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# Ectopic Expression of Rv0023 Mediates Isoniazid/Ethionamide Tolerance via Altering NADH/NAD$^+$ Levels in *Mycobacterium smegmatis*

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Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*Mtb*) accounts for nearly 1.2 million deaths per annum worldwide. Due to the emergence of multidrug-resistant (MDR) *Mtb* strains, TB, a curable and avertable disease, remains one of the leading causes of morbidity and mortality. Isoniazid (INH) is a first-line anti-TB drug while ethionamide (ETH) is used as a second-line anti-TB drug. INH and ETH resistance develop through a network of genes involved in various biosynthetic pathways. In this study, we identified Rv0023, an *Mtb* protein belonging to the xenobiotic response element (XRE) family of transcription regulators, which has a role in generating higher tolerance toward INH and ETH in *Mycobacterium smegmatis* (*Msmeg*). Overexpression of Rv0023 in *Msmeg* leads to the development of INH- and ETH-tolerant strains. The strains expressing Rv0023 have a higher ratio of NADH/NAD$^+$, and this physiological event is known to play a crucial role in the development of INH/ETH co-resistance in *Msmeg*. Gene expression analysis of some target genes revealed reduction in the expression of the *ndh* gene, but no direct interaction was observed between Rv0023 and the *ndh* promoter region. *Rv0023* is divergently expressed to *Rv0022c* (*whiB5*) and we observed a direct interaction between the recombinant Rv0023 protein with the upstream region of *Rv0022c*, confirmed using reporter constructs of *Msmeg*. However, we found no indication that this interaction might play a role in the development of INH/ETH drug tolerance.

**Keywords: XRE family of protein, *whiB5*, isoniazid resistance, ethionamide resistance, transcription regulation**

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## INTRODUCTION

Tuberculosis (TB) remains a major cause of death worldwide and the leading cause by a single infectious agent (World Health Organisation, 2018). Even though the disease can be cured and managed by several multidrug regimens, the emergence of multidrug-resistant (MDR) TB is proving to be a major challenge for complete eradication of the disease. Worldwide, MDR TB constitutes 3.5% of new TB cases and 18% of previously treated cases (World Health Organisation, 2018). To overcome these challenges and to better counter resistance in *Mycobacterium tuberculosis* (*Mtb*), understanding the mechanisms and deciphering the pathways majorly responsible for generating resistance are greatly required.