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Title:	Characterization of an acid inducible lipase Rv3203 from Mycobacterium tuberculosis H37Rv
Authors:	Narang, D. (/jspui/browse?type=author&value=Narang%2C+D.)
Keywords:	Lipase Rv3203 Acidic stress Mycobacterium Tetrahydrolipstatin
Issue Date:	2014
Publisher:	Springer
Citation:	Molecular Biology Reports, 41(1), pp.285-296.
Abstract:	The Rv3203 (LipV) of Mycobacterium tuberculosis (Mtb) H37Rv, is annotated as a member of Lip family based on the presence of characteristic consensus esterase motif 'GXSXG'. In vitro culture studies of Mtb H37Ra indicated that expression of Rv3203 gene was up-regulated during acidic stress as compared to normal whereas no expression was observed under nutrient and oxidative stress conditions. Therefore, detailed characterization of Rv3203 was done by gene cloning and its further expression and purification as his-tagged protein in microbial expression system. The enzyme was purified to homogeneity by affinity chromatography. It demonstrated broad substrate specificity and preferentially hydrolyzed p-nitrophenyl myristate. The purified enzyme demonstrated an optimum activity at pH 8.0 and temperature 50 °C. The specific activity, K _m and V _{max} of enzyme was determined to be 21.29 U mg ⁻¹ protein, 714.28 μM and 62.5 μmol ml ⁻¹ min ⁻¹ , respectively. The pH stability assay and circular dichroism spectroscopic analysis revealed that Rv3203 protein is more stable in acidic condition. Tetrahydrolipstatin, a specific lipase inhibitor and RHC80267, a diacylglycerol lipase inhibitor abolished the activity of this enzyme. The catalytic triad residues were determined to be Ser50, Asp 180 and His203 residues by site-directed mutagenesis.
Description:	Only IISERM authors are available in the record.
URI:	https://link.springer.com/article/10.1007%2Fs11033-013-2861-3 (https://link.springer.com/article/10.1007%2Fs11033-013-2861-3) http://hdl.handle.net/123456789/3077 (http://hdl.handle.net/123456789/3077)
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