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THSTI scientists develop novel tools that could turn off bad genes in bacteria

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With their research funded by the Department of Biotechnology (DBT), two scientists at the THSTI which is a part of the Faridabad biocluster, have independently created tools that have successfully addressed the challenge of studying genes of various diseases causing bacteria including the tuberculosis causing mycobacterium.

Dr Bhabatosh Das, assistant professor at the Center for Human Microbial Ecology, THSTI, and his team have successfully built a delivery tool technically called "vector" for introducing and controlling any gene that they wish to study in a range of bacteria.

The scientific intelligence involved in the building of this vector termed as pBD32 (BD is abbreviation for Bhabatosh Das) is the selection of DNA from various sources and bringing them together into one delivery system. pBD32 has a piece of DNA termed as XBS selected from a virus, which in nature was known to attack the cholera bacteria and with the help of this DNA fragment goes and sits inside the bacterial chromosome. Dr Das believed that XBS would come handy to place any gene of interest in other bacterial chromosome to turn the gene "on" or "off". Scientifically this is termed as a stable, broad host range tightly regulated expression system that as described by Dr Das and his team in their Journal of Bacteriology Publication (Volume: 196, Number: 23, December 2014, Pages - 4071 4080) could be used in several bacteria like Vibrio cholerae (causing Cholera) and Klebsiella pneumoniae (causing Pneumonia), Salmonella enterica (causing Typhoid fever), Escherichia coli (lives in human gut).

Scientists working across the world like USA, Germany, UK and Japan are using this genetic tool for functional studies in the drug discovery field and highly appreciated the development with encouraging compliments.

When it comes to the tuberculosis causing bacterium Mycobacterium tuberculosis, nothing is ever enough as this bacterium has successfully made it difficult for the scientists' to study its essential genes. None of the tools developed till date has been proved to be greatly efficient in elucidating essential gene function in TB. Turning "off" the essential genes is a primary

approach but is a major challenge as most of the delivery tools do not achieve finding the exact place where the essential gene sits in the mycobacterial genome and do not have efficient blocking mechanism for causing loss of function.

Dr Nisheeth Agarwal, assistant professor at the Vaccine and Infectious Disease Research Center, THSTI, and his team have tackled it by improvising the CRISPRi (Clustered Regularly Interspaced Short Palindromic Repeat Interference) tool for disrupting the mycobacterial genes in a cost effective manner. This tool employs a foreign protein dCas9 borrowed from other bacteria and tweaked by his team to work in mycobacteria, and a stretch of oligonucleotide fragment termed as guide RNA (sgRNA) designed to pair with the gene in the mycobacteria that the scientist would like to disrupt.

Dr Agarwal's team has successfully engineered specific guide RNAs-dCas9 complex for many essential genes of the mycobacteria and have disrupted them from becoming functional. This tool is simple, cost effective and more importantly quicker considering that the tuberculosis causing mycobacteria takes a long time to culture under laboratory conditions and precious time is lost in the absence of quick manipulative tools. More than 50 genes could be inactivated in a short span of six months using this tool in Dr. Agarwal's laboratory. This will enable identifying new pathways unique to M.tuberculosis growth and design drugs against it in a significantly greater pace. The study was published in Nature Communications on 25th February, 2015 and has received worldwide acclaim from the community of eminent TB researchers.