Progress Report for R&D Projects [Year 03] *

Section-A: Project Details

A1. Project Title: Integrating Genome scale metabolic analysis of model plant pathogen Ralstonia solanacearum with RNAseq and fluxomics

A2. DBT Sanction Order No. & Date: BT/PR16361/NER/95/192/2015, 18-10-2016

A3. Name of Principal Investigator: Dr. Siddhartha Sankar Satapathy
Dr. Shyam K Masakapalli

Name of Co-PI/Co-Investigator: Prof. Suvendra Kumar Ray
Dr. Tulika Srivastava

A4. Institute: Tezpur University (NER)
Indian Institute of Technology Mandi (Non-NER)

A5. Address with Contact Nos. (Landline & Mobile) & Email:

- **Siddhartha Sankar Satapathy** (PI), Associate Professor, Dept. of Computer Science & Engineering, Tezpur University, Napaam-784028, ssankar@tezu.ernet.in, Phone: 03612275117, 9435979648
- **Shyam K Masakapalli** (PI), Associate Professor, School of Basic Sciences Indian Institute of Technology Mandi – 175005, shyam@iitmandi.ac.in, Phone: 8628088505
- **Suvendra Kumar Ray** (Co-PI), Professor, Department of Molecular Biology and Biotechnology, Tezpur University, Napaam-784028. suven@tezu.ernet.in, Phone: 3712275406
- **Tulika P Srivastava** (Co-PI), Professor, School of Basic Sciences, Indian Institute of Technology Mandi – 175005, tulika@iitmandi.ac.in, Phone: 01905237922

A6. Total Cost: 57.40 lakh

A7. Duration: 3 years + 6 months (no cost extension)

A8. Approved Objectives of the Project:

- NER (Tezpur University)
  1. To reconstruct and validate genome-scale models of *R. solanacearum* strains from available draft genome sequences.
  2. To use the combination of metabolite profiling, biomass compositional analysis, co-utilization of different substrates to understand the metabolic potential and limitations of *R. solanacearum*.
  3. Constraint based flux analysis of *R. solanacearum*
  4. RNAseq transcriptome sequencing under normal and mimicked nutritional regimes
- Non-NER (IIT Mandi)
  1. To define the precise metabolic flux phenotypes of R. solanacearum under different nutritional regimes.
  2. Analysis of RNAseq whole transcriptome data, to map the gene expression profiles under different conditions using advanced tools.
  3. Integration of RNAseq, FBA and/or 13C flux analysis data into the genome scale metabolic models as constraints leading to improved modelling studies.

A9. Specific Recommendations made by the Task Force (if any): Nil

Section-B: Scientific and Technical Progress

B1. Progress made against the Approved Objectives, Targets & Timelines during the Reporting Period (1000-1500 words for interim reports; 2500-3500 words for final report; data must be included in the form of up to 3 figures and/or tables for interim reports; up to 7 figures and/or tables for final reports):

Objective: To reconstruct and validate genome-scale models of *R. solanacearum* strains from available draft genome sequences.

Reconstruction of central carbon metabolism:

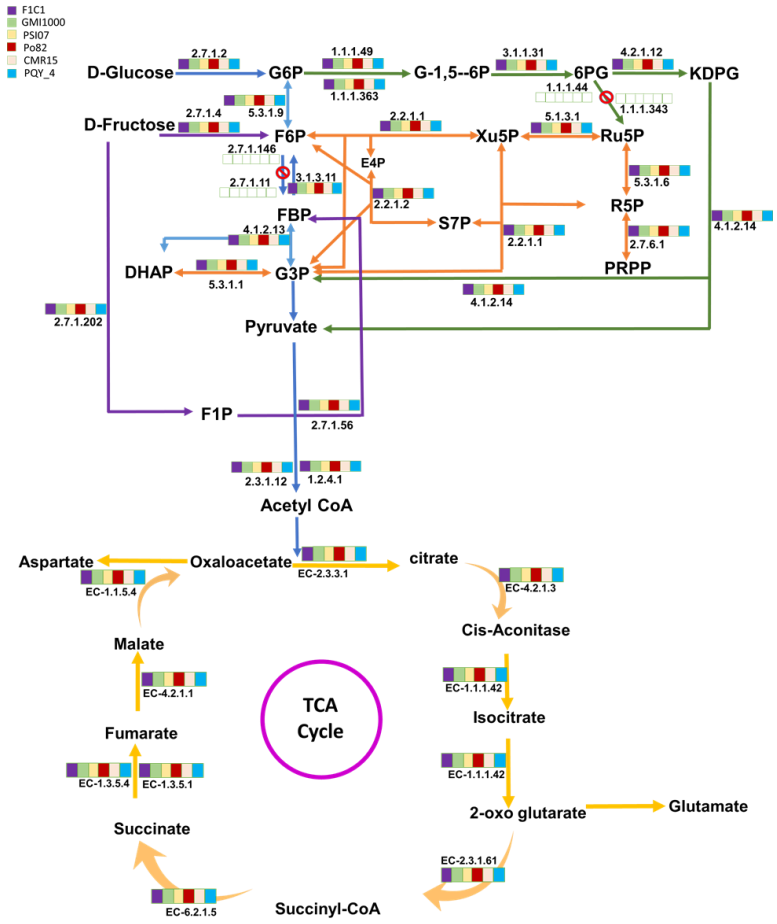

Figure 1. Comparative pathway analysis of five different *Ralstonia solanacearum* strains (GMI1000, PSI, Po82, CMR and FQY) along with F1C1. An inhouse developed pipeline (named G2KO) tool was used to retrieve KO numbers of Ralstonia strains from KEGG. KEGG mapper was used to further map pathways based on presence and absence of annotated genes. Central carbon metabolic pathways mainly glycolysis, Entner-Doudoroff (ED), pentose phosphate pathway and TCA cycle was mapped to check glucose oxidation in *Ralstonia solanacearum* strains. Enzymes are represented by enzyme commission (EC) numbers and color coding for different strains. The absence of any color indicate the absence of enzyme in that strain. Reactions between metabolites in the pathways, namely, glycolysis, ED pathway, pentose phosphate pathway, fructose metabolic pathway and TCA cycle are shown in blue, green, brown, purple and yellow colored arrows respectively.

The chromosomal and megaplasmid sequences of a novel strain of *Ralstonia solanacearum* f1c1 (isolated from Chilli host) was subjected to detailed genome annotation using latest tools available. Genome annotation has provided detailed insights of *R. solanacearum,* mainly central carbon metabolism. GHOSTX inbuilt in KEGG database was used for function prediction of genes, which provided KEGG Orthology (KO) numbers to the annotated sequences. Based on functional information, genome scale model of central carbon metabolic pathways was reconstructed. The central metabolic pathways mainly glycolysis, Entner-Doudoroff (ED) pathway, pentose phosphate pathway and TCA cycle were reconstructed using KEGG Orthology (KO) numbers by KEGG pathway mapper, confirmed the completeness of ED, TCA and pentose phosphate pathway whereas some regulatory enzymes of glycolysis and oxidative pentose phosphate pathway were absent. The five strains of *Ralstonia solanacearum* namely, GMI1000, PYQ_4, Po82, CMR15 and PSI07 which belongs to different phylotypes, Phylotype I, I, II, III and IV respectively were selected for comparative pathway analysis. Their central metabolic pathways mainly glycolysis, Entner-Doudoroff (ED) pathway and pentose phosphate pathway were reconstructed. Similar results were found in comparative pathway analysis (Figure 1). In case of glycolysis all strains lack an important regulatory enzyme phosphofructokinase-1, which indicates the absence of glucose oxidation by glycolytic pathway. The link between ED pathway and pentose phosphate pathway is also missing by the absence of phosphogluconate dehydrogenase enzyme which indicates the absence of oxidative pentose phosphate pathway (Figure 1).

Objective: Stable isotope (13C) feeding experiments for validation of genome based model reconstruction and evaluating the feasibility of metabolic flux analysis studies in *Ralstonia solanacearum*

Cell growth and maintenance: F1C1 Culture was maintained in BG medium (Kumar et al., 2013) in 50 ml falcon tubes at 28°C on an orbital shaker running at 180 r.p.m. For pathway mapping experiment cells were grown in BG medium for 24 hrs untill the OD_{600} reaches to 1. Cells were pelleted down by using centrifuge at 7000rpm for 5 minutes. Cells were washed twice with sterile water and redistributed in minimal medium supplemented with labelled isotopes (0.5%w/v). The composition of minimal medium (Ray et al., 2015; Plener et al., 2010) is as follows (gL^{-1}): $FeSO_4$-$7H_2O$, 1.25×10^{-4}; $(NH_4)2SO_4$, 0.5; $MgSO_4$-$7H_2O$,0.05; KH_2PO_4, 3.4. The pH was adjusted to 7.0 with KOH. Cells were grown in minimal media with different combinations of labelled isotopes ([1-^{13}C], [1-^{13}C]&[$^{13}C_6$] (60:40), 40%[$^{13}C_6$], [12C]; Figure2). Cells were harvested at steady state condition (18hrs post culture; Figure 3).

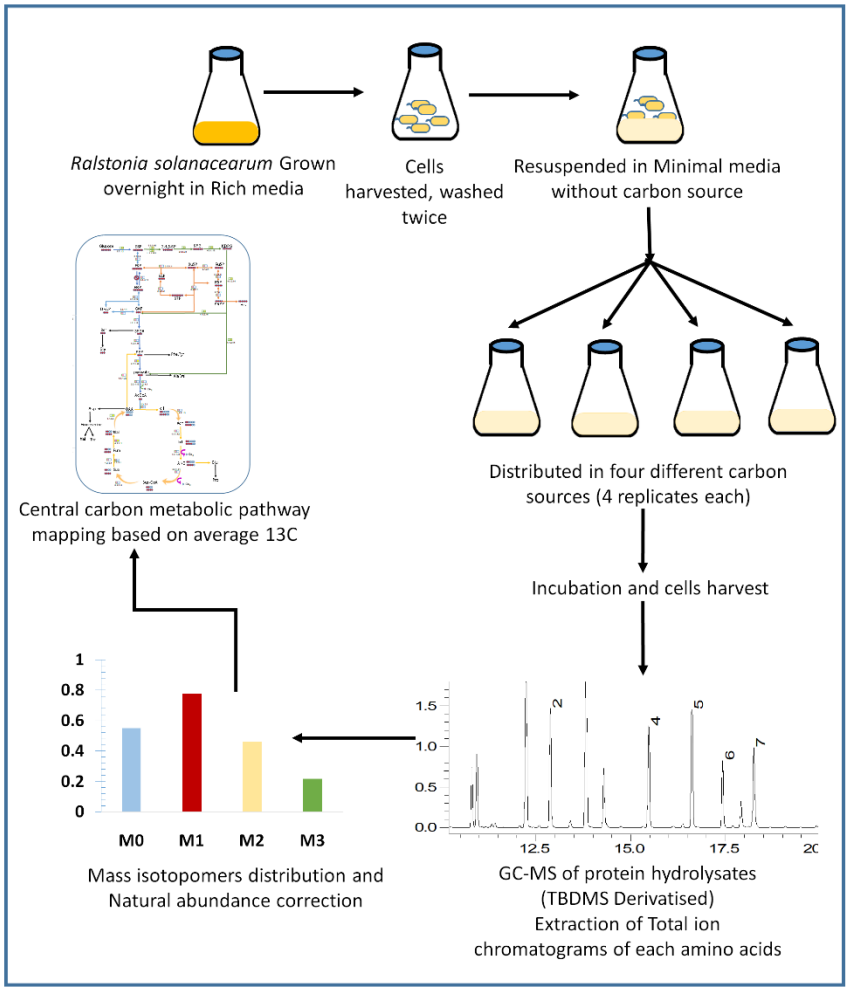

Figure 2. Experimental work flow opted for growing *Ralstonia solanacearum* in rich and minimal media (Kumar et al., 2013), followed by 13C feeding and GC-MS data analysis towards central metabolic pathway mapping. Ralstonia cells were grown overnight in rich media till optical density (OD_{600}) reaches to 1. Cells were harvested by centrifugation at 7000rpm and washed twice with water. Isotopic tracers i.e. 40%[$^{13}C_6$]glucose and [$^{1\text{-}13}C_6$]glucose was then fed to the cells along with ^{12}C substrates as control (four replicates each). Cells were harvested after 18hr of inoculation and were acid hydrolysed using 6M HCl. Protein hydrolysates were derivatised by TBDMS (Tertiary butyl dimethyl silane) derivatization for GC-MS analysis. The TIC (Total Ion Chromatograms) of all amino acids were corrected for natural isotope correction and validation was based on average ^{13}C in unlabelled fragments. Validated amino acid fragments were then used to map the central metabolic pathways.

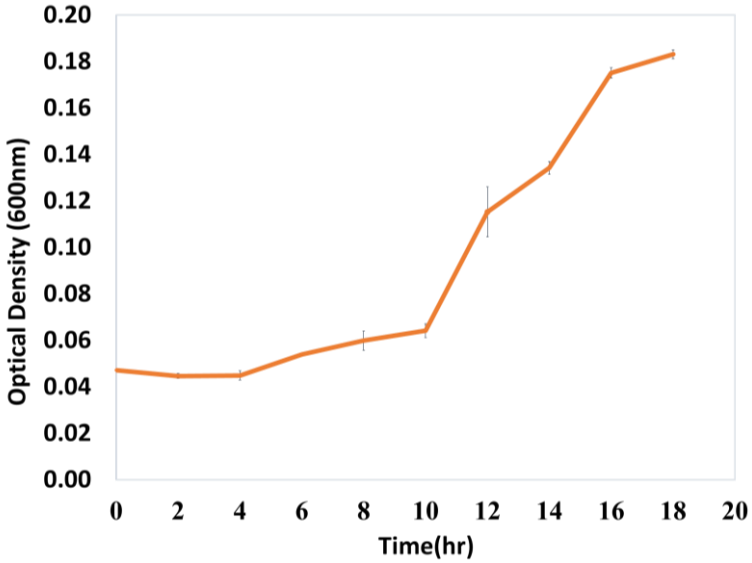

Figure 3. The growth curve of *R. solanacerum* cells in minimal media containing 0.5% (w/v) glucose as carbon source. Cells were harvested in the mid exponential phase around 18hr post inoculation.

Acid hydrolysis and Derivatization of amino acids: Cell pellets (2 mg) were acid hydrolysed by suspending them in 500 µL of 6M HCl for 18 hr at 100°C to release the component amino acids (Antoniewicz et al., 2007). 50 µL acid hydrolysate was then dried in speed-vacuum to ensure complete removal of water. The amino acids extracts were derivatised by TBDMS (Shree et al., 2018). To obtain the TBDMS derivatives, the dried samples were first dissolved in 30 µl of pyridine and incubated at 37^{o}C, shaking at 900 rpm for 30 min. Then 50 µl of MtBSTFA + 1% t-BDMCS (N-methyl-N-(t-butyldimethylsilyl) trifluoroacetamide + 1% t-butyl-dimethylchlorosilane, Regis Technologies Inc*)* was added and incubated the samples at 60^{o}C with shaking at 900 rpm for 30 min on a thermomixer. The derivatised samples were centrifuged for 10 min at 13000 rpm to pellet any insoluble material and transfer the supernatant to glass vials for GC-MS and sealed with a septum cap.

Gas chromatography-mass spectrometry: The GC-MS measurements were performed on an Agilent 7890B GC, electron impact ionisation (70 eV) equipped with an Agilent HP 5-ms ultra-inert column (Agilent 19091S-433UI, 30m x 250µm x 0.25 µm dimentions) at the facility in BioX, IIT Mandi. In GC-MS, 1 µl sample volume was taken for injection. The initial oven temperature was constant at 120^{o}C for 5 min, then a 4^{o}C /min ramped to 270^{o}C, held for 3 min, then a 20^{o}C /min ramped to 320^{o}C and held for 1 min. The carrier gas (Helium) flow was maintained at 1.3 ml min-1. The spectra were recorded with a scanning range of 25 to 600 mz^{-}1 for a total run time of 49 mins. MassHunter (Agilent Technologies, USA) was used to control the data acquisition parameters (both GC separation and mass spectrometry) during all the sample runs. The 15 amino acids with their respective elution time (min) and mass ions (m/z) are as follows (Table 1; Figure 4).

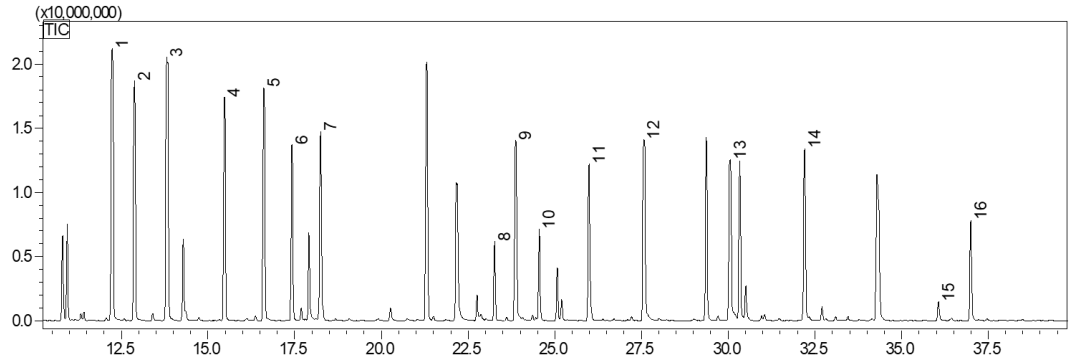

Figure 4. Total ion chromatogram of GC-MS from protein hydrolysate of *R. solanacearum* indicating the presence of 15 amino acids and some other cell wall components. Y-axis represents abundance(unit) and X-axis represents time

Table 1. 15 Amino acids with their respective elution time, derivative and specific ions (m/z) obtained from GC-MS

Peak No	Elution time	Amino acid	Derivative	Specific ions (m/z)				
1	12.292	Alanine	2TBDMS	158	232	260	317	302
2	12.958	Glycine	2TBDMS	218	246	144	288	303
3	13.923	3-hydroxybutyric acid	2TBDMS	159	189	233	275	
4	15.622	Valine	2TBDMS	186	260	288	345	
5	16.793	Leucine	2TBDMS	200	274	302	344	
6	17.626	Isoleucine	2TBDMS	200	274	302	344	
7	18.479	Proline	2TBDMS	184	258	286		
8	23.639	Methionine	2TBDMS	218	292	320	302	362
9	24.262	Serine	3TBDMS	288	302	362	390	432
10	24.965	Threonine	3TBDMS	302	376	404	417	
11	26.436	Phenylalanine	2TBDMS	243	308	336	378	
12	28.068	Aspartic acid	3TBDMS	316	390	418	302	432
13	30.615	Glutamic acid	3TBDMS	272	330	432	358	404
14	32.826	Lysine	3TBDMS	329	431	488	302	473
15	36.810	Histidine	3TBDMS	196	338	413	440	
16	37.756	Tyrosine	3TBDMS	364	438	466	302	

Metabolite identification and mass isotopomer data handling The raw GC-MS spectra need to be baseline corrected at first for accurate assessment of mass isotopomer distributions in metabolites. The raw files from GC-MS was baseline corrected using

MetAlign software (Lommen, 2012) with its default parameters. Metabolite identification was done using NIST (National Institute of Standards and Technology, Maryland). The intensity of the mass ions of each amino acid fragment (Masakapalli et al., 2014) was obtained by using Agilent chemstation software. The MIDs of each fragment ion obtained from individual scans and the averaged mass spectra were corrected for the presence of naturally occurring heavy isotopes attached to the carbon backbone of the derivative using mass correction software IsoCor (Millard et al., 2012). The average 13C abundances (%) of amino acids pointed to the activities of the key metabolic pathways.

Mapping of central metabolic pathways retro biosynthetically from labelled amino acid fragments:

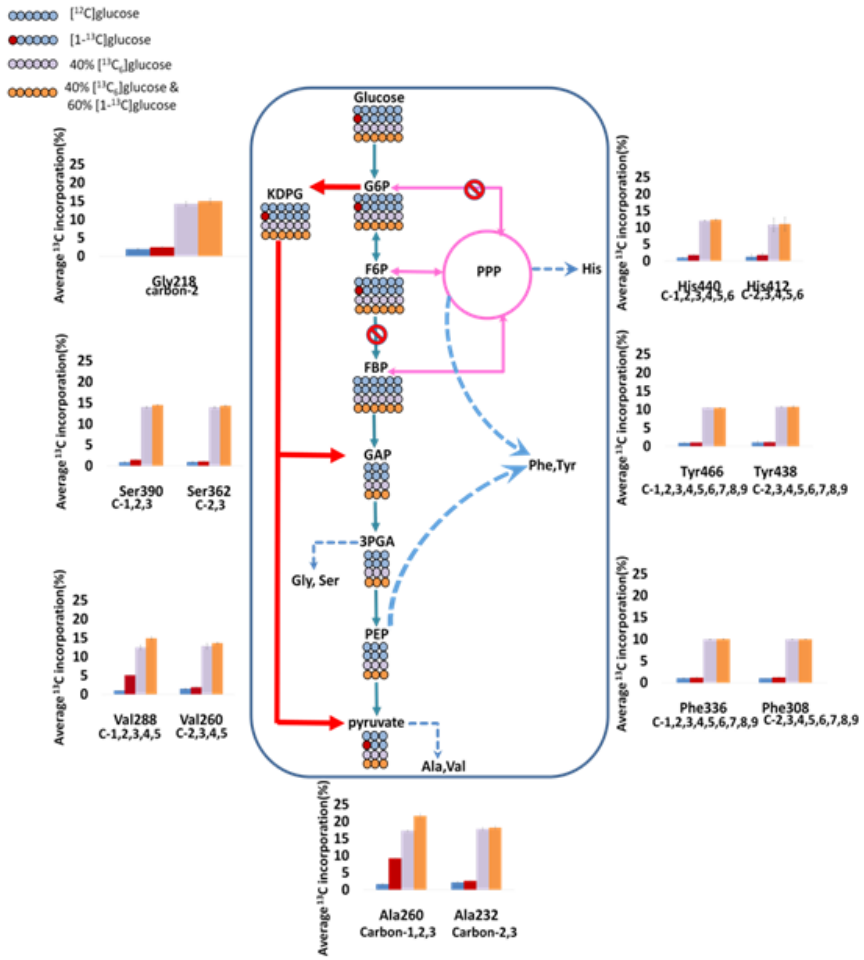

Figure 5. ^{13}C label incorporation in the mass isotopomers (M-57 & M-85 fragments) of amino acids when cells were fed with [^{12}C] glucose (blue bars), [1-^{13}C] glucose (red bars), 40%[$^{13}C_6$] glucose (yellow bars) and [1-^{13}C] & [$^{13}C_6$] glucose (green bars). The distribution of average ^{13}C in amino acids highlights the metabolic pathway activities and carbons retained in amino acid fragments further confirmed the pathway activities. The metabolites in the pathways are shown in black color whereas the reactions, namely glycolysis, ED- pathway, pentose phosphate pathway and amino acids are presented in blue, red, pink and dotted blue color respectively.

Average ^{13}C incorporation label in representative amino acid fragments retrobiosynthetically report on central metabolite precursors and highlights the metabolic pathway activities (Figure 5). When cells were fed with [1-^{13}C] glucose, only alanine and valine has 13C incorporation whose precursor is pyruvate. There is no ^{13}C enrichment in serine and glycine fragments, therefore we can exclude any contribution of glycolytic pathway in the formation of ser or its precursor 3-PGA. There is high enrichment (9%) of ^{13}C label in [M-57] fragment of ala which retained carbon 1,2&3, indicates the activity of ED pathway. The [M-85] fragment of ala retain only C2 and C3 has no ^{13}C incorporation which indicate that pyruvate is labelled at C1 position. Isotopic Labeling of pyruvate at C1 indicates the activity of ED pathway while at C3 position is the indication of glycolysis. When the cells were fed with [$^{13}C_6$], the ^{13}C enrichment is similar (17%,13%) in [M-57] and [M-85] fragment (17%,13%) of ala and val respectively, while there is more ^{13}C incorporation in [M-57] fragment (21.6%,15%) than [M-85] fragment (18%, 14%) in ala and val respectively, when fed with a mixture of [1-^{13}C] & [$^{13}C_6$] glucose. 13C enrichment in the other amino acid fragments is same (Phe-10%, tyr-11%, His-12%, Gly-14, Ser-14%) when the cells fed with [$^{13}C_6$] glucose and a mixture of [1-13C] & [$^{13}C_6$] glucose.

RNAseq under three nutrient conditions: F1C1 Culture was grown in BG medium (Kumar et al., 2013), Minimal media (Ray et al., 2015; Plener et al., 2010) and tomato xylem mimicking media, in 50 ml falcon tubes at 28°C on an orbital shaker running at 180 r.p.m. untill the OD_{600} reaches to 1. Cells were pelleted down by using centrifuge at 7000rpm for 5 minutes. Cells were washed twice with sterile water to remove media components. Total RNA under three conditions were isolated and their concentrations were checked using nanodrop spectrophotometer (Table 2). RNA was also run on agarose gel to check its integrity (Figure 6). RNASeq analysis is ongoing with Xcelris company to get differential gene expression data.

Table2. RNA concentrations in three media conditions

Sample	Conc. (ng/uL)	260/280	260/230
MM	14.60	2.25	.89
MK	29.55	2.02	.98
RICH	157	2.17	1.15

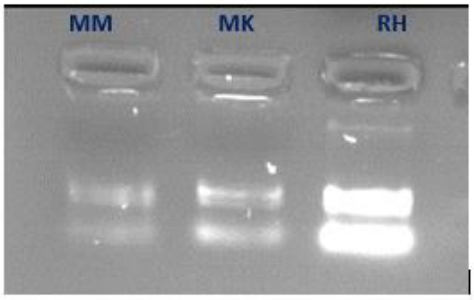

Figure 6. Isolated total RNA run on agarose gel where MM (minimal media), MK (mimicking media) and RH (rich media)

The 13C flux analysis using biomass constraints to obtain the flux map of *R. solanacearum* under minimal media requirements

Biomass estimation of *R. solanacearum* under minimal media condition is ongoing to obtain flux map of *R. solanacearum.*

The 13C feeding experiments to obtain the flux map of *R. solanacearum* under tomato xylem mimicking media conditions

To obtain flux map under tomato xylem mimicking conditions, 13C feeding experiments with different 13C substrates (13C glucose and 13C glutamine) has completed. Data analysis is ongoing to map central carbon metabolic pathways under tomato xylem mimicking conditions.

B2. Summary and Conclusions of the Progress made so far (minimum 100 words, maximum 200 words):

- Reconstruction of central carbon metabolic pathways has been successfully achieved
- We revealed the detailed metabolic network features of *R. solanacearum* strains (belonging to four phylotypes) with focus on central metabolic pathways.
- We successfully mapped the central carbon metabolic pathways using 13C tracer feeding experiment to validate the reconstructed pathways.
- We observed and experimentally validated that R. *solanacearum* strains lack glycolysis pathway for glucose oxidation as one major regulatory enzyme PFK1 is absent in *Ralstonia solanacearum* strains and use ED pathways as main glucose oxidation route.
- The progress achieved with the successful annotation of the genome (megaplasmid as well as chromosome) and the reconstruction and validation of central metabolic pathways of *R. solanacearum*. We aim to explore if Flux Balance Analysis can be done with the biomass constraints we already obtained.
- We publish research article in reputed peer reviewed journals and the project allowed us to present our work in international conferences.

B3. Details of New Leads Obtained, if any:

- We decoded for the first time that *R. solanacearum* follows ED pathway as major route for glucose oxidation rather than glycolysis and oxidative pentose phosphate pathway
- In continuation with the functional annotation of the genes of the two replicons: chromosome and megaplasmid of the of *Ralstonia solanacearum* f1c1 genome, a python based web portal is developed which is lunched in a web server (Figure 1) available in the department of Comp. Sc. & Engg., Tezpur University at http://14.139.219.242:8009/.

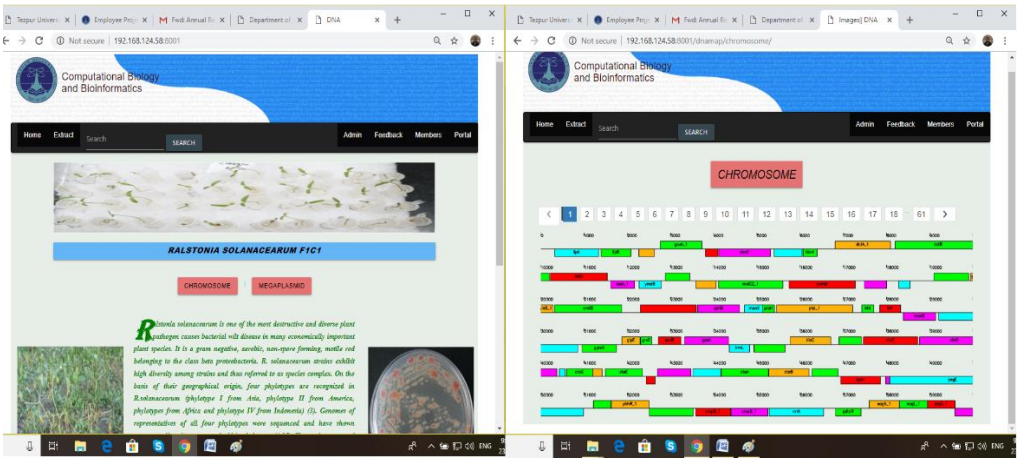

Figure 1: Home page of the web portal providing Chromosome and megaplasmid of the of *Ralstonia solanacearum* f1c1 genome

B4. Details of Publications, technology developed & Patents, if any emanated from the project:

1. Sen P, Kurmi A, Ray SK, Satapathy SS (2022) Machine learning approach identifies prominent codons from different degenerate groups influencing gene expression in bacteria. Genes to Cells 27(10):591-601.
2. Sen P, Ruksana A, Deka RC, Feil EJ, Ray SK, Satapathy SS (2022) Stem region of tRNA genes favors transition substitution towards keto bases in bacteria. J Mol Evol 90(1):114–123.
3. Aziz R, Sen P, Beura PK, Das S, Tula D, Dash M, Namsa ND, Deka RC, Feil EJ, Satapathy SS, Ray SK (2022) Incorporation of transition to transversion ratio and nonsense mutations, improves the estimation of the number of synonymous and non-synonymous sites in codons. DNA Res. 2022 Jun 25;29(4):dsac023.
4. Kabyashree K, Kumar R, Sen P, Satapathy SS, Ray SK (2020) Ralstonia solanacearum preferential colonization in the shoot apical meristem explains its pathogenicity pattern in tomato seedlings. Plant pathology 69(7):1347-1356.
5. Jyoti P, Shree M, Joshi C, Prakash T, Ray SK, Satapathy SS, Masakapalli SK (2020) The Entner-Doudoroff and nonoxidative pentose phosphate pathways bypass glycolysis and the oxidative pentose phosphate pathway in Ralstonia solanacearum. mSystems 5:e00091-20. https://doi.org/10.1128/mSystems.00091-20.

B5. Benefits gained:

- Scientific & Technical expertise gained:
  a. Gained knowledge on metabolic pathway databases & associated tools and techniques
  b. Gained knowledge on genome sequencing and gene annotation, associated software, the underlying scientific principles and computational algorithms
  c. Gained experience in developing web portal with genomic data
- No. of NER manpower (including PI & staffs) trained in the Non-NER Institute:
  a. JRF:04, PI: 01 and Co-PI:01
- No. of visits by Non-NER Researchers to NER Institutes and vise-versa:
  a. NER Researchers to Non-NER Institutes (IIT Mandi): 02
  b. Non-NER Researchers to NER Institutes (Tezpur University): 01
- Training in any new techniques, if any
  a. metabolic pathway analysis, genome sequencing and gene annotation

Section-C: Details of Grant Utilization#

C1. Equipment Acquired or Placed Order with Actual Cost:

Sl. No.	Instruments	Equipment Price without VAT (in Rupees)	Cost including VAT & Discount (in Rupees)
1	HP color printer	35,840.00	37,990.00
2	Dell laptop	83,600.00	88,616.00
3	Dell workstation	2,45,000.00	2,59,700.00
4	Projector	54,450.00	62,618.00
		Total	4,48,924.00

C2. Manpower Staffing and Expenditure Details:

Tezpur University:

1st year (in Rupees)	2nd year (in Rupees)	3rd year (in Rupees)	4th year* (in Rupees)	Total (in Rupees)
56,234.00	2,65,484.00	2,75,000.00	26,400.00	6,23,118.00

*No Cost 6-month extension

C3. Details of Recurring Expenditure:

Tezpur University:

	1st year (in Rupees)	2nd year (in Rupees)	3rd year (in Rupees)	4th year* (in Rupees)	Total (in Rupees)
Consumables	00,000.00	2,50996.00	3,98063.00	2,69,520.00	9,18579.00
Travel	00,000.00	43,384.00	57,708.00	41,908.00	1,43000.00
Contingency	14,206.00	24,656.00	36,074.00	44,637.00	1,19573.00
Overhead	1,19,400.00	59,375.00	62,500.00	53,725.00	2,95000.00

C4. Financial Requirements for the Next Year with Justifications: Not Applicable

#Grant utilization details (UC&SE, Assets Certificate & manpower details) also required to be submitted separately as per the prescribed format

S. S. Satapathy

[Signature(s) of the Investigator(s)]

Instructions:

(i) All the information needs to be provided; otherwise the Progress Report will be treated as incomplete. In case of 'Nil' / 'Not Applicable' information, the same may be indicated.

(ii) In case of multicentre project, a combined Progress Report should be submitted incorporating the progress of all components. The Project Co-coordinator/ PI will be responsible for this.

*(iii) *Please indicate the reporting period [i.e. Year 1/2/3/4/5].*

(iv) Submission of Progress Report by the end of the 11th month of grant sanction is linked with further continuation of the project and timely release of funds for the next year.

Appendix-B

Utilisation Certificate

(for the financial year ending 31st March, 2021)

(Rs. in Lakhs)

1.	Title of the Project/Scheme: **"Integrating Genome scale metabolic analysis of model plant pathogen Ralstonia solanacearum with RNAseq and fluxomics"**	
2.	Name of the Organisation:	**Tezpur University**
3.	Principal Investigator:	**Dr. Siddhartha Sankar Satapathy**
4.	Deptt. of Biotechnology sanction order No. & date of sanctioning the project:	**BT/PR16361/NER/95/192/2015, Dt: 18/10/2016**
5.	Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given:	**Rs. 3.35789 lakhs**
6.	Amount received from DBT during the financial year (*please give No. and dates of sanction orders showing the amounts paid)*:	**Rs 0.00000 lakhs**
7.	Other receipts/interest earned, if any, on the DBT grants:	**Rs. 0.04486 lakhs (Interest earned)**
8.	Total amount that was available for expenditure during the financial year (Sl. Nos. 5,6 and 7):	**Rs 3.40275 lakhs**
9.	Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed):	**Rs. 1.00740 lakhs**
10.	Unspent balance refunded, if any *(Please give details of cheque No. Etc.):*	**Rs. *2.39535* lakhs**
11.	Balance amount available at the end of the financial year:	***'Not Applicable'***
12.	Amount allowed to be carried forward to the next financial year vide letter No. & date:	***'Not Applicable'***

1. Certified that the amount of **Rs. 1.00740 lakhs** mentioned against col. 9 has been utilised on the project/scheme for the purpose for which it was sanctioned and that the balance of **Rs. 2.39535 lakhs** remaining unutilized at the end of the year has been surrendered to Govt. (vide No. _____ dated _____)/~~will be adjusted towards the grants-in aid payable during the next year~~.

2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. (Cash Book)
2. (Ledgers)
3. (Vouchers)
4. (Bank Statements)
5.

(PROJECT INVESTIGATOR)

Dr. Sankar Satapathy
Assoc. Professor
Department of Computer Science & Engg.
Tezpur University

(FINANCE OFFICER)

Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)

Registrar
Tezpur University

(To be countersigned by the DBT Officer-in-charge)

Appendix-C

Statement of Expenditure referred to in para 9 of the Utilisation Certificate

Showing grants received the Department of Biotechnology and the expenditure incurred during the period from 1st April, 2020 to 31st March, 2021.

(Rs. in lakhs)

	Unspent balance carried forwarded from previous year	Grant received from DBT during the year	Other receipt/interest earned if any on the DBT grant	Total of Colm. (2+3+4)	Expenditure (excluding commitments) incurred during the Year	Balance (5-6)	Remarks
1	2	3	4	5	6	7	8
1. Non-Recurring							
(i) Equipments (Rs)	0.00076	0.00000		0.00076	0.00000	0.00076	
2. Recurring							
(i) Human Resource	1.31882	0.00000		1.31882	0.00000	1.31882	
(ii) Consumables	0.39421	0.00000		0.39421	0.00000	0.39421	
(iii) Travel	0.04565	0.00000	Total interest	0.04565	0.04565	0.00000	
(iv) Contingency	0.72877	0.00000	earned:	0.72877	0.42450	0.30427	
(v) Overheads	0.53725	0.00000		0.53725	0.53725	0.00000	
(vi) Interest	0.33243	----	0.04486	0.37729	----	0.37729	
Total:	**3.35789**	**0.00000**	**0.04486**	**3.40275**	**1.00740**	**2.39535**	

(PROJECT INVESTIGATOR)

Siddhartha Sankar Satapathy
Assoc. Professor
Department of Computer Science & Engg.
Tezpur University

(FINANCE OFFICER)

14-10-22

Finance Officer
Tezpur University

14/10/22

(HEAD OF THE INSTITUTE)

Registrar
Tezpur University

Appendix-A

Details of Assets acquired wholly or substantially out of Govt. grants (April 1 2017 to March 31, 2018)

Name of the Sanctioning Authority:		**DBT, Govt. of India, New Delhi**
1.	Sl. No.	**BCIL/NER-BPMC/2016/**
2.	Name of the Grantee Institution:	**Tezpur University**
3.	No. & Date of sanction order:	**BT/PR16361/NER/95/192/2015 dt. 18.10.2016**
3.	Amount of the sanctioned grant:	**Rs. 4,49,000.00**
4.	Brief purpose of the grant:	**"Integrating Genome scale metabolic analysis of model plant pathogen *Ralstonia solanacearum* with RNAseq and fluxomics"**
5.	Whether any condition regarding the right of ownership of Govt. in the Property or other assets acquired out of the grant was incorporated in the grant-in-aid sanction order:	**Yes**
7.	Particulars of assets actually credited or acquired:	**Rs. 4,48,924.00**
8.	Value of the assets as on **31/03/2018:**	**Rs. 4,48,924.00**
9.	Purpose for which utilized at present:	**For the project**
10.	Encumbered or not	**Not**
11.	Reasons, if encumbered	**NA**
12.	Disposed of or not	**Not**
13.	Reasons and authority, if any, for Disposal	**NA**
14.	Amount realized on disposal	**NA**
15.	Remarks	**Assets are at good, functional conditions.**

(PROJECT INVESTIGATOR)

(FINANCE OFFICER)

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* List of equipment purchased indicating the item wise costs may please be provided.

List of equipment purchased

Serial No.	Instruments	Status	Equipment Price without VAT (in Rupees)	Cost including VAT & Discount (in Rupees)
1	HP color printer	Installed	35,840.00	**37,990.00**
2	Dell laptop	Procured	83,600.00	**88,616.00**
3	Dell workstation	Installed	2,45,000.00	**2,59,700.00**
4	Projector	Installed	54,450.00	**62,618.00**
			Total	**4,48,924.00**

S.S.Sarkar 21/01/2019

(PROJECT INVESTIGATOR)

(FINANCE OFFICER)

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