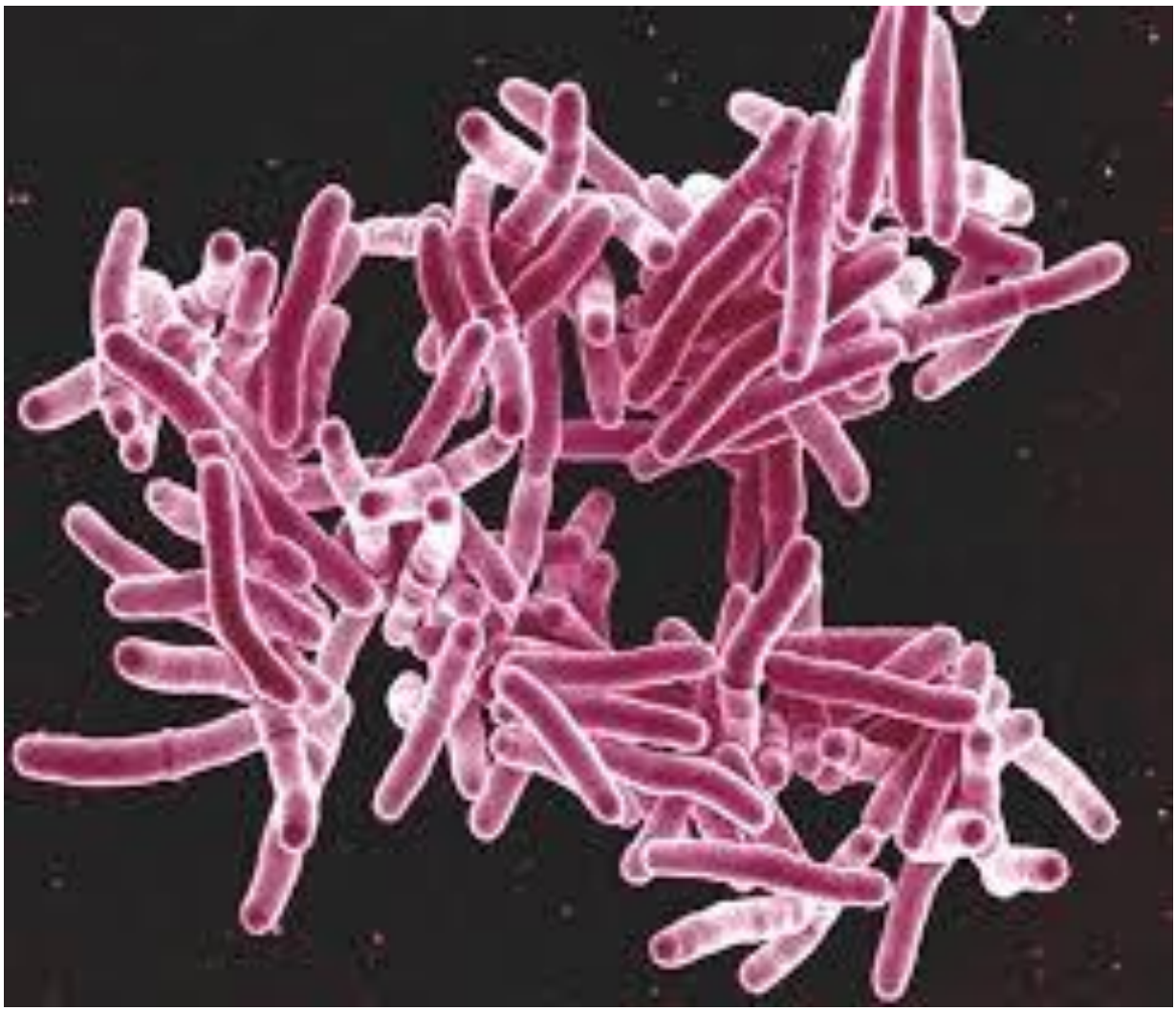
DBT-CDFD researchers decode how TB causing bacteria evades immunity



New Delhi, June 23: *Mycobacterium tuberculosis* (*M. tuberculosis*), which causes tuberculosis (TB) disease, has evolved several adaptive skills and evasion mechanisms to escape the defence system of the body and continue to survive within what are called macrophages. Normally, macrophages engulf invading bacteria and kill and digest them with the help of an inter-cellular organelle called lysosome. The process involves the production of reactive oxygen species (ROS) or reactive nitrogen species (NO). This creates an oxidative stress, which then leads to the destruction of the bacteria. In the case of *Mycobacterium tuberculosis*, however, this does not happen. It has evolved strategies to avoid oxidative stress caused by NO/ROS.

A team of researchers at the Department of Biotechnology's Centre for DNA Fingerprinting And Diagnostics (DBT-CDFD), Hyderabad have now got an insight into the nefarious strategy. They have identified a protein that seems to limit the oxidative stress mediated by reactive oxygen species (ROS) or reactive nitrogen species (NO). It belonged to a family of secretory protein of *M. tuberculosis* called PPE. The protein named PPE2has a monopartite nuclear localization signal (NLS), a DNA binding domain and an eukaryotic SH3 domain. PPE2 is translocated into the macrophage nucleus *via* the classical importin α/β pathway to interact with a GATA-binding site overlapping with the TATA box of *inos* promoter and inhibit NO production. PPE2 enhances the survival of the bacilli in macrophages as well as in a mice infection model.

In addition to having NLS and DNA binding domain, bioinformatics study revealed the presence of eukaryotic like SH3 domain and a PxxP motif in PPE2. PPE2 interacted with $p67^{phox}$ subunit of NADPH oxidase in the cytosol and hindered the migration of cytosolic subunits $p47^{phox}$ and $p67^{phox}$ from cytosol to membrane, resulting in faulty assembly of NADPH oxidase complex and inhibition of ROS production. Further, to investigate the role of SH3-like domain and PxxP motif in PPE2 mediated ROS inhibition, we mutated conserved residues in SH3-like domain to alanine (Y209A, W236A, and P249A), deleted a PxxP motif (Δ540-543), in PPE2. We observed that W236A mutation could not inhibit ROS generation, also, it failed to inhibit PPE2-$p67^{phox}$ interaction. This suggested that W236 residue in SH3 like domain of PPE2 is probably crucial for PPE2-$p67^{phox}$ interaction.

These studies suggest that PPE2 may be an important target for the development of novel drugs against *M. tuberculosis*. PPE2 inhibits the production of NO and ROS in a very coordinated manner to diminish oxidative stress. Further studies on PPE2 can help expand on understanding of host and mycobacterial interactions and the role of oxidative stress in TB pathogenesis which may help in the development of new drugs to control tuberculosis.

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