

**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

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Dedicated

to

My beloved Parents

and

MetSysBio lab



INDIAN INSTITUTE OF TECHNOLOGY MANDI

MANDI- 175001 (H.P.), INDIA

www.iitmandi.ac.in

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



INDIAN INSTITUTE OF TECHNOLOGY MANDI

MANDI- 175001 (H.P.), INDIA

www.iitmandi.ac.in

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

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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% [$^{13}\text{C}_6$] 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* (phosphoglucosomerase) 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 ¹³C glucose (99%[1-¹³C]-, or 99%[1,2-¹³C]-, or 40%[¹³C₆]-) or ¹³C xylose (99%[1-¹³C]-, or 40%[¹³C₅]-) or ¹³C glutamate (99%[¹³C₅]) has comprehensively mapped central carbon metabolism. ¹H NMR analysis confirmed that *Xoo* cells oxidise xylose/glucose and glutamate in appreciable amounts, while no utilisation of methionine was observed. With 40% [¹³C₆] glucose or 40% [¹³C₅] xylose the activity of PPP (based on histidine average ¹³C labelling), and the TCA cycle (based on labelling in Asp, Glu, Pro and Lys) was confirmed qualitatively. The ¹³C 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%[¹³C₅] 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 ¹³C 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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