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Molecular engineering of the mycobacteriophage endolysin and holin proteins for the development of high virulence phages

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Mycobacteriophages rapidly infect and kill mycobacteria. The peptidoglycan hydrolase, Lysin A (LysA), coded by one of the most potent mycobacteriophages, D29, carries two catalytic domains at its N-terminus and a cell wall binding domain at its C-terminus. Our bioinformatics data suggested that not all mycobacteriophages harbor lysozyme-like catalytic domain (LD) in their endolysins. Hence, we explored the importance of LD of LysA in phage physiology. We had previously identified an R198A substitution that causes inactivation of LD, when it is present alone on a polypeptide. We now show that upon incorporation of an identical mutation (i.e. R350A) in the full-length LysA, the protein demonstrates substantially reduced activity *in vitro*, even in the presence of N-terminal catalytic domain, and has poor mycobacterial cell lysis ability when it is expressed in *M. smegmatis*. However, a mutant D29 phage harbouring this substitution ($D29^{R350A}$) in its LysA protein shows a significant increase in the burst size and the plaque diameter. Furthermore, we show that the holin-coding gene of D29 phage is "somewhat" dispensable for the survival of phage. The holin knock-out of D29 shows delayed lysis with low phage titre. Taken together, our data show the importance of an intact LD region in D29 LysA PG hydrolase and the holin protein. These results also indicate an evolutionary advantage over other phages that lack such domainin their endolysins.While it remains to be seen if an introduction of LD in the endolysin of mycobacteriophages that originally lack it will make these phages more virulent, the holin-less D29 is being pursued further for the development of mutant phages with engineered holin protein for the development of hypervirulent phages.