AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Spectrum

LETTER TO THE EDITOR

Mycobacterium abscessus Tetracycline-Modifying Monooxygenase *MAB_1496c* Appears Not to Be Sufficient to Cause Resistance to Tetracycline When Expressed in *Mycobacterium smegmatis*

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KEYWORDS tetracycline, mycobacteria, antibiotic resistance, *Mycobacterium*, drug resistance mechanisms, *Mycobacterium abscessus*

Recently, a gene in *Mycobacterium abscessus* (*MAB_1496c*) coding for a FAD-binding monooxygenase protein was described (1, 2). It was shown that the protein bears resemblance to tetracycline destructases (TetX) from other bacteria (3), that deletion of the gene rendered *M. abscessus* substantially more sensitive to tetracycline, and that this resistance was unrelated to the *whiB7* pathway (4, 5). The protein was also shown to modify tetracycline *in vitro* by monooxygenation and to be inhibited by a low concentration of the tetracycline analog anhydrotetracycline (ATc) (1, 6). These findings suggested that the protein coded by *MAB_1496c* can induce tetracycline resistance in those mycobacteria that are naturally more sensitive to tetracycline than *M. abscessus*, such as *M. smegmatis* and *M. tuberculosis* (7, 8). This could be used for research and biotechnology purposes, such as *in vitro* positive selection (9, 10).

We therefore opted to examine whether the recombinant expression of *MAB_1496c* (MabTetX protein) in *M. smegmatis* would substantially increase the bacterium's MIC to tetracycline, to the point where it could be used for *in vitro* selection. We cloned *MAB_1496c* into the blunt HpaI site in pDB32—a multicopy, kanamycin-selected, episomal vector (11). The gene was PCR amplified from *M. abscessus* genomic DNA (gDNA) using the following primers: forward, 5′-ACAGTGGTGATCGCCGGGGCCGGCC-3′; reverse, 5′-CATCTAGACAACACGGGCGAG ATA-3′. Cloning into the HpaI site of this vector places the gene in frame with a hemagglutinin (HA) tag at the N terminus and under a constitutive mycobacterial optimized promoter (MOP). As the native promoter of *MAB_1496c* is regulated by *MAB_1497c* (5), a *tetR*-like transcription regulator (and is induced by tetracycline), placing the recombinant construct under a constitutive promoter avoids problems resulting from regulation of expression. The genetic structure of the construct is shown in Fig. 1A for clarity. Correct in-frame cloning with the HA tag was confirmed by Sanger sequencing, and the plasmid (pDB451) was electroporated into wild-type (WT) *M. smegmatis* MC^2 155 to produce mDB335. The expression of the full-length MabTetX protein was verified by Western blotting using anti-HA antibodies (Abcam), showing a protein at the expected size of 53.2 kDa (Fig. 1B). However, when we tested the TetX-expressing mutants for tetracycline sensitivity, we found no difference between their MICs and that of WT *M. smegmatis* with an empty vector on either 7H10 agar plates (Fig. 1Ci) or in 7H9/glycerol broth (Fig. 1Cii). The MIC also remained well below that of WT *M. abscessus* (Fig. 1D). Of note, when we examined the MIC to tetracycline in the *E. coli* bacteria used for the cloning (DH5-α), we again found bacteria with the plasmids to have the same MIC to tetracycline as "WT" *E. coli* bacteria, with the empty control vectors (data not shown).

To summarize, despite the convincing results of the carefully performed experiments reported in the original description (1), it appears that the full picture is more complex,

Editor Gyanu Lamichhane, Johns Hopkins University School of Medicine

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The authors declare no conflict of interest.

Published 27 July 2022

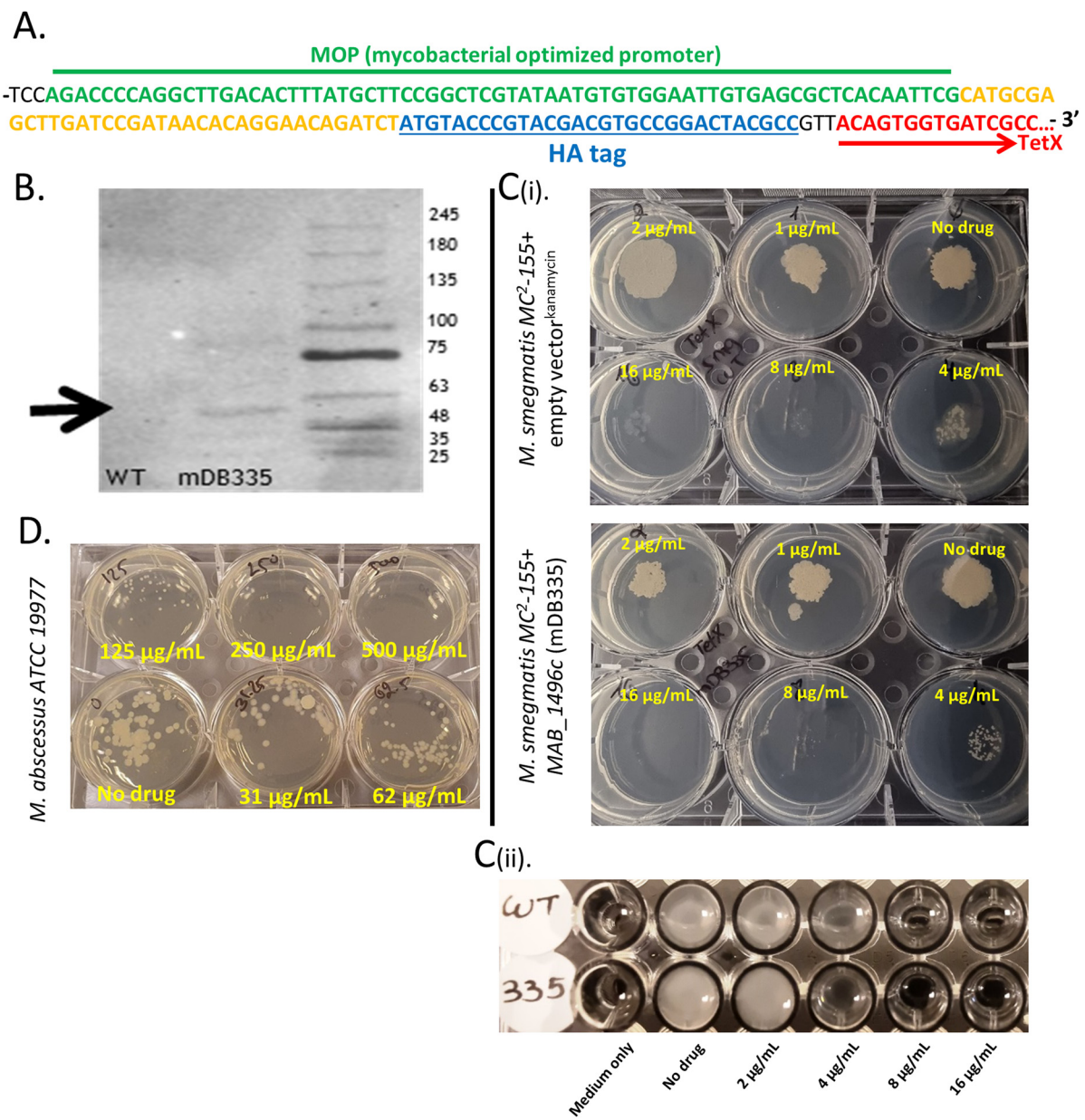

FIG 1 (A) Genetic sequences of the MOP promoter (green) and untranslated region (UTR) (yellow) driving the expression of an HA-tagged (blue) TetX protein (red). (B) Western blot demonstrating the full-length (53.2-kDa) HA-TetX protein in mDB335 in comparison to WT bacteria. (Ci) Approximately 250 CFU of *M. smegmatis*$^{\text{empty kana vector}}$ (top) or mDB335 (bottom) was plated onto 7H10 agar plates with increasing concentrations of tetracycline. (Cii) WT *M. smegmatis* and *M. smegmatis* mDB335 were grown in 7H9/glycerol broth with the designated concentrations of tetracycline. (D) *M. abscessus* ATCC 19977 was plated onto 7H10 agar plates with the designated concentrations of tetracycline.

and expression of the MAB_1496c protein is, by itself, not sufficient to induce tetracycline resistance in *M. smegmatis*. This could be due to a requirement for another, yet unidentified cofactor, low activity of the protein in the *M. smegmatis* context, retained antimicrobial activity of the oxygenated tetracycline product against *M. smegmatis*, or misfolding of the protein in *M. smegmatis*, resulting in a non- or poorly functional, albeit full-size, protein.

ACKNOWLEDGMENT

We thank Eyal Gur, Ben Gurion University, for technical assistance.

REFERENCES

1. Rudra P, Hurst-Hess K, Lappierre P, Ghosh P. 2018. High levels of intrinsic tetracycline resistance in Mycobacterium abscessus are conferred by a tetracycline-modifying monooxygenase. Antimicrob Agents Chemother 62:e00119-18. https://doi.org/10.1128/AAC.00119-18.

2. Yang W, Moore IF, Koteva KP, Bareich DC, Hughes DW, Wright GD. 2004. TetX is a flavin-dependent monooxygenase conferring resistance to tetracycline antibiotics. J Biol Chem 279:52346–52352. https://doi.org/10.1074/jbc.M409573200.
3. Forsberg KJ, Patel S, Wencewicz TA, Dantas G. 2015. The tetracycline destructases: a novel family of tetracycline-inactivating enzymes. Chem Biol 22:888–897. https://doi.org/10.1016/j.chembiol.2015.05.017.
4. Hurst-Hess K, Rudra P, Ghosh P. 2017. Mycobacterium abscessus WhiB7 regulates a species-specific repertoire of genes to confer extreme antibiotic resistance. Antimicrob Agents Chemother 61:e01347-17. https://doi.org/10.1128/AAC.01347-17.
5. Luthra S, Rominski A, Sander P. 2018. The role of antibiotic-target-modifying and antibiotic-modifying enzymes in Mycobacterium abscessus drug resistance. Front Microbiol 9:2179. https://doi.org/10.3389/fmicb.2018.02179.
6. Park J, Gasparrini AJ, Reck MR, Symister CT, Elliott JL, Vogel JP, Wencewicz TA, Dantas G, Tolia NH. 2017. Plasticity, dynamics, and inhibition of emerging tetracycline resistance enzymes. Nat Chem Biol 13:730–736. https://doi.org/10.1038/nchembio.2376.
7. Brown-Elliott BA, Nash KA, Wallace RJ. 2012. Antimicrobial susceptibility testing, drug resistance mechanisms, and therapy of infections with nontuberculous mycobacteria. Clin Microbiol Rev 25:545–582. https://doi.org/10.1128/CMR.05030-11.
8. Park S, Kim S, Park EM, Kim H, Kwon OJ, Chang CL, Lew WJ, Park YK, Koh W-J. 2008. In vitro antimicrobial susceptibility of Mycobacterium abscessus in Korea. J Korean Med Sci 23:49–52. https://doi.org/10.3346/jkms.2008.23.1.49.
9. Yang F, Njire MM, Liu J, Wu T, Wang B, Liu T, Cao Y, Liu Z, Wan J, Tu Z, Tan Y, Tan S, Zhang T. 2015. Engineering more stable, selectable marker-free autoluminescent mycobacteria by one step. PLoS One 10:e0119341. https://doi.org/10.1371/journal.pone.0119341.
10. Tomioka H, Namba K. 2006. Development of antituberculous drugs: current status and future prospects. Kekkaku 81:753–774. (In Japanese.)
11. Barkan D, Liu Z, Sacchettini JC, Glickman MS. 2009. Mycolic acid cyclopropanation is essential for viability, drug resistance, and cell wall integrity of Mycobacterium tuberculosis. Chem Biol 16:499–509. https://doi.org/10.1016/j.chembiol.2009.04.001.