DATE
Nov 19, 2022,

Identification of a mycobacterial hydrazidase, an isoniazid-hydrolyzing enzyme

Arata Sakiyama, Department of Bacteriology, Osaka City University Graduate School of Medicine

E-mail: d19ma003@st.osaka-cu.ac.jp

Abstract

Background: Isoniazid (INH), a first-line anti-tuberculosis drug, shows strong bactericidal effect on *Mycobacterium tuberculosis*. However, INH cannot be used for infection with nontuberculous mycobacteria (NTM) because of their inherent resistance to this drug, the mechanism of which is still unclear. Here, we focus on an enzyme that hydrolyzes INH to isonicotinic acid and hydrazine. This enzyme was first reported in 1962 as a possible INH resistance factor of *M. avium*. Despite its importance, no studies have attempted to reveal its identity. In this study we aimed to identify an INH-hydrolyzing enzyme from one of NTM species as the first step toward revealing its physiological role.

Methods: *M. smegmatis* MC^{2}155, a model strain for studying mycobacteria, was chosen as the subject for our study. The strain was grown in Middlebrook 7H9 or M9-based liquid media at 37°C with shaking. To determine INH-hydrolyzing activity of cells grown under different conditions, cell-free extracts were incubated with INH at 37°C for 3 hours and then isonicotinic acid formed was measured using an ultra performance liquid chromatography system. The enzyme responsible for INH hydrolysis was purified by ammonium sulfate precipitation and column chromatography and identified by peptide mass fingerprinting. Additionally, we investigated the INH-degradation activity of some mycobacteria, including the laboratory strains *M. avium* 104, *M. intracellulare* ATCC 13950^T, and *M. bovis* BCG Pasteur, and the clinically isolated strains *M. abscessus and M. kansassii*. We used 7H9 without INH as a growth media for these strains. The INH-degrading activity of some mycobacteria, assessed using hydrazine as an index using colorimetric methods.

Results: We first evaluated INH-hydrolyzing activity of *M. smeg-matis* grown in several different media. We confirmed that *M. smeg-matis* grown in 7H9 indeed has an activity to hydrolyze INH, albeit very weak. Notably, the activity increased 43-fold when cells were cultured in an INH-containing M9-based media and was suppressed to the basal level by the addition of 18 mM (1 g l^{-1}) NH_4Cl to this culture. We succeeded in purifying the enzyme from cell-free extracts and revealing its identity as PzaA, an enzyme known as pyrazinamidase/nicotinamidase whose physiological role remains unknown. To investigate whether and how *pzaA* is distributed among mycobacteria, we searched the NCBI database for homologs of PzaA. We found that INH-degrading enzymes are widely distributed in mycobacteria.

Discovery of INH-induced INH-degrading Activity in *M. smegmatis*

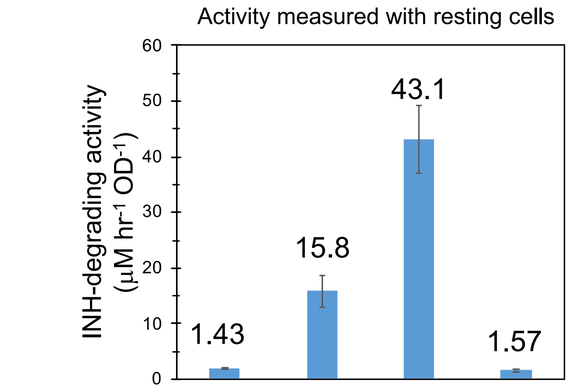

Fig 1. Effect of synthetic media additives on the isoniazid (INH)-hydrolyzing activity of *Mycobacterium smegmatis*. *M. smegmatis* MC^{2}155 cells grown in 7H9 medium were transferred to synthetic media containing the indicated components and incubated for 8 h. The activities of resting cells were normalized by optical density. Bars indicate standard deviations (N = 3).

Purification of INH-degrading Enzyme from *M. smegmatis*

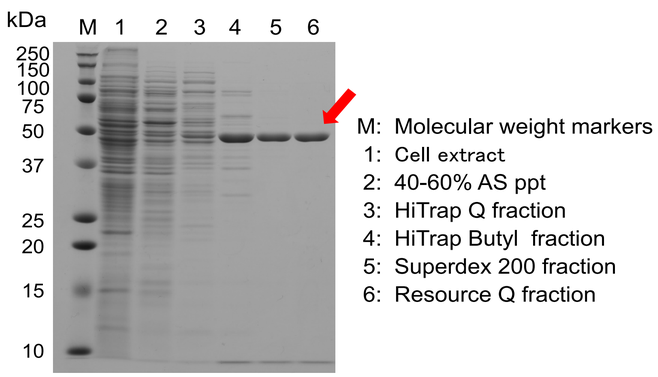

Fig 2. Purification of the isoniazid (INH)-hydrolyzing enzyme from *Mycobacterium smegmatis* MC^{2}155. An image of a CBB-stained SDS-PAGE gel is shown. The arrow indicates the purified enzyme.

Identification of INH-degrading Enzyme

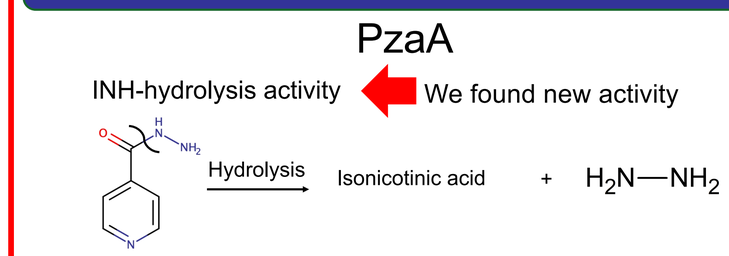

Fig 3. The analysis of the purified material revealed that the enzyme was an amidase named PzaA , known to hydrolyze pyrazinamide and nicotinamide. For the first time, we showed PzaA is involved in the degradation of INH by *M. smegmatis*.

Distribution of PzaA homologs in mycobacteria

Fig2. Phylogenetic tree of mycobacteria generated using 16S rRNA gene sequences. Asterisks indicate species that have a gene orthologous to *pzaA*. The bar represents 0.020 substitutions per site.

Conclusions: We have successfully identified the INH-hydrolyzing enzyme of *M. smegmatis* as PzaA, revealing its previously unknown secondary function. To our knowledge, this is the first and only example of an identified bacterial INH hydrolase.