# Mapping central carbon metabolism of *Xanthomonas oryzae* and *Xanthomonas campestris* by integrating Metabolic Systems Biology approaches

A Thesis

Submitted by

**Manu Shree**

**(D15022)**

For the award of the degree of

**Doctor of Philosophy**

*Under the supervision of*

**Dr Shyam Kumar Masakapalli**

**(Associate Professor)**


**School of Basic Sciences**

**Indian Institute of Technology Mandi**

**Mandi, Himachal Pradesh, India -175005**

**November, 2019**

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Dedicated

to

My beloved Parents

and

MetSysBio lab

INDIAN INSTITUTE OF TECHNOLOGY MANDI
MANDI- 175001 (H.P.), INDIA
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## Declaration by the Research Scholar

I hereby declare that the entire work embodied in this Thesis is the result of investigations carried out by me in the ***School of Basic Sciences,*** Indian Institute of Technology Mandi, under the supervision of ***Dr Shyam Kumar Masakapalli,*** and that it has not been submitted elsewhere for any degree or diploma. In keeping with the general practice, due acknowledgements have been made wherever the work described is based on finding of other investigators.

Place: Mandi, Himachal Pradesh
Date:

Signature:
Name: Manu Shree

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## Declaration by the Research Advisor

I hereby certify that the entire work in this Thesis has been carried out by ***Manu Shree,*** under my supervision in the ***School of Basic Sciences,*** Indian Institute of Technology Mandi, and that no part of it has been submitted elsewhere for any Degree or Diploma.

Date:

Signature:

Name of the Guide: Dr Shyam Kumar Masakapalli
Associate Professor
School of Basic Sciences (BIO-X)
MetSysBio lab
IIT Mandi

# Acknowledgements

I am indebted to many people who have made my education, and this particular adventure called PhD, possible. First and foremost, my gratitude goes to my supervisor, Dr Shyam Kumar Masakapalli, who has given me an opportunity to avail PhD in the field of Fluxomics. A heartfelt thanks for all his patience, motivation, supervision, time and efforts. I sincerely thank you for giving me the freedom to explore different responsibilities (including the motivation for accepting the Research Secretary position at IIT Mandi) and the opportunity to explore multiple research areas (Genetic engineering, Metabolic engineering, Metabolomics and Fluxomics) at different places (ICGEB, NIPGR, and University of Bath). Being the first and foremost student of MetSysBio (MSBL) lab, I have mixed set of valuable experiences, which made me the kind of person I am today.

I am thankful to Dr. Ramesh Sonti (Director, NIPGR) and associated lab members (Dr. Hitendra, Chandan, Akansha) for providing us with the *Xanthomonas oryzae* strain and also for their suggestions whenever required. I am grateful to Dr. Tanmoy Samanta (Postdoctoral fellow in MSBL lab) for giving his time and efforts towards the completion of this work, specifically in providing every possible help in characterization and maintenance of *Xanthomonas campestris* strain. I also extend my special thanks to the doctoral committee members, Dr Prosenjit Mondal, Dr Aditi Haldar, Dr Viswanath Balakrishnan and Dr Tulika Prakash Srivastava for their time, valuable suggestions, discussions, general advices and continuous monitoring of my progress throughout the tenure of my PhD. I would also like to thank all the incredible scientists, Dr Ranjan Nanda (ICGEB), Dr Jagadish Kapughanti (NIPGR), Prof Suresh Nair (ICGEB), and Prof David Leak (University of Bath), I've had the privilege of working in these past four years. I am obliged to prof. David and all his lab members (Martyn Bennett, Ed Nesbitt, Christopher Ibenegbu, Emanuele Kenrick, Maria Vittoria Ortenzi, Andrew and Helen) for providing me an opportunity to learn and explore metabolic engineering aspects in *Geobacillus* for a period of four months in UK (United Kingdom).

I would like to acknowledge all the faculties, their lab group members, and technical staffs at Bio-X centre and AMRC (Advanced Material Research Centre), IIT Mandi for technical support and giving the access to use all lab instruments any time. I would like to pay special thankfulness, warmth and appreciation to the members of MSBL and

Medicinal Plant lab for making this journey successful. Thanks to Bandana, Dr. Reshma, Portia, and Jyotika for their contribution in maintaining the Medicinal plant lab environment quite happy and cheerful, totally a stress buster. Special thanks to Maneesh for all his help, wonderful criticism, arguments, scientific and non-scientific discussions that has always helped me to achieve confidence in my work. Many thanks to Chandrakant, Poonam and Yogesh, who were always there to help whenever required. All the past and present interns and MTech students who were/are associated with the MSBL lab were great to work with.

I would also like to thank my friends, who made my PhD journey a bit easier in every possible sense, Dr. Parvez, Dr Arpita, Dr. Inderjeet, and Dr. Shilpa. A big thanks to Dr. Parvez for always being the pillar of support and motivation throughout, that has helped me to reach to a stage where I am writing my PhD thesis instead of quitting it long back. I am thankful to Sharad, Paromita, Naina, Vineeth, Tanya, Subia, Ruchi, Akansha, Geetika, and Surbhi who helped me to make the most of living at IIT Mandi. Last but not the least, I thank my parents for always stressing the importance of my education and supporting me through my career goals. Their encouragement means more to me than they know. Finally, the unexpected support and patience of my fiancé, Aman that has helped me to carry through the later stages of my PhD. I am grateful to the lifelong lessons I have experienced in the beautiful Indian state of Himachal Pradesh.

Manu Shree

## ABSTRACT

*Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Xanthomonas campestris* pv. *campestris* (*Xcc*) are named among the top 10 bacterial phytopathogens with relevance to global crop loss due to the potential to cause diseases like bacterial leaf blight of rice, black rot of crucifers, cassava bacterial blight etc. *Xanthomonas campestris* owing to its ability to produce xanthan has been of interest as potential cell factory. On the other hand, there are huge gaps in our understanding of *Xanthomonas oryzae* metabolism. Deciphering the metabolic phenotypes of these phytopathogens under different nutritional regimes is of significance to understand their pathway regulations that would either support in their multiplication or induce virulence as a trade-off. The main objective of the thesis is to understand the precise metabolic phenotypes of *Xanthomonas campestris* pv. *campestris* (*Xcc*) strain NCIMB 5028 and *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strain BXO43 subjected to xanthan producing minimal media (XMD) or established plant mimicking media (XOM2) by integrating robust metabolic systems biology approaches of comparative genome, $^{13}C$ tracer based metabolic pathway mapping and $^{13}C$ Metabolic Flux Analysis ($^{13}C$ MFA).

First, the robust workflow of $^{13}C$ MFA and $^{13}C$ label analysis based upon amino acids mass isotopomer distributions using Gas Chromatography-Mass Spectroscopy (GC-MS) was adopted for *Xanthomonas* species. Second, the comparative analysis of the central metabolic pathways of 33 *Xanthomonas* strains using KEGG pathway mapper provided insights into the robustness of central metabolism. The gap identified from the annotations in relation to the missing genes in central metabolism (phosphoserine phosphatase, aspartate aminotransferase, phosphoglucoisomerase) were further validated by $^{13}C$ fingerprinting in *Xcc* and *Xoo*. Third, growth studies established that *Xcc* has the ability to substantially yield xanthan (72.2g xanthan/100g glucose), thereby supporting the need to understand its metabolic phenotypes. The metabolic flux phenotype of *Xcc* was established comprehensively by using parallel $^{13}C$ labelling approach with 99%[1-$^{13}C$] glucose, 99%[1,2-$^{13}C$] glucose, and a combination of 60% [1-$^{13}C$]+40% [$^{13}C_6$] glucose. The flux estimated (normalised to 100 uptake) through ED (Entner Doudoroff) pathway, glycolytic route, and PPP (Pentose phosphate pathway) was estimated to be 35.49%, 20.84 % and 9.18% respectively. The flux through anaplerotic reactions, supported by malate dehydrogenase and phosphoenolpyruvate carboxylase was 6% and 3% respectively. Also, TCA (9.3%) and glyoxylate cycle (8.3%) flux was found to contribute significantly in central metabolism.

Finally studies on *Xoo* BXO43 highlighted the potential lack of *pgi* (phosphoglucoisomerase) gene that typically supports glycolysis. Mapping the glucose/xylose oxidation pathways is anticipated to provide critical understanding of this important phytopathogen. *Xoo* fed with plant mimicking media XOM2 containing methionine, either $^{13}C$ glucose (99%[1-$^{13}C$]-, or 99%[1,2-$^{13}C$]-, or 40%[$^{13}C_6$]-) or $^{13}C$ xylose (99%[1-$^{13}C$]-, or 40%[$^{13}C_5$]-) or $^{13}C$ glutamate (99%[$^{13}C_5$]) has comprehensively mapped central carbon metabolism. $^{1}H$ NMR analysis confirmed that *Xoo* cells oxidise xylose/glucose and glutamate in appreciable amounts, while no utilisation of methionine was observed. With 40% [$^{13}C_6$] glucose or 40% [$^{13}C_5$] xylose the activity of PPP (based on histidine average $^{13}C$ labelling), and the TCA cycle (based on labelling in Asp, Glu, Pro and Lys) was confirmed qualitatively. The $^{13}C$ incorporation in serine (m/z- 390) and alanine (m/z-260) via glucose or xylose tracer reports on the activities of ED pathway and a potential alternative glucose oxidation route. When 100%[$^{13}C_5$] glutamate was fed with unlabelled glucose/xylose, anaplerotic route (OAA → PEP) and gluconeogenic cycle was found to be potentially active due to the significant incorporation of $^{13}C$ in histidine (6%) via non oxidative pentose phosphate pathway. The investigation suggests critical role of glutamate along with glucose/xylose in sustaining the *Xoo* metabolic activities. The outcomes of current thesis have provided robust strategies that can be adopted for qualitative and quantitative metabolic analysis of phytopathogens whose metabolism is poorly understood. The metabolic insights are anticipated to provide vital cues in extending future investigations in decoding the plant host-phytopathogen metabolic crosstalk.

# Abbreviations

| | |
|---|---|
| **CI** | Confidence Intervals |
| **DSS** | 4,4-Dimethyl-4-Silapentane-1-Sulfonic Acid |
| **EI** | Electron Ionisation |
| **EMU** | Elementary Metabolite Unit |
| **FBA** | Flux Balance Analysis |
| **GC-MS** | Gas Chromatography Mass Spectrometry |
| **KEGG** | Kyoto Encyclopaedia of Genes and Genomes |
| **m/z** | Mass by charge ratio |
| **MC** | Monte Carlo |
| **MeOX-HCl** | Methoxamine Hydrochloride |
| **MFA** | Metabolic Flux Analysis |
| **MID** | Mass Isotopomer Distribution |
| **Isocor** | Natural isotope abundance correction software |
| **MSTFA** | N-Methyl-N-Trimethylsilyl N-Trifluoroacetamide |
| **NIST** | National Institute of Standards and Technology, Maryland |
| **NMR** | Nuclear Magnetic Resonance |
| **OxPPP** | Oxidative Pentose Phosphate Pathway |
| **PPP** | Pentose Phosphate Pathway |
| **TBDMS** | N-Methyl-N-Tertiary-Butyl Dimethylsilane |
| **TIC** | Total Ion Count |

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