Final Report of the work done on the Major Research Project

(From 01/04/2013 to 31/03/17)

F. No. 42-168/2013(SR)

Metagenomic analysis of rhizosphere diversity of *Crocus sativus* grown in Kashmir

Submitted To

THE SECRETARY
UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI-110002

Submitted by

PROF. JYOTI VAKHLU

SCHOOL OF BIOTECHNOLOGY

UNIVERSITY OF JAMMU

BABA SAHEB AMBEDKAR ROAD, JAMMU TAWI

JAMMU-180006

23236351, 23232701, 23237721, 23234116
23235733, 23232317, 23236735, 23239437

विश्वविद्यालय अनुदान आयोग
बहादुरशाह जफर मार्ग
नई दिल्ली-110 002
UNIVERSITY GRANTS COMMISSION
BAHADURSHAH ZAFAR MARG
NEW DELHI-110 002

F. No. 42-168/2013 (SR)

21 MAR 2013

The Under Secretary (FD-III)
University Grants Commission
New Delhi-110002

Sub:- UGC support for the Major Research Project in Physical Sciences, Bio-Sciences, Maths , Medical, Agricultural Sciences and Engineering & Chemistry to University/College Teachers – Project entitled,

"Metagenomic anlaysis of rhizosphere diversity of crocus sativus grown in Kashmir"

Sir,

I am to refer to your letter forwarding the application of **Dr. Jyoti Vakhlu** of your institution for financial assistance under the above scheme and to convey the Commission's approval & sanction an on account grant of **Rs. 7,54,800/- (Rupees: seven lakh fifty four thousand eight hundred only)** to the Registrar, **University of Jammu, [redacted], Jammu-180006, J&K** in r/o Major Research Project of **Dr. Jyoti Vakhlu,** Department of **Biotechnology** for the period of **3** years w.e.f. **1.4.2013** as detailed below:-

S.No	ITEMS	AMOUNT APPROVED	GRANT RELEASED AS Ist INSTALMENT	Category
A.	Non - Recurring		0	GEN
1.	Books & Journals	nil		
2.	Equipment ()	nil		
B.	Recurring			
1.	Honorarium to Retd. Teacher @ Rs. 12, 000/- p.m.	nil		
2.	Project Fellow @14,000/- p.m. for initial 2 years and Rs. 16,000/- p.m. from the third year onwards.	5,28,000/-		
3.	Chemical/ Glassware / Consumable	5,00,000/-	7,54,800/-	
4.	Hiring Services	1,50,000/-		
5.	Contingency .	30,000/-		
6.	Travel/Field Work	60,000/-		
7.	Special Need	nil		
8.	Overhead Charges @ Rs. 10% approved recurring Grant (Except Travel & Field Work)	1,20,800/-		
	Total (A + B)	13,88,800/-	7,54,800/-	

The acceptance Certificate in prescribed format (Annexure-1 available on the UGC web-site) may be sent to the undersigned within one month from the issue of the award letter failing which the project may be treated as cancelled.

If the terms & conditions are acceptable, as per guideline which are available on UGC web-site www.ugc.ac.in the Demand Draft/ Cheque being sent may be retained. Otherwise the same may be returned in original to the UGC by Registered Post in variably with in 15 days from the receipt of the Demand Draft/Cheque in favour of Secretary, UGC, New Delhi.

Principal Investigators should ensure that the statement of expenditure & utilization Certificate to the effect that the grant has been utilized for the purpose for which it has been sanctioned shall be furnished to the University Grants Commission in time.

The first instalment of the grant shall comprise of 100% of the Non –Recurring including Over Head Charges, and 50% of the total Recurring grant.

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002
GEN

FD Diary No. 4999
Dated : 21.06.2016

F .No.42-168/2013 (SR)

August 2016

24 AUG 2016

The Under Secretary (FD-III)
University Grants Commission
Bahadur Shah Zafar Marg
New Delhi – 110002

Sir,

Sub: Release of Grant-in aid to **University of Jammu, Jammu - 180 006, Jammu and Kashmir** for the year 2016-17 under plan in respect of Major Research Project entitled **Metagenomic.....Kashmir"** awarded to **Dr. Jyoti Vakhlu, Department of Biotechnology,** tenure of project from **01.04.2013 to 31.03.2017.**

I am directed to convey the sanction of the University Grants Commission for payment of grant of **Rs. 5,42,240/- (Rupees Five Lakh Forty Two Thousand Two Hundred Forty Only)** as **2nd installment** for the year 2016-17 towards Major Research Project to **The Registrar, University of Jammu, Jammu - 180 006, Jammu and Kashmir** for the plan expenditure to be incurred during 2016-17.

I am also directed to say that the tenure of the above project has been extended by the UGC upto 31.03.2017 without any additional financial assistance for the extended period.

Name of the Item	Amount Allocated	Head of Account	Grant now Being Sanctioned	Grant already Released	Total Grant
Books & Journal	.....	3.A(56).35	.....	.....	.....
Equipment	.....		.....	.....	.....
Honorarium	.....	3.A(56).31	.....	.....	.....
Project fellow	5,28,000/-		2,11,200/-	2,64,000/-	4,75,200/-
HRA	10,5,600/-		95,040/-	.....	95,040/-
Chemicals	5,00,000/-		2,00,000/-	2,50,000/-	4,50,000/-
Contingency	30,000/-		12,000/-	15,000/-	27,000/-
Hiring Services	1,50,000/-		.....	75,000/-	75,000/-
Travel/field work	60,000/-		24,000/-	30,000/-	54,000/-
Overhead Charges	1,20,800/-		.....	1,20,800/-	1,20,800/-
Additional Grant	.....		.....	.....	.....
Total	13,94,400/-		5,42,240/-	7,54,800/-	12,97,040/-

1. The sanctioned amount is debit able to **Major Research Project head Sector 3.A(56).31** and is valid for payment during the financial year **2016-17** only.

2. The amount of the Grant shall be drawn by the Under Secretary (Drawing and Disbursing Officer) UGC on the Grants-in-aid bill and shall be disbursed to and credited to **The Registrar, University of Jammu, Jammu - 180 006, Jammu and Kashmir** through Electronic mode as per the following details:-

(a)	Bank Name & Address of Branch	**The Jammu & Kashmir Bank Ltd, Jammu and Kashmir Bank, New University Campus, Jammu – 180 006.**
(b)	Account No	**0345040160000002**
(c)	Type of Account : SB /Current /Cash Credit	**SB**
(d)	**IFSC Code**	**JAKA0CANAAL**
(e)	MICR Code	**180051018**
(f)	Whether Bank Branch is RTGS or NEFT enabled : RTGS / NEFT /Both	**YES**
(g)	Name & Address of Account Holder	**The Registrar, University of Jammu, Jammu - 180 006, Jammu and Kashmir.**

3. The Grant is Subject to the adjustment on the basis of Utilization Certificate in the prescribed proforma submitted by the University / Institution.

4. The University / Institution shall maintain proper accounts of the expenditure out of the Grants, which shall be utilized, only on the approved items of expenditure.

5. The University / Institution may follow the General Financial Rules, 2005 and take urgent necessary action to amend their manuals of financial procedures to bring them in conformity with GFRs, 2005 and those don't have their own approved manuals on financial procedures may adopt the provisions of GFRs, 2005 and instructions / guidelines there under from time to time.

6. The Utilization Certificate to the effect that the grant has been utilized for the purpose for which it has been sanctioned shall be furnished to UGC as early as possible after the close of current financial year.

7. The assets acquired wholly for substantially out of University Grants Commission's Grant shall not be disposed or encumbered or utilized for the purposes other than those for which the grants waayanands given without proper sanction of the UGC and should at any time the University ceased to function, such assets shall revert to the University Grants Commission.

8. A Register of Assets acquired wholly or substantially out of the grant shall be maintained by the University in the prescribed proforma.

9. The grantee institution shall ensure the utilization of grants-in-aid for which it is being sanctioned / paid. In case of non-utilization / part utilization thereof, simple interest @ 10% per annum, as amended from time to time on the unutilized amount from the date of drawal to the date of refund as per provisions contained in General Financial Rules of Govt. of India, will be charged.

10. The University / Institutions shall follow strictly the Government of India / UGC guidelines regarding implementation of the reservation policy [both vertical (for SC, ST & OBC) and horizontal (for persons with disability etc.)] in teaching and non-teaching posts.

11. The University / Institution shall fully implement the Official Language Policy of Union Government and comply with the Official Language Act, 1963 and Official Languages (Use for Official Purposes of the Union) Rules, 1976 etc.

12. The sanction is issued in exercise of the delegation of powers vide UGC Order No. 69/2014 [F.No.10-11/12 (Admn. IA & B)] dated 26/3/2014.

13. The University / Institution shall strictly follow the UGC Regulations on curbing the menace of Ragging in Higher Education Institutions, 2009.

14. The University / Institution shall take immediate action for its accreditation by National Assessment & Accreditation Council (NAAC).

15. The accounts of the University / Institution will be open for audit by the Comptroller & Auditor General of India in accordance with the provisions of General Financial Rules, 2005.

16. The annual accounts i.e. balance sheet, income and expenditure statement and statement of receipts and payments are to be prepared strictly in accordance with the Uniform Format of Accounting prescribed by Government.

17. The grantee institution shall remit the amount the grants-in-aid and/or interest through e-mode (RTGS/NEFT) directly to UGC account as per following bank details:-

Account Holder	Secretary, UGC, New Delhi-110 002
Name of Bank & Address	Canara Bank, UGC Office, New Delhi-110 002
A/C No.	**8627101002122**
Type of A/C	Savings
IFSC Code	CNRB0008627
MICR Code	110015170

18. An amount of **Rs. 6,43,873/-** out the grant of **Rs. 7,54,800/-**..... Sanctioned vide letter No. **F. No. 42-168/2013 (SR)** dated **21-03-2013** has been utilized by University/College/Institution for the purpose for which it was sanctioned. Utilization Certificate for **Rs .....** ...NIL..... has been entered at S. No..... now we may enter Utilization Certificate for **Rs...6,43,873/-** .....S. No.4172 and in the U.C. Register at page No..07..

19. Funds to the extent of Rs are available under the scheme or BE / RBE of the year 2016-17.

20. These issues with the concurrence of IFD vide **Diary No 398 (IFD) dated 22/04/2016.**

21. This issues with the approval of Joint Secretary (MRP) vide **Diary No. 48009 dated 13/05/2016.**

Your faithfully,

(G.S. AULAKH)
UNDER SECRETARY

Copy forwarded for information and necessary action for :-

1. **The Registrar, University of Jammu, Jammu - 180 006, Jammu and Kashmir.**
2. Office of The Finance Officer, General of Audit, Central Revenues, AGCR Building, I.P. Estate, New Delhi.
3. Accountant General, State Govt. of **University of Jammu, Jammu - 180 006, Jammu and Kashmir.**
4. **Dr. Jyoti Vakhlu, Department of Biotechnology University of Jammu, Jammu - 180 006, Jammu and Kashmir.**
5. Guard file.

(ARUN KUMAR SINHA)
(SECTION OFFICER)

Annexure-VIII

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI-110002

Annual/Final Report of the work done on the Major Research Project.

1. Project report No . : Final report
2. UGC Reference No. : **F. No.42-168/2013(SR) dated 21st march, 2013**
3. Period of report : 01/04/2013-31/03/17
4. Title of research project : **Metagenomic analysis of rhizosphere diversity of *Crocus sativus* grown in Kashmir**

5. (a) Name of Principal Investigator : Prof.JyotiVakhlu
   (b) Department : School of Biotechnology
   (c) University/college where the work has progressed : University of Jammu
6. Effective date of starting of project : 01/04/2013
7. Grant approved and expenditure incurred during the period of the report:

   (a) Total amount approved : Rs. 14,94,400/-
   (b) Total amount received : Rs. 12,97,040/-
   (c) Total expenditure : Rs. 12,79,352/-

(c) Report of work done : Report attached

SIGNATURE OF
PRINCIPAL INVESTIGATOR

DR. JYOTI VAKHLU
PI UGC PROJECT
Metagenomic Analysis of Rhizosphere ...
... Kashmir

Registrar
University of Jammu

REGISTRAR/PRINCIPAL

(Seal)

11/4/17

For RC Gupta & Co.
Chartered Accountants

R.C. GUPTA & Co. FRN-001198 N Jammu Chartered Accountants

M.No:-504307

UDIN:-23504307BGZGA07127

Annexure-IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI-110002

PERFORMA FOR THE SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1 Title of research project : **Metagenomic analysis of rhizosphere diversity of *Crocus sativus* grown in Kashmir**

2 Name and address of the principal investigator : Prof JyotiVakhlu, School of Biotechnology, University of Jammu. Jammu-180006.

3 Name and address of the institution : School of Biotechnology, University of Jammu, Baba SahebAmbedkar Road, Tawi, Jammu, Jammu and Kashmir 180006.

4 UGC approval letter no. and Date : **F. No. 42-168/2013(SR) dated 21st march, 2013**

5 Date of implementation : 01-04-2013

6 Tenure of project : 01-04-2013 to 31-03-2017

7 **Total grant allocated** : **Rs 14,94,400/-**

8 **Total grant received** : **Rs 12,97,040/-**

9 **Final expenditure** : **Rs 12,79,352/-**

10 **Title of the project** : **Metagenomic analysis of rhizosphere diversity of *Crocus sativus* grown in Kashmir**

11 **Objectives of the project:**

a) Amplification of bacterial phylogenetic relavent genes from metagenome of bulk soil, rhizosphere, endorhizosphere, cormosphere, endocormosphere of saffron growth in Kashmir
b) NGS of amplicon and bioinformatic analysis
c) Cataloguing of bacterial diversity of underground part of saffron

Whether objectives were achieved:

The progress has been according to the original plan of the work, however there is slight deviation in the objective of the proposed project.

13 Achievements from the project :

The work done under this project will be communicating for the publication in reputed journal (Manuscript preparation under progress).

14 Summary of the findings : Separate sheet attached.

15 Contribution to the society : The proposed work was utilized for training the M.Sc. students.The students were also taught the isolation and purification techniques related to molecular biology. This opportunity was also utilized in the developing the skills of the students in analyzing the sequencing data.

16 Whether any Ph.D. enrolled/produced : Nil

17 No. Of publications out of the project : Nil

SIGNATURE OF
PRINCIPAL INVESTIGATOR

DR. JYOTI VAKHLU
PI UGC PROJECT
Metagenomic Analysis of Rhizosphere ... in Kashmir

REGISTRAR/PRINCIPAL

Registrar
University of Jammu

(Seal) 11/4

12/4/23

(CO-INVESTIGATOR)

For RC Gupta & Co.
Chartered Accountants

RC GUPTA & Co. * Chartered Accountants * CA FRN-001198N Jammu

M.No:-504307

UDIN:-23504307BGZGAG7127

Annexure-V

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI-110002

STATEMENT OF EXPENDITURE IN RESPECT OF MAJOR/MINOR RESEARCH PROJECT

1. Name of principal investigator : Prof.JyotiVakhlu
2. Deptt. Of University of Jammu : School of Biotechnology, University of Jammu
3. UGC approval letter no. and Date : **F. No. 42-168/2013(SR) dated 21st march, 2013**
4. Title of the project : **Metagenomic analysis of rhizosphere diversity of *Crocus sativus* grown in Kashmir**
5. Effective date of starting of project : 01-04-2013
6. Period of expenditure : 01-04-2013 to 31-03-2017
7. Detail of expenditure :

Statement of expenditure

S.No.	Items	Amount approved	Amount received	Expenditure incurred	Balance
A. Non-recurring					
1.	Books and journals	NIL			
2.	Equipments	NIL			
B. Recurring					
1.	Contingency	30,000	27,000	24,400	2,600
2.	Travel/Field work	60,000	54,000	39,730	14,270
3.	Hiring services	1,50,000	75,000	74,910	90
4.	Chemicals	5,00,000	4,50,000	4,49,912	88
5.	Overhead charges	1,20,800	1,20,800	1,20,800	NIL
	TOTAL	**8,60,800**	**7,26,800**	**7,09,752**	**17,048**

C. Staff

Date of Appointment: 11th September 2013

S.No.	Items	Amount approved	Amount received	Expenditure incurred	Balance
1.	Project fellow: Rs. 14,000/- p.m for initial 2 years and Rs. 16,000/- p.m for the 3rd year and HRA	6,33,600	5,70,240	5,69,600	640
	TOTAL	**6,33,600**	**5,70,240**	**5,69,600**	**640**

1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.

2. If as a result of checks or audit objectives, some irregularity is noticed, later date, action will be taken to refund, adjust or regularize the objective amount.

3. Payment @ revised rates shall be made with arrears on the availability of additional funds.

4. Certified that the grant of **Rs. 12,97,040/-** received from the University Grants Commission under the scheme of support for minor research project entitled "**Metagenomic analysis of rhizosphere diversity of *Crocus sativus* grown in Kashmir**" vide UGC letter No. **F. No. 42-168/2013(SR)** dated **21st march, 2013**. Out of which **Rs. 12,79,352/-** has been utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission and the balance amount of **Rs. 17,688/-** shall be refunded **to UGC**.

SIGNATURE OF
PRINCIPAL INVESTIGATOR

DR. JYOTI VARHLU
PI UGC PROJECT
Matagenomic Analysis of Rh zosphere ...
... in Kashmir

Registrar
University of Jammu

REGISTRAR/PRINCIPAL
(Seal)

For RC Gupta & Co.
Chartered Accountants

R. GUPTA & Co. CA FRN-001198 N Jammu Chartered Accountants

M.No:- 504307

UOIN:- 23504307BGZGAG7127

REPORT OF THE WORK DONE:

1. Brief objective of the project :

   Cataloguing of bacterial diversity of underground part of saffron.

2. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication.

   We catalogued the bacterial diversity associated with underground part of *Crocus sativus* including rhizosphere, daughter and mother cormosphere. Comparing bacterial diversity of bulk soil we were able to find spatial variation in the bacterial diversity of different underground part of the saffron crop.

3. Has the progress been according to original plan of work and towards achieving theobjective.if not, state reasons.

   The progress has been according to the original plan of the work, however there is slight deviation in the objective of the proposed project.

4. Please indicate the difficulties, if any, experienced in implementing the project : **No.**

5. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet : ---

6. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done may also be sent to the Commission:
   Copies Attached.

7. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as

   (a) Manpower trained : One Project fellow
   (b) Ph. D. awarded : NO
   (c) Publication of results : 03
   (d) other impact, if and

DR. JYOTI VAKHLU
PI UGC PROJECT
Metagenomic Analysis of Rhizosphere ..
Grown in Kashmi

SIGNATURE OF THE
PRINCIPAL INVESTIGATOR

Annexure-IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI-110002

PERFORMA FOR THE SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1 Title of research project : **Metagenomic analysis of rhizosphere diversity of *Crocus sativus* grown in Kashmir**

2 Name and address of the principal investigator : Prof JyotiVakhlu, School of Biotechnology, University of Jammu. Jammu-180006.

3 Name and address of the institution : School of Biotechnology, University of Jammu, Baba SahebAmbedkar Road, Tawi, Jammu, Jammu and Kashmir 180006.

4 UGC approval letter no. and Date : **F. No. 42-168/2013(SR) dated 21st march, 2013**

5 Date of implementation : 01-04-2013

6 Tenure of project : 01-04-2013 to 31-03-2017

7 Total grant allocated : Rs 14,94,400/-

8 Total grant received : Rs 12,97,040/-

9 Final expenditure : Rs 12,79,352/-

10 **Title of the project** : **Metagenomic analysis of rhizosphere diversity of *Crocus sativus* grown in Kashmir**

11 Objectives of the project:

a) Amplification of bacterial phylogenetic relavent genes from metagenome of bulk soil, rhizosphere, endorhizosphere, cormosphere, endocormosphere of saffron growth in Kashmir
b) NGS of amplicon and bioinformatic analysis
c) Cataloguing of bacterial diversity of underground part of saffron

Whether objectives were achieved:

The progress has been according to the original plan of the work, however there is slight deviation in the objective of the proposed project.

13 Achievements from the project :

The work done under this project will be communicating for the publication in reputed journal (Manuscript preparation under progress).

14 Summary of the findings : Separate sheet attached.

15 Contribution to the society : The proposed work was utilized for training the M.Sc. students.The students were also taught the isolation and purification techniques related to molecular biology. This opportunity was also utilized in the developing the skills of the students in analyzing the sequencing data.

16 Whether any Ph.D. enrolled/produced : Nil

17 No. Of publications out of the project : Nil

SIGNATURE OF
PRINCIPAL INVESTIGATOR

DR. JYOTI VAKHLU
PI UGC PROJECT
Matagenomic Analysis of Rhizosphere ...
... in Kashmir

REGISTRAR/PRINCIPAL

Registrar
University of Jammu

(Seal) 11/4 14/4/23

(CO-INVESTIGATOR)

For RC Gupta & Co.
Chartered Accountants

RC GUPTA & Co. CA FRN-001498N Chartered Accountants

M.No:-504307
UDIN:-23504307BGZGAG7127

REPORT OF THE WORK DONE:

1. Brief objective of the project :

Cataloguing of bacterial diversity of underground part of saffron.

2. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication.

We catalogued the bacterial diversity associated with underground part of *Crocus sativus* including rhizosphere, daughter and mother cormosphere. Comparing bacterial diversity of bulk soil we were able to find spatial variation in the bacterial diversity of different underground part of the saffron crop.

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The progress has been according to the original plan of the work, however there is slight deviation in the objective of the proposed project.

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6. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done may also be sent to the Commission:
Copies Attached.

7. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as

(a) Manpower trained	:	One Project fellow
(b) Ph. D. awarded	:	NO
(c) Publication of results	:	03
(d) other impact, if and		

DR. JYOTI VAKHLU
PI UGC PROJECT
Metagenomic Analysis of Rhizosphere ..
Grown in Kashmir.

SIGNATURE OF THE
PRINCIPAL INVESTIGATOR

REPORT OF THE WORK DONE:

1. Brief objective of the project :

   Cataloguing of bacterial diversity of underground part of saffron.

2. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication.

   We catalogued the bacterial diversity associated with underground part of *Crocus sativus* including rhizosphere, daughter and mother cormosphere. Comparing bacterial diversity of bulk soil we were able to find spatial variation in the bacterial diversity of different underground part of the saffron crop.

3. Has the progress been according to original plan of work and towards achieving theobjective.if not, state reasons.

   The progress has been according to the original plan of the work, however there is slight deviation in the objective of the proposed project.

4. Please indicate the difficulties, if any, experienced in implementing the project : **No.**

5. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet : ---

6. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done may also be sent to the Commission: **Copies Attached.**

7. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as

   (a) Manpower trained : One Project fellow
   (b) Ph. D. awarded : NO
   (c) Publication of results : 03
   (d) other impact, if any

SIGNATURE OF THE
PRINCIPAL
INVESTIGATOR

SUMMARY:

We analysed bacterial microbiome from three separate below ground compartments of saffron which includes rhizosphere, mother cormosphere and daughter cormosphere taking bulk soil as a reference. The root-associated bacterial communities were sampled in vegetative phase of saffron life cycle because it is the phase when mother corm gives rise to daughter corm. The rhizosphere compartment was composed of soil tightly adhering the roots which cannot be easily shaken from the roots. The mother cormosphere compartment comprises of the outer sheath of the mother corm and similarly daughter corm sheath was taken as daughter cormosphere.

While comparing the bacterial diversity at phylum level we found that bulk soil was dominated by firmicutes (11%) followed by proteobacteria (10%) and actinobacteria (8%). Bacteriodetes (22%) and proteobacteria (21% and 23%) were dominant in both daughter cormosphere and rhizosphere followed by firmicutes (6.2% and 3%)and actinobacteria (5.4% and 3%) but mother corm was dominated by proteobacteria (25%)followed by bacteriodetes (14%) and acidobacteria (4%).

There are notable differences in the proportions of various generas across the samples. The rhizosphere and daughter cormosphere has greater percentage of Pedobacter, Chryseobacterium, Rhizobium and Serratia as compared to mother cormosphere, whereas Sphingomonas, Acidobacterium and Pseudomonas are depleted in rhizosphere and daughter cormosphere compared to mother cormosphere.

This study revealed significant difference in bacterialdiversity of bulk soil with all other samples i.e, - daughter corm, - rhizosphere and mother cormosphere by Principal coordinates (Unifrac) analysis. It was observed that bacterial diversity associated with rhizosphere was similar to daughter cormosphere and both were different from mother cormosphere

Material and methods:

Sample collection

For sampling, Kashmir (74º58'0"E, 34º1'30"N, 5173ft) of Jammu and Kashmir, India was selected during vegetative phase(January - May). The loose soil was removed by vigorously shaking of corms and then corm sheath was taken as cormosphere. The samples were properly labeled and transported to the laboratory in the autoclaved bags. The samples were stored at -20˚C for further metagenomic analysis.

Preparation of the sample

The corm sheath was separated from corms and further used for the isolation of metagenomic DNA using different protocols. The isolation of metagenomic DNA was done in triplicates for each sample with three different protocols, namely, Zhou *et al*. (1996), Wetcher*et al*. (2003) and Pang *et al*. (2008). The triplicates were subsequently pooled to get maximum diversity for each sample. The DNA was quantified using QubitdsDNA HS Assay kit.

Preparation of MiSeqAmplicon Library

The amplicon libraries were prepared using Nextera XT Index Kit (Illumina Inc.) as per the 16S Metagenomic Sequencing Library preparation protocol. Primers for the amplification of the V3-V4 hyper-variable region (Table-1) of 16S rDNA gene of Eubacteria and Archaea were designed in Xcelris NGS Bioinformatics Lab. These primers were synthesized in Xcelris**PrimeX**facility.

Table1: Primers for the amplification of the V3-V4 hyper-variable region

Sr.No.	Oligo Name	Oligo Sequence (5' to 3')	Length of primer	Product size (Approx.)
1	V3-Forward	CCTACGGGNGG CWGCAG	17	~ 460 bps
2	V4-Reverse	GACTACHVGGGG TATCTAATCC	21	

The amplicons with the Illumina adaptors were amplified by using i5 and i7 primers that add multiplexing index sequences as well as common adapters required for cluster generation (P5 and P7) as per the standard Illumina protocol. The amplicon libraries were purified by 1X AMpureXP beads and checked on Agilent DNA High Sensitivity chip on Bioanalyzer 2100 and quantified on fluometer by QubitdsDNA HS Assay kit (Life Technologies).

Quality was checked on 1% agarose gel and concentration of each sample was determined using Qubit® 2.0 fluorometer. The pooled metagenomic DNA samples were subjected to paired-end sequencing using IlluminaMiSeq platform 2 x 150bp chemistry at Xcelris Labs Ltd, Ahemdabad, India.Briefly,the paired-end sequencing libarary was prepared using IlluminaTruSeq Nano DNA HT Library preparation kit. The amplified libaray was analysed in Bioanalyser 2100 (Agilent Technologies) using High sensitivity (HS) DNA chip. After obtaining the mean peak size from the Bioanalyser profile, library was loaded onto NextSeq 500 for cluster generation and sequencing.

Sequence analysis

Sequences were analysed using QIIME software. QIIME is comprehensive software comprising of tools and algorithms such as FastTree for heuristic based maximum-likelihood phylogeny inference (Price et al., 2010), the RDP classifier for the assignment of taxonomic data using a naïve bayesian classifier (Wang et al., 2007). To process our data Stitching of the PE data into single end reads was done. All the sequences from all the samples were clustered into Operational Taxonomic Units (OTUs) based on their sequence similarity. OTUs are clusters of sequences, frequently intended to represent some degree of taxonomic relatedness done using uclust, each resulting cluster is typically represents a genus.A representative sequence from each OUT was picked for downstream analysis. Alignment of the sequences against the representative sequences is performed using PyNAST. Minimum length of 150 and a minimum percent identity of 75.0 was used for alignment purpose. Alignment was followed by assigning the taxonomy to microbial lineages. This was done by setting assignment_method to the RDP classification system and a confidence of 0.8. PCoA plots were also generated to visually depict the differences between the samples.

Result:

We analysed bacterial microbiome from three separate below ground compartments of saffron which includes rhizosphere, mother cormosphere and daughter cormosphere taking bulk soil as a reference (Fig. 1). The root-associated bacterial communities were sampled in vegetative phase of saffron life cycle because it is the phase when mother corm gives rise to daughter corm. The rhizosphere compartment compose of soil tightly adhered to the roots which cannot be easily shaken from the roots. The mother cormosphere compartment comprises of the outer sheath of the mother corm and similarly daughter corm sheath was taken as daughter cormosphere.

Total metagenomic DNA from the bacterial community of all the four samples was extracted using various protocols (Zhou et al. 1996,Wechter et al. 2003, and Pang et al. 2008) but only pang protocol yielded good quantity of DNA.

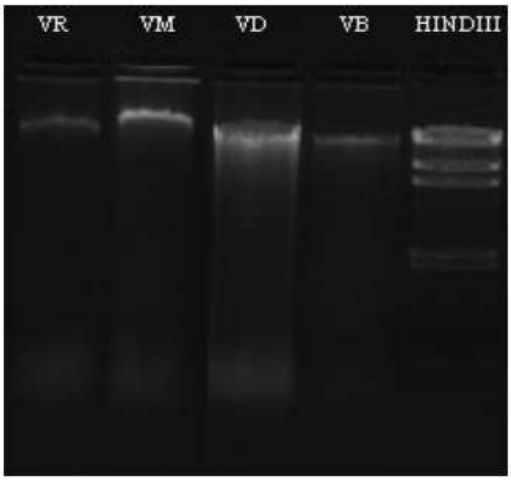

Fig1. **Quality check of gDNA in 0.8% Agarose gel**

The V3-V4 hyper-variable region of the 16S rDNA gene was amplified and sequenced using illumine 2 x 300 bp sequencing chemistry. Total of 4,562,387 high quality reads were obtained from all samples in range of 651,725-1,598,661read counts per sample. The sequences are uploaded on MG-RAST with following Id numbers

Sample no.	CODE	MG-RAST ID
1	VB_S15_L001	bc55f65f216d676d343538333830332e33
2	VM_S13_L001	08a86e01a66d676d343538333830302e33
3	VD_S14_L001	de6f0c26e26d676d343538333830312e33
4	VR_S12_L001	14abf2ace26d676d343538333734372e33

We observed the Bray–Curtis distances between samples using PCoA to determine how dissimilar the soil microbial communities were in each sample. While considering PCo1, Principal coordinates (Unifrac) analysis of bacterial communityof four samples reflects significant difference in bacterialdiversity of bulk soil (brown) with all other samples i.e, - daughter corm(red), - rhizosphere(green) and mother cormosphere (yellow). When PCo2 is considered, it was observed that rhizosphere was similar to daughter cormosphere and both were different from mother cormosphere(Fig. 3). This is also supported bootstrap-supported hierarchical cluster tree of four microbial communities under investigation showing that bulk form separate cluster. The tree also shows that daughter cormosphere and rhizosphere are more close and share a node (Fig. 4).

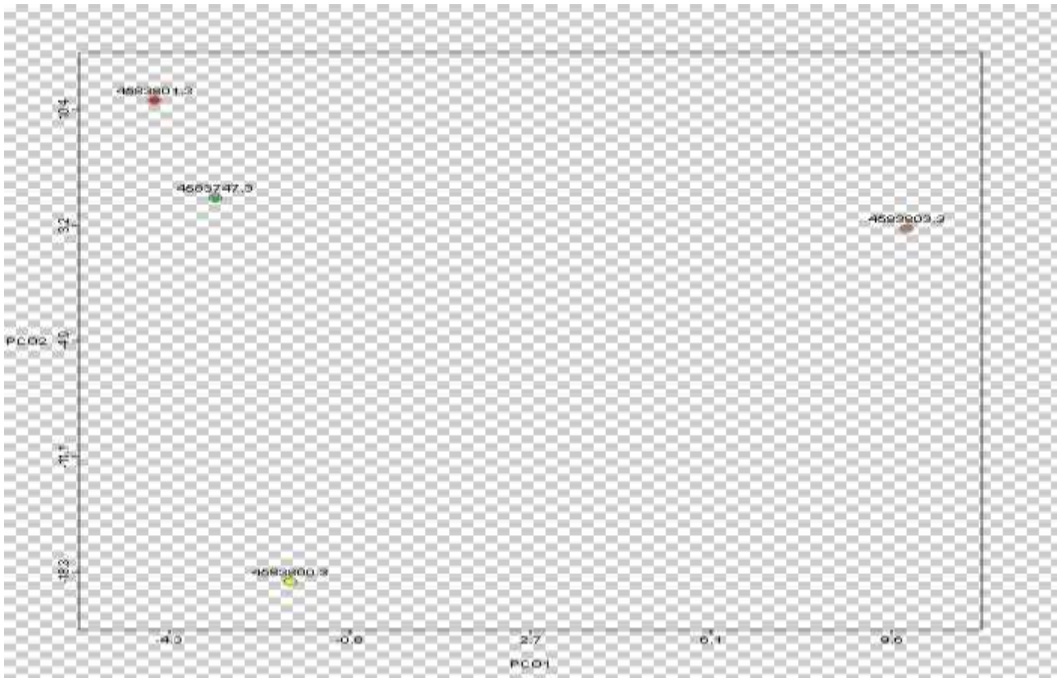

Fig. 3.PCoA plot [red- daughter corm, green- rhizosphere, yellow- mc, brown- bulk]

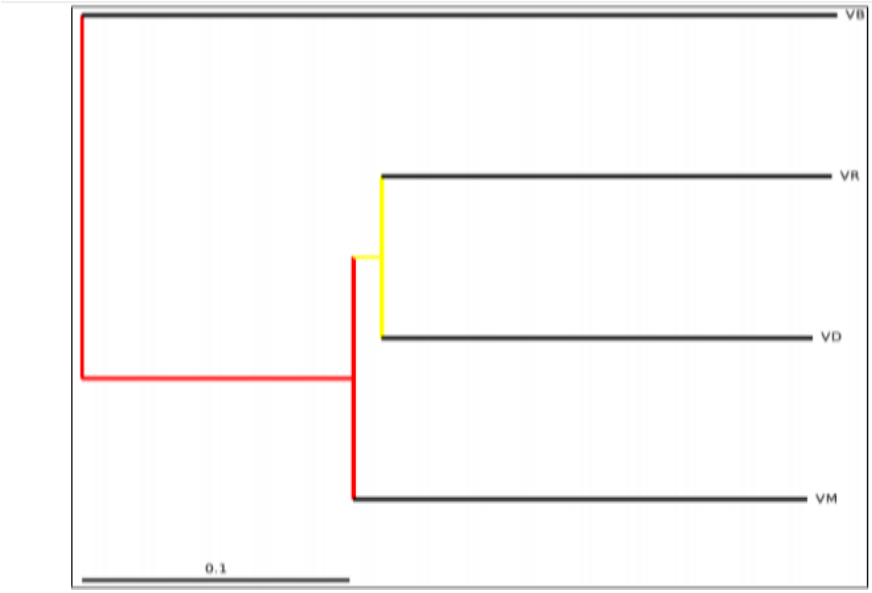

Fig.4 A visualization of bootstrap-supported hierarchical cluster tree.

The figure shows the tree with internal nodes colored with red signifying 75-100% support, yellow signifying 50-75%, green indicating 25-50%, and blue for < 25% bootstrap support. The bar at the bottom of the tree indicates a length corresponding to 0.1 which indicates nucleotide substitutions per site

At phylum level, bulk soil was dominated by firmicutes(11%) followed by proteobacteria(10%) and actinobacteria(8%). Bacteriodetes (22%)and proteobacteria(21% and 23%) were dominant in both daughter cormosphere and rhizosphere followed by firmicutes (6.2% and 3%)and actinobacteria(5.4% and 3%) but mother corm was dominated by proteobacteria (25%)followed by bacteriodetes(14%) and acidobacteria(4%) (Fig: 5).

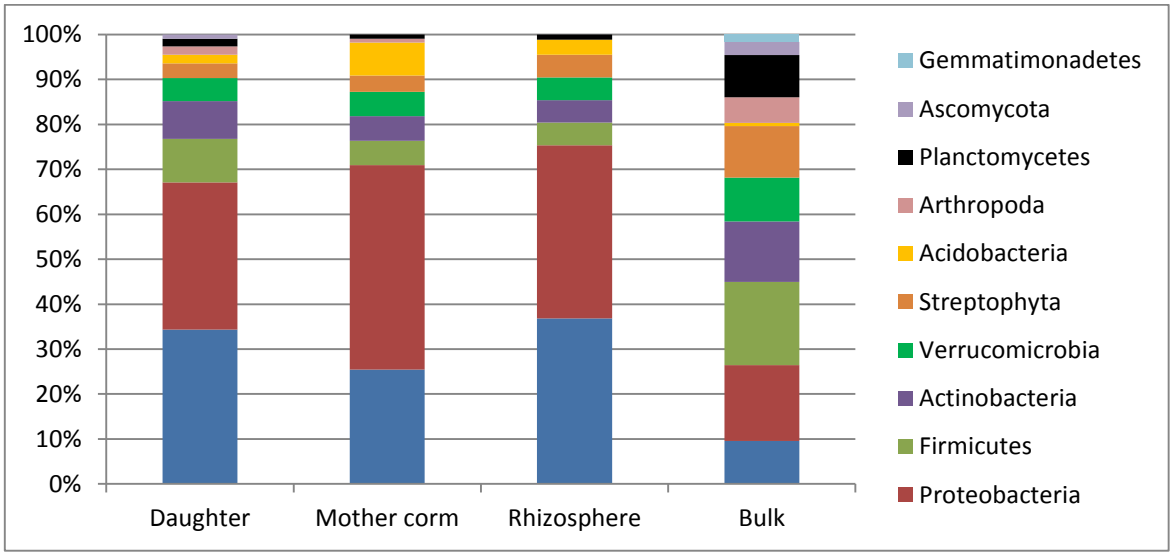

Fig 5: Phylum level distribution across all four samples

There are notable differences in the proportions of various generas across the samples(Fig. 6). The rhizosphere and daughter cormosphere has greater percentage of Pedobacter, Chryseobacterium, Rhizobium and Serratia as compared to mother cormosphere, whereas Sphingomonas, Acidobacterium and Pseudomonas are depleted in rhizosphere and daughter cormosphere compared to mother cormosphere.

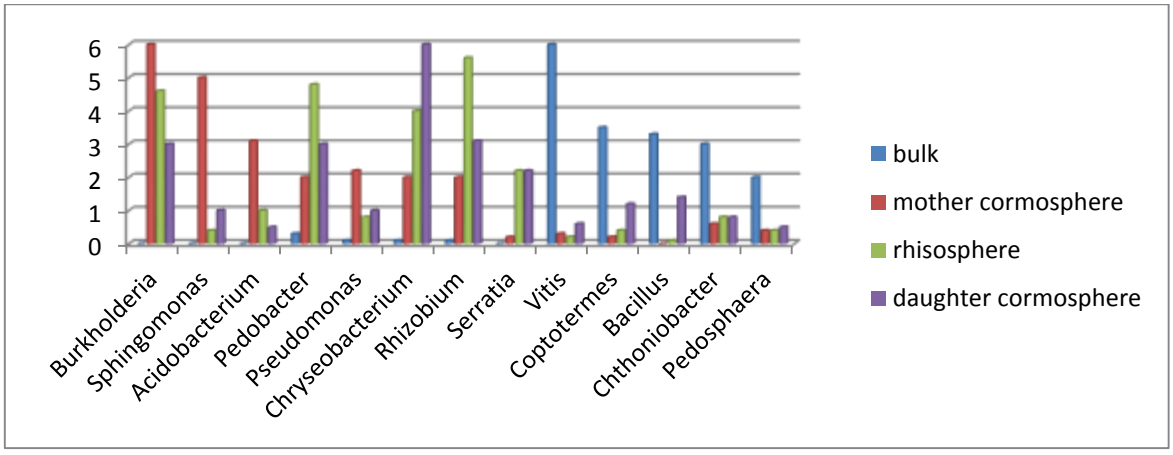

Fig.6. *Differences in the proportions of various generas across the samples*

This study revealed significant difference in bacterialdiversity of bulk soil with all other samples i.e, - daughter corm, - rhizosphere and mother cormosphere by Principal coordinates (Unifrac) analysis. It was observed that bacterial diversity associated with rhizosphere was similar to daughter cormosphere and both were different from mother cormosphere

PLOS | ONE

RESEARCH ARTICLE

Comparative Metagenomics Reveal Phylum Level Temporal and Spatial Changes in Mycobiome of Belowground Parts of *Crocus sativus*

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Abstract

Plant-fungal associations have been explored by routine cultivation based approaches and cultivation based approaches cannot catalogue more than 5% of fungal diversity associated with any niche. In the present study, an attempt has been made to catalogue fungal diversity associated with belowground parts i.e. rhizosphere and cormosphere, of *Crocus sativus* (an economically important herb) during two growth stages, using cultivation independent ITS gene targeted approach, taking bulk soil as reference. The 454 pyrosequencing sequence data analysis suggests that the fungal diversity was niche and growth stage specific. Fungi diversity, in the present case, was not only different between the two organs (roots and corm) but the dominance pattern varies between the cormosphere during two growth stages. *Zygomycota* was dominant fungal phylum in the rhizosphere whereas *Basidiomycota* was dominant in cormosphere during flowering stage. However in cormosphere though *Basidiomycota* was dominant phylum during flowering stage but *Zygomycota* was dominant during dormant stage. Interestingly, in cormosphere, the phyla which was dominant at dormant stage was rare at flowering stage and vice-versa (*Basidiomycota*: Flowering = 93.2% Dormant = 0.05% and *Zygomycota*: Flowering = 0.8% Dormant = 99.7%). At genus level, *Rhizopus* was dominant in dormant stage but was rare in flowering stage (*Rhizopus*: Dormant = 99.7% Flowering = 0.55%). This dynamics is not followed by the bulk soil fungi which was dominated by *Ascomycota* during both stages under study. The genus *Fusarium*, whose species *F. oxysporum* causes corm rot in *C. sativus*, was present during both stages with slightly higher abundance in roots. Interestingly, the abundance of *Rhizopus* varied a great deal in two stages in cormosphere but the abundance of *Fusarium* was comparable in two growth stages (Bulk soil Flowering = 0.05%, Rhizosphere Flowering = 1.4%, Cormosphere Flowering = 0.06%, Bulk soil Dormant = 2.47% and cormosphere dormant = 0.05%). This is the first report on the fungal diversity associated with the root of

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ORIGINAL RESEARCH
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Microbiome Fingerprint as Biomarker for Geographical Origin and Heredity in *Crocus sativus*: A Feasibility Study

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Host–microbiome interactions are specific and not random, making them defining entities for the host. The hypothesis proposed by various researchers earlier, that both plants and animals harbor specific inheritable core microbiome, is being augmented in the present study. Additionally, a case for using microbial fingerprint as a biomarker, not only for plant identification but also as a geographical indicator, has been investigated, taking *Crocus sativus*, saffron, as a study material. *Crocus sativus*, a monogenetic herb, on account of its male sterility and vegetative propagation, is reported to lack genome based molecular markers. Cormosphere microbiome (microbiome associated with corm) has been compared across three geographical locations, in two continents, to identify the core and unique microbiome, during the vegetative phase of its growth. Microbiome analysis done at phylum and genus level, using next generation sequencing technology, revealed that cormosphere at three locations harbored common phyla. At genus level, 24 genera were found common to all three geographical locations, indicating them to be part of the core microbiome of saffron. However, there were some bacterial genera unique to Kashmir, Kishtwar, and Morocco that can be used to develop microbial markers/geographical indicators for saffron grown in these regions. This is a preliminary study, indicating that the location specific bacterial community can be used to develop microbial barcodes but needs further augmentation with high coverage data from other saffron growing geographical regions.

Keywords: barcodes, microbiome, microbial fingerprint, biomarker, cormosphere, saffron

INTRODUCTION

Molecular markers are important tools for plant genome analysis, crop improvement, and development of barcodes for authentic plant identification (Mishra et al., 2016). The development of molecular markers relies on genetic variation in the plants; however, plants lacking genetic variation due to asexual reproduction harbor unidentified or poor resolution molecular markers. *Crocus sativus* is one amongst such plants that lack genetic variations. *Crocus sativus*, commonly known as saffron, is an autumn-flowering

Bacillus-Mediated-Induced Systemic Resistance (ISR) Against *Fusarium* Corm Rot

2

Shanu Magotra, Deepika Trakroo, Sneha Ganjoo, and Jyoti Vakhlu

Abstract

Fungi constitute the largest group of plant pathogens responsible for a range of serious plant diseases wherein fungal rot is a major disease associated with the pre- and post-harvest produce of plants. One of the most widely spread rot-causing fungi is *Fusarium* spp. that include *F. oxysporum*, *F. graminearum*, and *F. solani* that infect bulbs, tubers, rhizomes, and corms and lead to the decomposition of the tissue and finally death of the plant. Under low temperature, fungal infection usually remains dormant which under favorable climatic conditions converts in to disease. As there is a large decline in the annual yield of the crop plant due to *Fusarium* rots, so this has been an issue of concern since long. Earlier only chemical pesticides were used to control these infections but due to their ill effects on soil fertility, the focus has shifted to the use of biological control agents (BCA). Among BCA, a group comprised of bacilli, pseudomonads, and actinomycetes, together with nonpathogenic organisms *Fusarium*, *Trichoderma*, and *Streptomyces*, played an important role against phytopathogens. BCA helps in plant disease control and growth mainly by two methods: (i) secretion of antimicrobial compounds and (ii) induction of systemic resistance in plants. *Bacillus* species have been very effective BCA due to their ability to produce heat and desiccation-resistant spores and to withstand high temperature, unfavorable pH, lack of nutrients or water, and the ease of stable formulation preparation. This species can display almost all the mechanisms of a bio-control and bio-stimulation/fertilization agent.

2.1 Introduction

Plants by virtue of being capable of fixing solar energy are the main players in a complex food web together with their ability to innate defensive capacity against pests and pathogens.

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