Mycobacterium marinum mmar_2318 and *mmar_2319* are Responsible for Lipooligosaccharide Biosynthesis and Virulence towards *Dictyostelium*

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Background

Myobacteria possess a unique lipid-rich cell wall that is important in directing host-pathogen interactions and confers resistance to many therapeutic agents. *Mycobacterium marinum* can cause a systemic tuberculosis-like infection in fish, ectotherms and human, a process that involves persistent growth within macrophages. *Dictyostelium*, a free-living amoeba, serves as a macrophage-like system for studying bacteria-host interactions. A well-established model system using *Dictyostelium discoideum* was introduced for studying the interactions between phagocytes and bacteria

Material & Method

We constructed a *M. marinum* mutant library (1728 transposon mutants of the NTUH-M6094 strain) by transposon mutagenesis and used a *Dictyostelium* phagocytosis plaque screening model to identify genetic loci involved in *M. marinum* virulence. The unmarked deletion and complementation of target genes were generated. Then their virulence to *Dictyostelium*, colony morphology, glycolipid profile, as well as entry/replication inside *Dictyostelium* and mammalian macrophages (J774a.1 and THP-1) were analyzed.

Conclusion

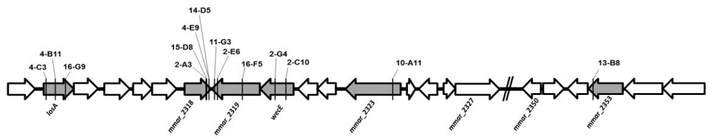
Although *mmar_2319* has been reported to involve LOS biosynthesis in a previous study, we identified a new gene, *mmar_2318* that is also involved in the biosynthesis of LOS. Deletion of *mmar_2318* or *mmar_2319* both exhibits reduction of virulence towards *Dictyostelium*, and increased entry into THP-1 cells.

Result

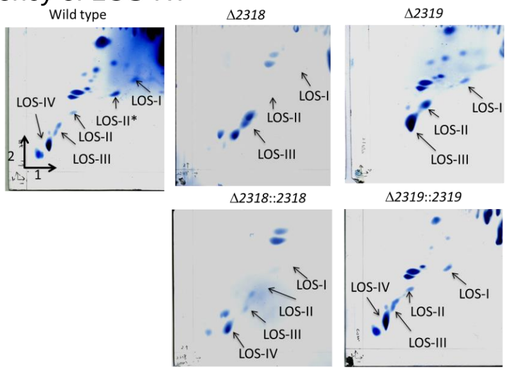
The screening identified a total of 30 mutants permissive for *Dictyostelium* growth. These mutants revealed interruptions in 20 distinct loci.

Mutant No.	Genes inserted by transposon	Putative function	homologues in M. tuberculosis H37Rv
8-H11	MMAR0328	secreted antigen 85-C	Rv0129c
12-E12	MMAR0838	hypothetical protein	
14-C12	MMAR0932	PPE family protein	PPE24
14-F4	MMAR1514	PPE family protein, PPE51_1	PPE51
12-B3, 12-E1	upstream of MMAR1594 and MMAR1595	MMAR1594: PE-PGRS family protein MMAR1595:O-methyltransferase	PE-PGPS55 Rv3767c
15-B4	MMAR1639	PPE family protein	PPE8
12-C12	MMAR1877	conserved transmembrane transport protein	
4-B11, 4-C3, 16-G9	MMAR2313	losA, glycosyltransferase	Rv1500
14-D5, 2-A3, 4-E9, 15-D8	MMAR2318	conserved hypothetical protein	Rv1502
16-F5, 11-G3, 2-E6	MMAR2319	conserved hypothetical transmembrane protein	
2-C10, 2-G4	MMAR2320	wecE, pyridoxal phosphate-dependent enzyme	
10-A11	MMAR2323	conserved hypothetical transmembrane protein	
13-B8	MMAR2353	UDP-glycosyltransferase	Rv1524
12-E11	upstream of MMAR2684	PPE family protein	PPE32
18-G4	MMAR3183	hypothetical alanine rich protein	
18-D7	upstream of MMAR3375	conserved hypothetical protein	
17-A5, 18-H5, 12-A1	MMAR4263	conserved hypothetical protein	
13-G8	MMAR4621	PPE family protein	PPE8
5-H1	MMAR4630	membrane-bound C-5 sterol desaturase	Rv1814

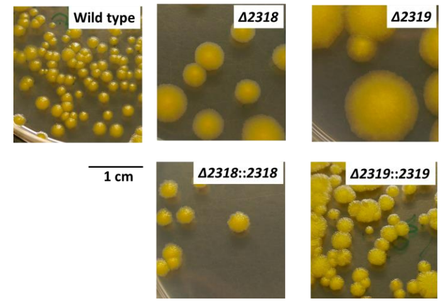
Of the 20 loci, six genes (*losA*, *mmar_2318*, *mmar_2319*, *wecE*, *mmar_2323* and *mmar_2353*) were located in the lipooligosaccharide (LOS) synthesis cluster.



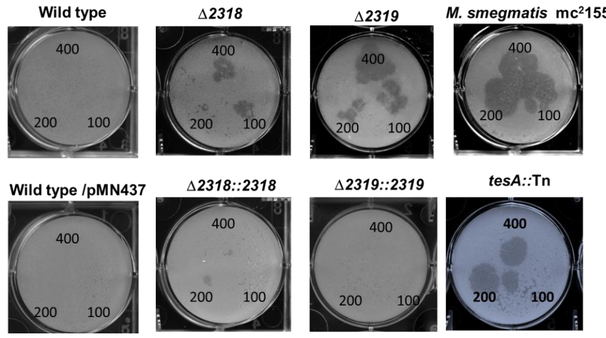
Two-dimensional thin-layer chromatography (2D-TLC) glycolipid profiles revealed that deletion of *mmar_2318* or *mmar_2319* resulted in the accumulation of LOS-III and deficiency of LOS-IV.



The *mmar_2318* or *mmar_2319* deletion mutants also exhibited rough colony morphology and bigger colony size in comparison with wild type.



Deletion and complementation of *mmar_2318* or *mmar_2319* confirmed that these genes both contributed to virulence towards *Dictyostelium* but not entry and replication inside *Dictyostelium*.



Co-incubation with a murine macrophage cell line J774a.1 or PMA-induced human monocytic cell line THP-1 demonstrated that *mmar_2318* or *mmar_2319* deletion mutant could grow in macrophages, and their initial entry rate was not affected in J774a.1 but significantly increased in THP-1.