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Identification of a novel peptidoglycan hydrolase CwlM in *Mycobacterium tuberculosis*

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Abstract

Mycobacterium tuberculosis is a major global pathogen whose threat has increased with the emergence of multidrug-resistant strains. The cell wall of *M. tuberculosis* is thick, rigid, and hydrophobic, which serves to protect the organism from the environment and makes it highly impermeable to conventional antimicrobial agents. There is little known about cell wall autolysins (also referred to as peptidoglycan hydrolases) of mycobacteria. We identified an open reading frame (Rv3915) in the *M. tuberculosis* genome designated *cwlM* that appeared consistent with a peptidoglycan hydrolase. The 1218-bp gene was amplified by PCR, cloned and expressed in *E. coli* strain HMS174(DE-3), and its gene product, a 47-kDa recombinant protein, was purified and partially characterized. Purified CwlM was able to lyse whole mycobacteria, release peptidoglycan from the cell wall of *Micrococcus luteus* and *Mycobacterium smegmatis*, and cleave *N*-acetylmuramoyl-L-alanyl-D-isoglutamine, releasing free *N*-acetylmuramic acid. These results indicate that CwlM is a novel autolysin and identify *cwlM* as the first, to our knowledge, autolysin gene identified and cloned from *M. tuberculosis*. CwlM offers a new target for a unique class of drugs that could alter the permeability of the mycobacterial cell wall and enhance the effectiveness of treatments for tuberculosis.
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1. Introduction

Mycobacterium tuberculosis, the microbe that causes tuberculosis (TB), is the leading cause of death from infection worldwide. In recent years, multidrug-resistant strains have emerged as a new threat in both developed and developing countries, and are strongly associated with mortality in AIDS patients. The *M. tuberculosis* cell envelope is unusually thick, rigid, and waxy, making it impermeable to many conventional antimicrobial drugs and thereby limiting the number of agents effective against tuberculosis [1–3]. Bacterial peptidoglycan hydrolases, also called autolysins, solubilize the bacterial cell wall by hydrolyzing specific bonds in cell wall peptidoglycan (Fig. 1). These enzymes, which are classified into glycosidases (including muramidases and glucosaminidases), amidases and peptidases [4,5], have been proposed to have roles in bacterial surface growth, cell division [6,7] and bacterial pathogenesis, including adhesion and invasion of host cells [8–12].

Genes encoding cell wall autolysins have been extensively studied in many bacteria [9,13–25]; however, little is known about the autolysins of mycobacteria, including *M.*

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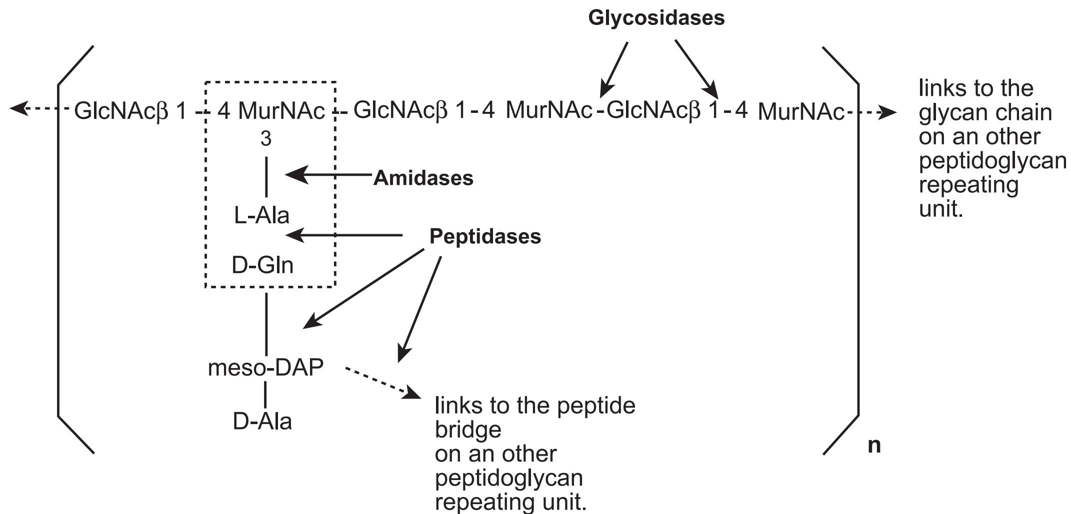

Fig. 1. Structure of bacterial cell wall peptidoglycan, and the proposed cleavage sites of autolysins. *meso*-DAP, *m*-diaminopimelic acid; [ ]$_n$ represents the basic repeating unit of peptidoglycan; solid arrows indicate the cleavage sites of typical autolysins including *N*-acetylmuramoyl-L-alanine amidase; dashed arrows indicate cross-links to adjacent peptidoglycan units; the structure inside the box is MAG, the substrate used for determination of the enzymatic products.

tuberculosis. In 1977, two autolysins, an amidase and an endopeptidase, were described in *Mycobacterium smegmatis* [26]; however, their physical, biochemical, and enzymatic properties were not defined. Li et al. [27] described the partial characterization of an autolysin from *Mycobacterium phlei*. We have previously reported that ethambutol, an anti-TB drug, stimulates an endogenous enzymatic activity that cleaves the cell wall of *M. smegmatis*. In the current study, we identified an open reading frame *cwlM* (Rv3915, accession number: AAK48399) in the *M. tuberculosis* genome [28,29], which had 31% homology and 21% identity with the *cwlB* gene of *Bacillus subtilis* [20] (Fig. 2).

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Mtub CwlM    1                   MP------SPRREDGD-------------------ALRCG--  15
Bsub CwlB    1 LRSYIKVLTMCFLGLILFVPTALADNSVKRVGGSNRYGTAVQISKQMYSTASTAVIVGGS  60
                                 .*      * .*  *                    *.  *

Mtub CwlM   16 DRSAAVTEIRAALTALGMLDHQEED-----------LTTGRNVALELFDAQLDQAVRAFQ  64
Bsub CwlB   61 SYADAISAAPLAYQKNAPLLYTNSDKLSYETKTRLKEMQTKNVIIVGGTPAVSSNTANQI 120
                 . *..    *      *     *               .** .      .

Mtub CwlM   65 QHRGLLVDGIVGEATYRALKEASYRLGARTLYHQFGAPLYGDDVATL------------- 111
Bsub CwlB  121 KSLGISIKRIAGSNRYDTAARVAKAMGATSKAVILNGFLYADAPAVIPYAAKNGYPILFT 180
               .  *. .  * *   * .    .  .** .     .  ** *  * .

Mtub CwlM  112 ---------QARLQDLGFYTGLVDGHFG---LQTHNALMSYQREYG-----LAADGICGP 154
Bsub CwlB  181 NKTSINSATTSVIKDKGISSTVVVGGTGSISNTVYNKLPSPTRISGSNRYELAANIVQKL 240
                         . ..* *  . .* *  *       * * *  *  *     ***  .

Mtub CwlM  155 ETLRSLYFLSS------RVSGGSPHAIREEELVRSSGPKLS------------------- 189
Bsub CwlB  241 NLSTSTVYVSNGFSYPDSIAGATLAAKKKQSLILTNGENLSTGARKIIGSKNMSNFMIIG 300
                   *  ..*        ..* .  * . . *. . *  **

Mtub CwlM  190 ------------------GKRIIIDPGRGGVDHGLIAQGPAGPISEADLLWDLASRLEGR 231
Bsub CwlB  301 NTPAVSTKVANQLKNPVVGETIFIDPGHGDQDSGAIGNG----LLEKEVNLDIAKRVNTK 356
                                *  * ****.*  * * * .*    . * ..  *.* *.  .

Mtub CwlM  232 MAAIGMETHLSRPTNRSPSDAERAATANAVGADLMISLRCETQTSLAANGVASFHFGNSH 291
Bsub CwlB  357 LNASGALPVLSRSNDTFYSLQERVNKAASAQADLFLSIHANANDSSSPNGSETYYDTTYQ 416
              . * *    *** .    *  **   * .  *** .*... .. * . **  ..   . .

Mtub CwlM  292 GSVSTIGRNLADFIQREVVARTGLRDCRVHGRTWDLLRLTRMPTVQVDIGYITNPHDRGM 351
Bsub CwlB  417 AANS---KRLAEQIQPKLAANLGTRDRGVKTAAFYVIKYSKMPSVLVETAFITNASDASK 473
               . *   . **. **  . *  * **  *.  .  ... ..**.* *.  .***  *

Mtub CwlM  352 LVSTQTRDAIAEGILAAVKRLYLLGKNDRPTGTFTFAELLAHELSVERAGRLGGS      406
Bsub CwlB  474 LKQAVYKDKAAQAIHDGTVSYYR                                      496
              *  .  .*  *. *       *
```

Fig. 2. Alignment of the deduced amino acid sequence of *M. tuberculosis cwlM*, the putative cell wall autolysin, with that of *B. subtilis cwlB*, a known cell wall autolysin. Identical residues are represented by "*" and conservative substitutions by ".". The alignment was produced using the ClustalW program.

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