain, M145. Growth of strains in 96-well plates was measured via absorbance (OD_{600}) using a Bio-Tek Multi-Detection Microplate Reader Synergy HT over a period of 48 hours. Readings were taken every 15 minutes.

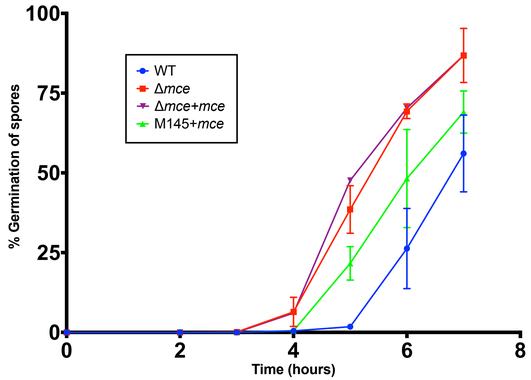

Fig. 2: Germination of WT and mutant *S. coelicolor* spores

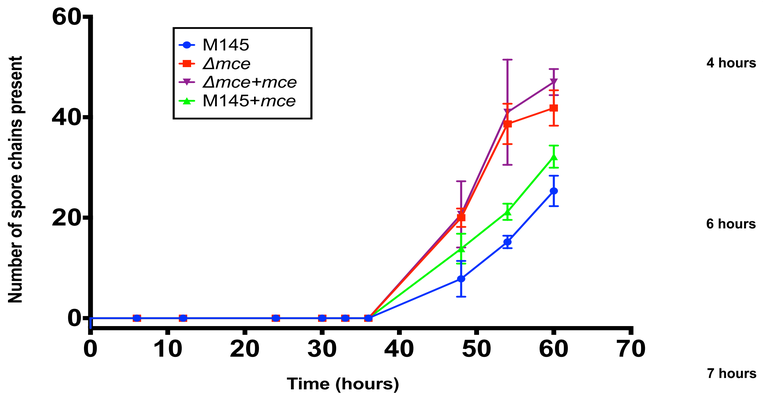

Fig. 3: Spore chain production of WT and mutant *S. coelicolor*

Deletion of the *mce* operon in *S. coelicolor* results in precocious germination and sporulation

Deletion of the *mce* operon was shown to produce an altered spore envelope in *S. coelicolor*. SEM images of Δ*mce* spores showed spore envelopes were wrinkled and more prone to collapse, with appendages upon the spore chain indicative of precocious germination[4]. A germination assay was carried out as described by Haiser *et al*, 2009[5]. Results of the assay showed that spores of the Δ*mce* mutant display precocious germination, producing germ-tubes earlier than WT *S. coelicolor* spores. To determine whether precocious germination led to precocious sporulation impression mount assays were performed and showed that the Δ*mce* strain displayed precocious germination when compared to M145 *S. coelicolor.* Strains were grown on mannitol-soya flour agar and phase contrast images were taken using a Nikon Eclipse TE-2000S inverted microscope equipped with a 100x objective.

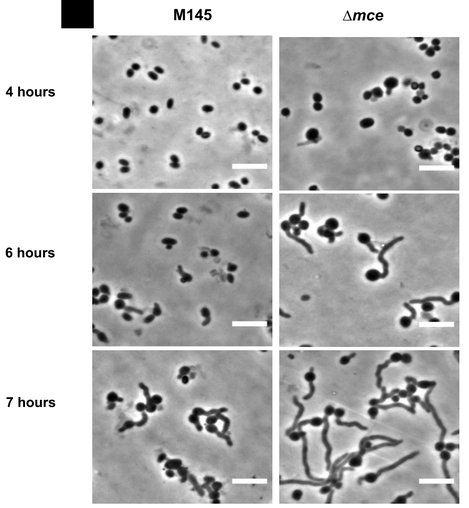

Fig. 4: Spores of the Δ*mce* mutant produce germ-tubes earlier than M145 spores.

Phase contrast images of M145 and Δ*mce* spores taken using a Nikon Eclipse TE-2000S microscope equipped with 100x objective.

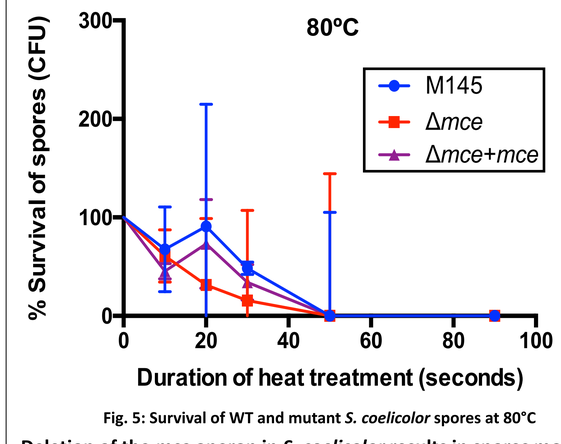

Fig. 5: Survival of WT and mutant *S. coelicolor* spores at 80°C

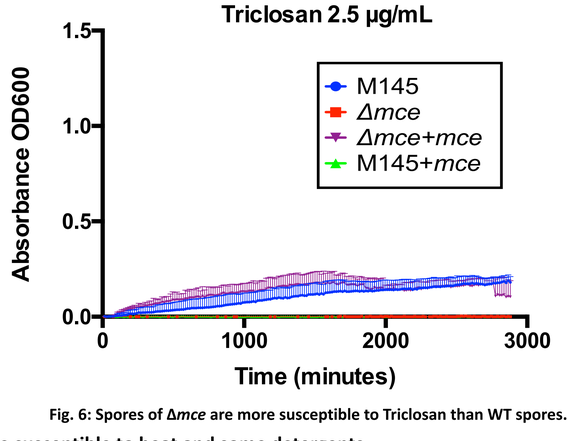

Fig. 6: Spores of Δ*mce* are more susceptible to Triclosan than WT spores.

Deletion of the *mce* operon in *S. coelicolor* results in spores more susceptible to heat and some detergents

As spores of the Δ*mce* mutant possess an altered spore envelope, which is the protective layer, it was hypothesised that Δ*mce* spores may be more susceptible to heat. Spores were exposed to a range of temperatures with results of heat kill assays showing Δ*mce* spores did not display heat activation and were more susceptible to heat then WT spores. Spores of the Δ*mce* mutant were also more susceptible to triclosan, lauryl sulfobetaine, capryl sulfobetaine and myristyl sulfobetaine.

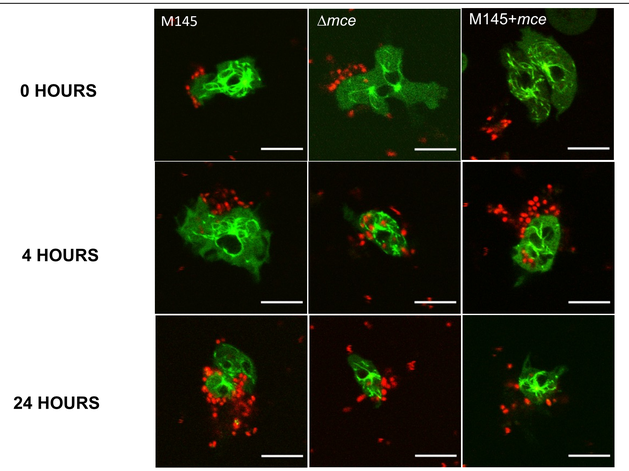

Fig. 7: 125,000 GF AX2-GFP-tubA *Dictyostelium* with 1.8 x 10^8 M145, Δ*mce*, or M145+*mce* spores expressing mCherry, in 1mL Sorensen's buffer. Images were taken using an Olympus FV1000 confocal microscope with a 60X objective. Excitation of 488nm and 534 simultaneously. Scale bar represents 5 nm.

Hypervirulence of the Δ*mce* mutant towards *Acanthamoeba* had been previously observed due to what appeared to be precocious germination inside the food vacuole[4]. Co-culture of *S. coelicolor* spores and *Dictyostelium discoideum* showed that Δ*mce* spores were not virulent towards *Dictyostelium* due to the rapid rate of *Dictyostelium* phagocytosis.

References

1. Casali, N. and Riley, L. W. (2007) 'A phylogenomic analysis of the Actinomycetales mce operons', BMC Genomics. doi: 10.1186/1471-2164-8-60. 2. Isom, G. L. et al. (2020) 'Ltr8 Structure Reveals a Tunnel for Lipid Transport across the Bacterial Envelope', Cell. doi: 10.1016/j.cell.2020.03.030. 3. Nazarova, E. V. et al. (2019) 'The genetic requirements of fatty acid import by mycobacterium tuberculosis within macrophages', eLife. doi: 10.7554/eLife.43621.
4. Clark, L. C. et al. (2013) 'Mammalian cell entry genes in Streptomyces may provide clues to the evolution of bacterial virulence', Scientific Reports. doi: 10.1038/srep01109. 5. Haiser, H. J., Yousef, M. R. and Elliot, M. A. (2009) 'Cell wall hydrolases affect germination, vegetative growth, and sporulation in Streptomyces coelicolor', Journal of Bacteriology. doi: 10.1128/JB.00767-09.