Protects Ecosystems and Biodiversity

Manipal University Jaipur features an expansive campus that has evolved into a dynamic environment for biodiversity conservation. The institution emphasizes the importance of native plant landscaping, the creation of wildlife habitats, and the implementation of sustainable land management practices, thereby serving as a model for surrounding communities. Central to the university's mission is a dedication to research and innovation. Faculty members, researchers, and students at Manipal University Jaipur are committed to exploring ecosystems, examining endangered species, and formulating conservation strategies. The university is actively involved in practical conservation initiatives, having launched restoration and rehabilitation projects in partnership with local conservation groups. These initiatives aim to restore degraded ecosystems, promote the planting of native species, and eliminate invasive species that pose a threat to biodiversity. Manipal University Jaipur has taken significant steps to establish and manage protected natural reserves. These reserves provide safe havens for local flora and fauna, enabling species to flourish in a secure environment. They also function as outdoor classrooms, offering students valuable experiential learning opportunities. Students are integral to the university's biodiversity preservation efforts, as they are encouraged to spearhead and participate in various conservation projects, ranging from tree planting initiatives to wildlife surveys. This active engagement enhances the effectiveness of these projects and cultivates a strong sense of environmental stewardship among the student body. Additionally, Manipal University Jaipur fosters connections with the wider community through public outreach initiatives, nature walks, workshops, and educational campaigns, thereby raising awareness about biodiversity conservation and motivating individuals and communities to become involved in these efforts. Manipal University Jaipur stands as a formidable force in the field of biodiversity conservation, characterized by its academic proficiency, research potential, and a vibrant community of enthusiastic young individuals. Through a combination of research endeavors, practical initiatives, student engagement, public awareness campaigns, and collaborative partnerships, the university plays a pivotal role in the preservation, enhancement, and expansion of current ecosystems and their diverse biological wealth.

MANIPAL UNIVERSITY
JAIPUR

Faculty of Management and Commerce

Department of Business Administration

Societal Connect Activity on

Bird Nest Installation

NOVEMBER 30, 2023

Head
Department of Business Administration
Manipal University Jaipur

1. **Introduction of the Event**

Introduction of the Event: School of Business and Commerce organized a activity to install bird nests in the nearby village on November 30, 2023. 5 students and 1 faculty member participated in the campaign. The event took place in nearby village of Manipal university.

2. **Objective of the Event**

The primary objective of the event was to promote environmental awareness and conservation by actively contributing to the well-being of local bird populations. Through the installation of bird nests, the aim was to create a sustainable habitat for birds in the nearby village, fostering biodiversity and ecological balance.

3. **Beneficiaries of the Event**

The beneficiaries of the event included the local bird species in the nearby village. By providing suitable nesting spaces, the initiative sought to enhance the living conditions for birds, contributing to the overall ecosystem health. Additionally, the participating students gained hands-on experience in environmental stewardship.

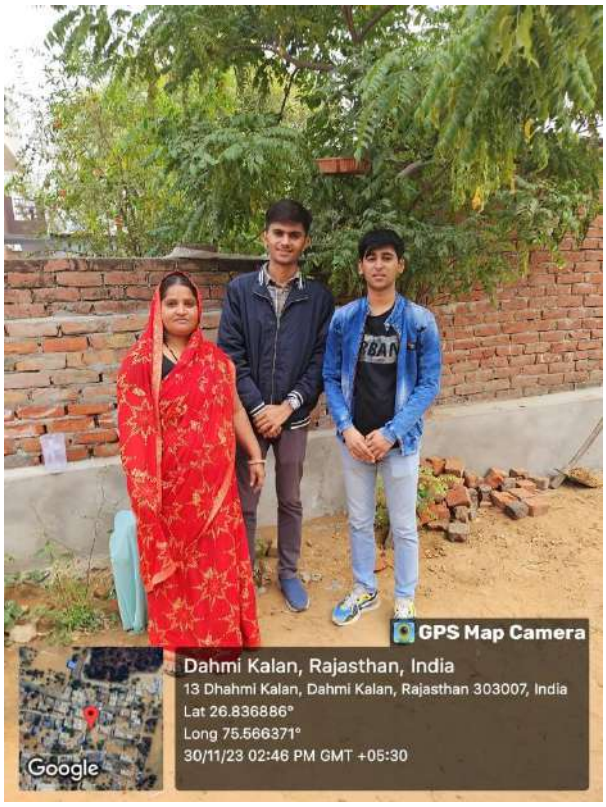
4. **Details of the Guests**

The event was laid by the students of BBA.

5. **Brief Description of the event**

The activity involved the installation of bird nests in the nearby village of Manipal University, with students and faculty members actively engaging in the process. Participants worked together to strategically place the nests, considering the local ecology and the needs of various bird species. The event not only contributed to the local environment but also provided a unique learning experience for the students, emphasizing the importance of hands-on conservation efforts. Overall, the initiative aimed to create a positive impact on the local ecosystem while instilling a sense of environmental responsibility among the participants.

6. **Photographs**

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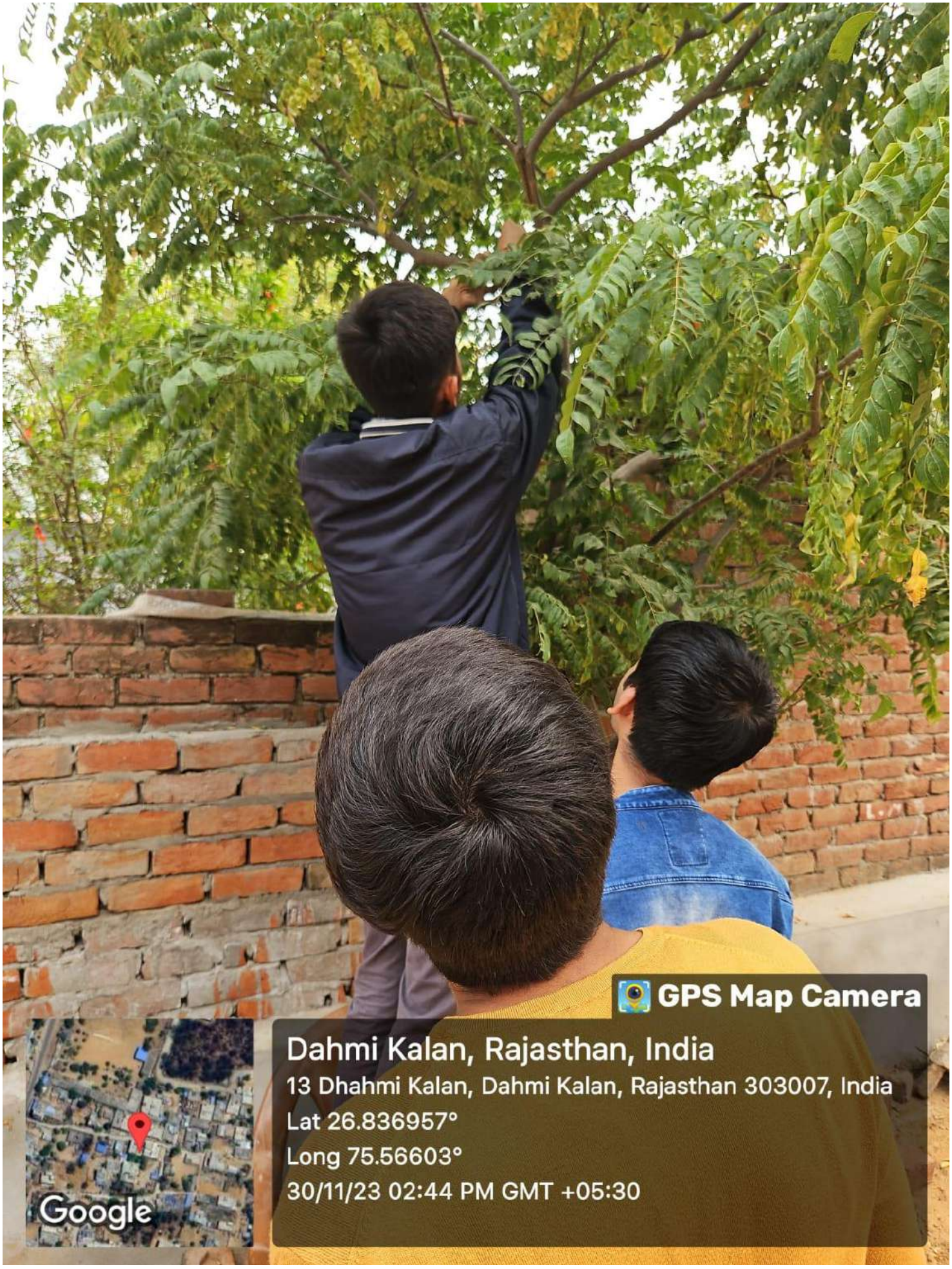
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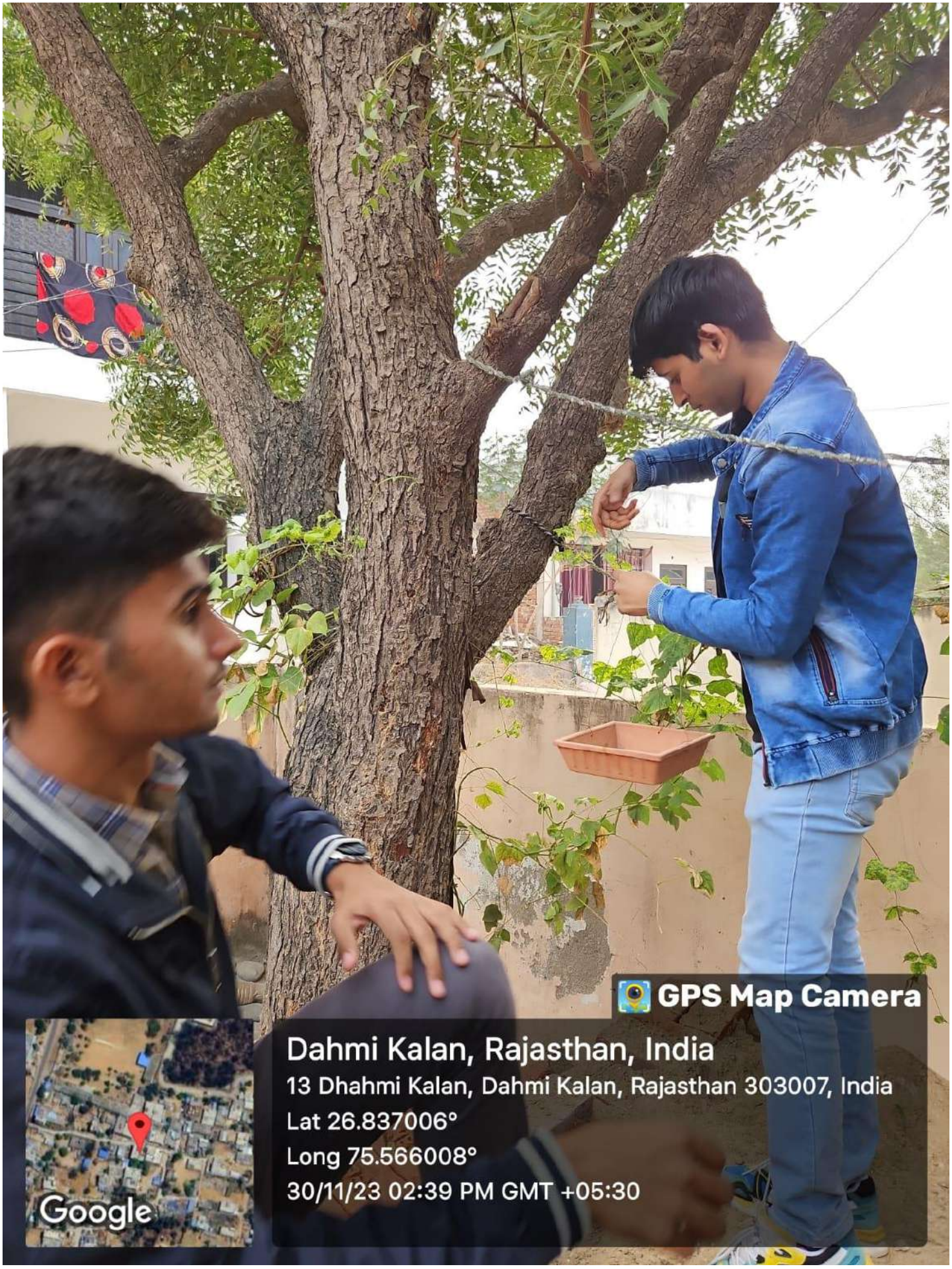
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GPS Map Camera
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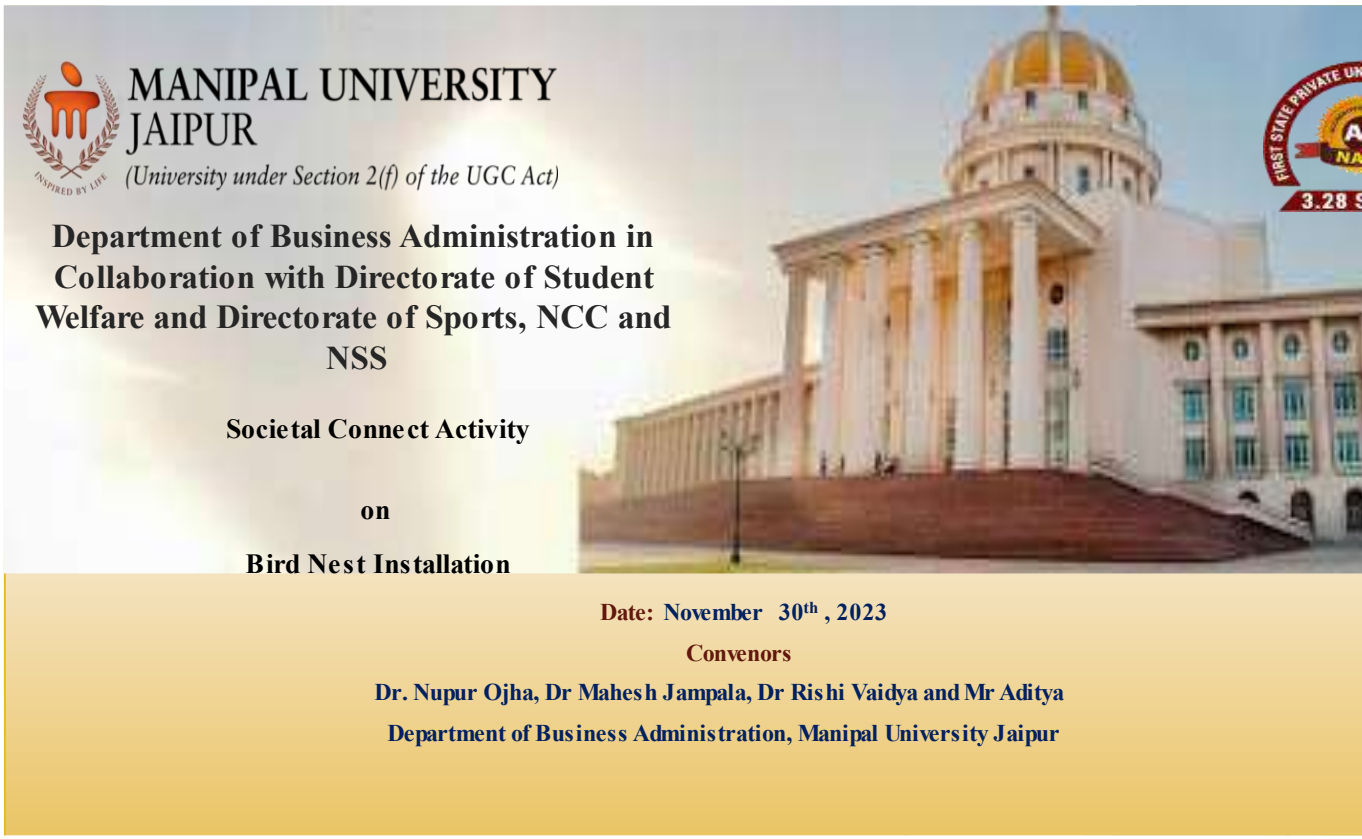
7. Brochure or creative of the event



8. Schedule of the Event

The event took place on November 30, 2023

9. Attendance of the Event

Sr. No	Name of Institution	Registration Number/ Employee Code	Attendee Name
1	Manipal University Jaipur	MUJ0099	Dr. Mahesh Jampala
2	Manipal University Jaipur	MUJ1538	Dr Rishi Vaidya
3	Manipal University Jaipur	MUJ0623	Dr. Nupur Ojha
4	Manipal University Jaipur	MUJ1490	Mr. Aditya Dhiman
5	Manipal University Jaipur	23FM10BBA00204	DINESH CHOUDHARY
6	Manipal University Jaipur	23FM10BBA00200	VANSH MULCHANDANI
7	Manipal University Jaipur	23FM10BBA00214	GOPAL BISHNOI
8	Manipal University Jaipur	23FM10BBA00215	AKSHAT SHARMA
9	Manipal University Jaipur	23FM10BBA00216	KHUSHWANT SANKHLA
10	Manipal University Jaipur	23FM10BBA00205	AYUSHMAN GUPTA

Head
Department of Business Administration
Manipal University Jaipur

Minutes of the Meeting 22nd BOS of Department of Law

17th May, 2023

The 22nd BOS meeting of the Department of Law was held as per the following schedule: -

Date & Time: May 17th, 2023 at room no. 208 in 2AB

Mode: Offline at 2 o'clock, room no. 208 2AB

Members of the BoS are as follows: -

1. Dr. Sony Kulshrestha-Chairperson
2. Prof. Jayaram ER-Special Invitee
3. Prof. Vijaylaxmi Sharma- Special Invitee
4. Prof. Richa Arora- Internal Member
5. Prof. Nitu Bhatanagar- Ex-Officio Member
6. Prof. Mridul Srivastava- External Member
7. Mr. Abhay Jain (ADJ)- Industry Member
8. Dr. Susruta Samanta- Member, Directorate of Academics
9. Dr. Ashu Maharshi- Convener

The following agenda points were placed before the BOS: -

Agenda Points:

A. Discussion:

Item I: Approval of SWAYAM/NPTEL courses for the Even and Odd sessions ie. Jan-Dec, 2023 to be included in CWS of all programs. Annexure I

Item II: Approval of revamped course structure and first year syllabi of existing programs BALLB(H), BBALLB(H), LLB and LLM (Corporate and Commercial Law) and (Criminal and Security Law) and course structure and syllabi of new programs ie. B.Com. LLB(H), B.Sc. LLB(H) and LLM (Intellectual Property Law) to be introduced and implemented from AY 2023-24. Annexure II

Item III: Approval of the course codes and syllabi of courses to be introduced in Department of Business Administration and Department of Commerce ie. School of Business and Commerce. Annexure III

Item IV: Approval of course codes and syllabi of first-degree courses offered by SBC for BBALLB(H) and BCom.LLB(H) programs, SHSS for BALLB(H) program and FoS for BSc.LLB(H) program. Annexure IV

Item V: Approval of Vision and Mission of the Department of Law in line with the Vision and Mission of the University. Annexure V

Item VI: Approval of CWS for Odd semester i.e. August to December 2023. Annexure VI

Item VII: Approval of Value Added courses to be introduced in AY 2023-24. Annexure VII

Item VIII: Discussion on the result of the Even Semester (Jan-May 2023) and scopes for improvements. Annexure VIII

Item IX: Discussion on PO attainment, individual course attainment and consequent discussion on the scopes for improvement. Annexure IX

Item X: Program Advisory Committee to be formed in the School of Law Department for course outcomes, Program outcomes preparations. Annexure X

B. Ratification:

1. Approval of Minutes of Meeting of 21st BoS meeting held on 25th January 2023 in the previous academic session.

C. Miscellaneous:

Any other matter with the permission/ suggestion of the Chair.

Following are the recommendations of BoS members:

Item I: Board discussed the SWAYAM/NPTEL courses for the Even and Odd sessions ie. Jan-Dec. 2023 to be included in CWS of all UG programs and recommended to be placed in FB for the approval.

Item II: Board discussed the revamped course structure for BALLB(H), BBALLB(H), B.Com. LLB(H), B.Sc. LLB(H), LLB and LLM (All branches) and syllabi of the First year to be introduced and implemented from AY 2023-24 and recommended to be placed in FB for the approval.

Item III: Approval was given by the Board to the syllabi of courses (with course codes) to be introduced in the Department of Business Administration and Department of Commerce ie. School of Business and Commerce and recommended to be placed in FB for the approval.

Item IV: Board approved the course codes and syllabi of first-degree courses provided by SBC for BBALLB(H) and BALLB(H) programs, SHSS for BALLB(H) programs, and FOS for B.Sc.LLB(H) program and recommended to be placed in FB for the approval.

Item V: Board discussed the Vision and Mission of the Department of Law in line with the Vision and Mission of the University and recommended to be placed in FB for the approval.

Item VI: Board discussed and approved the CWS for the Odd semester i.e. August to December 2023.

Item VII: Board discussed and approved the Values Added courses to be introduced in AY 2023-24.

Item VIII: The Board deeply discussed and analysed the result of the Even Semester (Jan-May 2023) and its scopes for improvements.

Item IX: Thorough discussion was taken place on PO attainment, individual course attainment and consequent discussion on the scopes for improvement.

Item X: Board discussed and gave its approval for the constitution of Program Advisory Committee to be formed in the School of Law Department for course outcomes, Program outcomes preparations.

B. Ratification:

1. Board approved the Minutes of Meeting of 21st BoS meeting held on 25th January 2023 in the previous academic session

These points are deliberated, and suggestions given by the members are incorporated.

for

Dr. Sony Kulshrestha
Head, Department of Law

Enclosures: -
1. Signature sheet of the Members
2. All relevant documents

MANIPAL UNIVERSITY
JAIPUR
(University under Section 2(f) of the UGC Act)

Manipal University Jaipur
Faculty of Law
Attendance Sheet
22nd BoS Meeting, Department of Law

Dated: May 17, 2023 (Wednesday),

Time: 1:30 PM Onwards

Venue: Room No. 208, 2nd Floor, 2AB.

The following Members are present: -

Sr. No.	Name	Signature
1	Dr. Sony Kulshrestha Head, Department of Law **Chairperson**	Sony Kulshrestha 17/5/2023
2	Prof. (Dr.) Jayaram E. R. Dean, Faculty of Law **Special Invitee**	
3	Prof. (Dr.) Vijaylaxmi Sharma Director, School of Law **Special Invitee**	Vijaylaxmi 17/05/23
4	Dr. Richa Arora Director, SHSS **Internal Member**	17/5/23
5	Prof. (Dr.) Nitu Bhatnagar, Registrar, MUJ **Ex-Officio Member**	
6	Prof. Mridul Srivastava Consultant Law Courses, Dr. Bhimrao Ambedkar Law University, Jaipur Former Dean, FoA&L, MUJ **External Member**	Consent taken through mail
7	Mr. Abhay Jain (ADJ) Senior Advocate, Rajasthan High Court **Industry Member**	Consent taken through mail
8	Dr. Susruta Samanta Directorate of Academics **Member**	17/5/23
9	Dr. Ashu Maharshi Associate Professor, Faculty of Law **Convener**	17/5/23

Biodiversity Protection , Farmers And Breeders Rights

By Prof. Padmavati Manchikanti, Prof. Narendran Thiruthy | IIT Kharagpur

ABOUT THE COURSE:
Protecting biological diversity has traditionally been associated with the lives and cultures of communities living in any specified region. More than half of the world's medicines are derived from plants and as such humans have used biodiversity for many purposes. There has been tremendous overexploitation of genetic resources by industries etc., leading to their reduction and associated effects on the environment and the human life. Following the formation of WTO as well as introduction of TRIPS, biodiversity representing 'knowledge' has received a new definition under intellectual property. Initial discussions at the Earth summit at Rio culminating in the Convention on Biodiversity has led to the realization of greater protection of genetic resources and the need for access as well as benefit sharing. Biodiversity Governance is directly connected to the sustenance of all major industry sectors, livelihood of rural population and the survival of mankind. The international, regional and domestic laws in this area have undergone significant transition in the last two decades. As a well-crafted topic equally balanced between its theoretical and practical realms, this course intends not only to create an awareness and knowledgebase about the regime of biodiversity protection, but also to enlighten the learner about the scope and ambit in pursuing one's career options in the same spectrum. This course aims at touching base on the essential areas of compliance with domestic and global Biodiversity Governance Regulations, regulatory and approval mechanisms operating in this area, and the procedure and practice nuances which can help in crafting a career in compliance practice.

INTENDED AUDIENCE: Undergraduate and postgraduate students of Biotechnology, Life science subjects & law, Legal practitioners at various courts of law, corporate legal professionals, Legal professionals working in various governmental/ regulatory services, IPR enthusiasts who want expand their horizons of knowledge.

PREREQUISITES: Preferably Graduate/Post Graduate Student of Biotechnology, Life science, Public Policy and Law. PhD Students also can take this who research on environmental and biodiversity matters

INDUSTRY SUPPORT: All Bio-based Industry Sectors – Agriculture / Biotechnology/ Pharma / Fertilizers & Pesticides / Cosmetics & Fragrances/ Foods& Beverages and AYUSH Industry.

Summary

Course Status :	Completed
Course Type :	Core
Duration :	8 weeks

Category :	○ Law
Credit Points :	2
Level :	Undergraduate/Postgraduate
Start Date :	24 Jul 2023
End Date :	15 Sep 2023
Enrollment Ends :	07 Aug 2023
Exam Registration Ends :	21 Aug 2023
Exam Date :	24 Sep 2023 IST

Note: This exam date is subjected to change based on seat availability. You can check final exam date on your hall ticket.

This is an AICTE approved FDP course

Course layout

Week 1: Concept and Scope of biodiversity protection

1. Concept and Scope of biodiversity protection
2. Types of biodiversity, mega-biodiverse centers,
3. Type of bio-resources, conservation mechanisms
4. International resources/centers of conservation
5. Traditional Resource rights, ecosystem measures

Week 2: Protection of Biological diversity: International mandate

1. Overview of International framework
2. Convention on Biodiversity - Objectives and Articles
3. International Regime on ABS
4. Biodiversity and Climate Change

5. Biobanks – Governance issues

Week 3: Protection of Biological Diversity -Indian position

1. The Biological Diversity Act, 2002

2. Regulatory authorities in India – NBA & SBB

3. Biodiversity Management Committees

4. People Biodiversity Registers

5. ABS Regulation and Benefit Sharing Procedures in India

Week 4: CBD, TRIPS and other treaties relevant to biodiversity protection – Interrelationship and Developments

1. Trade regime and Biodiversity

2. Comparison of Biodiversity Laws of countries

3. TRIPS-CBD relation

4. CBD and relation to other international treaties related to environment and organization of related bodies

5. Interrelationship and new Developments

Week 5: Biodiversity and Intellectual Property Rights

1. Biodiversity and Interface with IPR

2. Challenges related to Bio piracy - Case Studies

3. Patenting Biodiversity – Recent trends and Developments

4. Disclosure Requirements in Patent – Comparative Perspective

5. Regulatory Law – Comparative Perspective

Week 6: Plant Breeding and breeders' right v the farmers' right

1. Concept, Definitions and Criteria for Plant Variety Protection.

2. Protection of Plant Varieties and Farmers' Right 2001 - Major provisions of the Act

3. Plant Variety protection in US, EU, Japan, China etc.,

4. International Union for protection of new plant varieties (UPOV)

5. Farmers' Rights – other country models

Week 7: Biodiversity Governance and Compliance Procedures – Comparative Perspective

1. Principles of Biodiversity Governance

2. Compliance Procedures and Linkage with IPR

3. Compliance Procedures under International Framework

4. Compliance Procedures in India

5. Compliance Procedures in EU

Week 8: Biodiversity and Human Wellbeing

1. Biodiversity and Interrelationship with Life

2. Sustainable Development Agenda

3. Biodiversity, ecosystem functioning, ecosystem services

4. Biodiversity and Human Happiness

5. Nature Protects if She is Protected

Books and references

1.Genetic resources, traditional knowledge and the law. Eds. Evanson.C. Kamau, Gerd Winter
2.The governance of genetic information. Who decides? Eds. Heather Widdows and Caroline Mullen
3.Plant Variety and Farmer Rights Act 2001, India
4.Intellectual Property Rights in Agricultural biotechnology. 2nd Edition. F. H. Erbisch and K. M. Maredia. CABI Publishing.
(This is only an indicative list. Instructors will share specific course material)

Instructor bio

Prof. Padmavati Manchikanti

IIT Kharagpur

Prof. Padmavati Manchikanti With more than sixteen years of teaching and research experience, Prof. M. Padmavati teaches the subjects of Patent Law, Patent Procedure and Drafting, Biodiversity Law and TK protectionto undergraduate and master students of law at IIT Kharagpur. Her primary area of research includes Intellectual Property and Commercialization of recombinant and herbal drugs and Drug Regulation, Biodiversity Law, studies on implementation of IP. Prior to joining IIT Kharagpur, she was a Senior Scientist at Monsanto Research Center, Bangalore where she coordinated invention disclosure activities. She has many research as well as consultancy projects, from Ministry of Human Resource Development, DST etc,. She has been awarded the Microsoft–Young Faculty Scholarship in Intellectual Property. She is an Advisor to the IPR Cell, IIT Kharagpur. She is the Course Coordinator of the KIRAN-IPR Program at IIT Kharagpur. She has guided research of masters as well as doctoral students in the area of biodiversity implementation in India and works on the protection of TK in India. She has been an Observer to the 2018 COP meeting of the Convention on Biological Diversity and is a regular contributor for the CBD report for the Year Book of International Environmental Law. She is also an Editorial Board member of Journal of Intellectual Property Rights (JIPR) and Journal of Integrative Medicine. She has been an invited speaker at various international as well as national conferences.

Prof. Narendran Thiruthy

Prof. Narendran Thiruthy is Assistant Professor in Rajiv Gandhi School of Intellectual Property Law, Indian Institute of Technology, Kharagpur. Before joining the faculty of IIT Kharagpur, he served as the head for IPR Section in National Biodiversity Authority, Government of India. He has both theoretical expertise and administrative experience in the field of IPR and Biodiversity governance. Dr.Narendran coordinated the 'Module on Biodiversity Governance' for IFS Probationers in Indira Gandhi National Forest Academy, Dehradun for the years 2018, 2019, 2020 & 2021. He was the officer in-charge of coordinating the India Biodiversity Awards for IBA 2018 & IBA 2021 cycles and the NBA-UNDP Biodiversity Samrakshan Internship Program in 2019-20 & 2020-21. He has delivered many lectures on Intellectual Property Rights and Biodiversity Law in various forums including in United Nations CBD events. He has also represented the government in many bilateral and multilateral meetings. Narendran's research explores the intersection of law, technology and environment. His research areas include IP Philosophy, Protection of Cultural Property, IP procedure, IP Business models, New Technology Developments and IP, Theories of Creativity, Biodiversity Governance etc. He is currently exploring the challenges of DSI for biodiversity governance and Business models for open collaborative research

Course certificate

The course is free to enroll and learn from. But if you want a certificate, you have to register and write the proctored exam conducted by us in person at any of the designated exam centres.

The exam is optional for a fee of Rs 1000/- (Rupees one thousand only).

Date and Time of Exams: **24 September 2023** Morning session 9am to 12 noon; Afternoon Session 2pm to 5pm.

Registration url: Announcements will be made when the registration form is open for registrations.

The online registration form has to be filled and the certification exam fee needs to be paid. More details will be made available when the exam registration form is published. If there are any changes, it will be mentioned then.

Please check the form for more details on the cities where the exams will be held, the conditions you agree to when you fill the form etc.

CRITERIA TO GET A CERTIFICATE

Average assignment score = 25% of average of best 6 assignments out of the total 8 assignments given in the course.
Exam score = 75% of the proctored certification exam score out of 100

Final score = Average assignment score + Exam score

YOU WILL BE ELIGIBLE FOR A CERTIFICATE ONLY IF AVERAGE ASSIGNMENT SCORE >=10/25 AND EXAM SCORE >= 30/75. If one of the 2 criteria is not met, you will not get the certificate even if the Final score >= 40/100.

Certificate will have your name, photograph and the score in the final exam with the breakup.It will have the logos of NPTEL and IIT Kharagpur .It will be e-verifiable at nptel.ac.in/noc.

Only the e-certificate will be made available. Hard copies will not be dispatched.

Once again, thanks for your interest in our online courses and certification. Happy learning.

NPTEL Online Certification

(Funded by the MoE, Govt. of India)

This certificate is awarded to

ADITYA VIDYARTHI

for successfully completing the course

Biodiversity Protection, Farmers and Breeders Rights

with a consolidated score of **57** %

Online Assignments	19.17/25	Proctored Exam	38.27/75

Total number of candidates certified in this course: **286**

Jul-Sep 2023

(8 week course)

Prof. Haimanti Banerji
Coordinator, NPTEL
IIT Kharagpur

Indian Institute of Technology Kharagpur

swayam

Roll No: NPTEL23LW0654290071 To verify the certificate No. of credits recommended: 2 or 3

Elite

NPTEL Online Certification

(Funded by the MoE, Govt. of India)

This certificate is awarded to

DAMINI CHAUHAN

for successfully completing the course

Biodiversity Protection, Farmers and Breeders Rights

with a consolidated score of **60** %

Online Assignments	17.25/25	Proctored Exam	42.33/75

Total number of candidates certified in this course: **286**

Jul-Sep 2023

(8 week course)

Prof. Haimanti Banerji
Coordinator, NPTEL
IIT Kharagpur

Indian Institute of Technology Kharagpur

FREE ONLINE EDUCATION swayam शिक्षित भारत, उन्नत भारत

Roll No: NPTEL23LW06S45262839

To verify the certificate

No. of credits recommended: 2 or 3

Elite

NPTEL Online Certification

(Funded by the MoE, Govt. of India)

This certificate is awarded to

KHUSHBOO RATHORE

for successfully completing the course

Biodiversity Protection, Farmers and Breeders Rights

with a consolidated score of **69** %

Online Assignments	20.25/25	Proctored Exam	48.98/75

Total number of candidates certified in this course: **286**

Jul-Sep 2023

(8 week course)

Prof. Haimanti Banerji
Coordinator, NPTEL
IIT Kharagpur

Indian Institute of Technology Kharagpur

FREE ONLINE EDUCATION swayam शिक्षित भारत, उन्नत भारत

Roll No: NPTEL23LW06S45260404

To verify the certificate

No. of credits recommended: 2 or 3

Elite

NPTEL Online Certification

(Funded by the MoE, Govt. of India)

This certificate is awarded to

RASHI GOYAL

for successfully completing the course

Biodiversity Protection, Farmers and Breeders Rights

with a consolidated score of **71** %

Online Assignments	17.46/25	Proctored Exam	53.57/75

Total number of candidates certified in this course: **286**

Jul-Sep 2023

(8 week course)

Prof. Haimanti Banerji
Coordinator, NPTEL
IIT Kharagpur

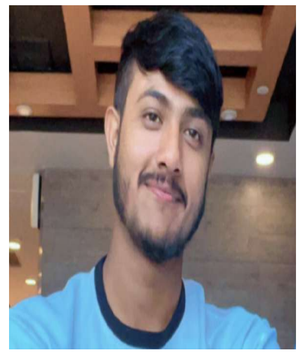
Indian Institute of Technology Kharagpur

FREE ONLINE EDUCATION swayam शिक्षित भारत, उन्नत भारत

Roll No: NPTEL23LW06S45260179

To verify the certificate

No. of credits recommended: 2 or 3



NPTEL Online Certification

(Funded by the MoE, Govt. of India)

This certificate is awarded to

RAVI DUBEY

for successfully completing the course

Biodiversity Protection, Farmers and Breeders Rights

with a consolidated score of **44** %

Online Assignments	13.38/25	Proctored Exam	30.62/75

Total number of candidates certified in this course: **286**

Jul-Sep 2023

(8 week course)

Prof. Haimanti Banerji
Coordinator, NPTEL
IIT Kharagpur

Indian Institute of Technology Kharagpur

FREE ONLINE EDUCATION swayam शिक्षित भारत, उन्नत भारत

Roll No: NPTEL23LW06S35260806

To verify the certificate

No. of credits recommended: 2 or 3

Elite

NPTEL Online Certification

(Funded by the MoE, Govt. of India)

This certificate is awarded to

ROHINI

for successfully completing the course

Biodiversity Protection, Farmers and Breeders Rights

with a consolidated score of **66** %

Online Assignments	17.25/25	Proctored Exam	48.98/75

Total number of candidates certified in this course: **286**

Jul-Sep 2023

(8 week course)

Prof. Haimanti Banerji
Coordinator, NPTEL
IIT Kharagpur

Indian Institute of Technology Kharagpur

FREE ONLINE EDUCATION swayam शिक्षित भारत, उन्नत भारत

Roll No: NPTEL23LW06S35290128

To verify the certificate

No. of credits recommended: 2 or 3

Elite

NPTEL Online Certification

(Funded by the MoE, Govt. of India)

This certificate is awarded to

SHASHANK FAUZDAR

for successfully completing the course

Biodiversity Protection, Farmers and Breeders Rights

with a consolidated score of **60** %

Online Assignments	17.54/25	Proctored Exam	42.33/75

Total number of candidates certified in this course: **286**

Jul-Sep 2023

(8 week course)

Prof. Haimanti Banerji
Coordinator, NPTEL
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with a consolidated score of **52** %

Online Assignments	17.83/25	Proctored Exam	33.68/75

Total number of candidates certified in this course: **286**

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(8 week course)

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Faculty of Art & Low (FOA&L)
External Marks Award List (LA3105)

ACADEMIC YEAR : 23-24 **ACADEMIC SESSION** : JUL-NOV 2023

PROGRAM CODE : INT-001 **BRANCH/SPECIALIZATION** : BA LLB (HONS)

COURSE CODE : LA3105 **COURSE NAME** : INTERNATIONAL LAW

SECTION : A **SEMESTER** : V

FACULTY NAME : AYUSHI RAGHUWANSHI (MUJ1493)

S.No	Reg. No	Student Name	Internal Marks (Max - 60)	External Marks (Max - 40)	Total Marks (Max - 100)	Rev Grade
1	211301003	APRAJITA DALAL	41	21	62	C
2	211301005	HITESHI VYAS	56	37	93	A+
3	211301008	MAHENSHI MAHENDRASINGH CHAUHAN	56	28	84	B
4	211301009	GEETIKA TYAGI TYAGI	0	0	0	F
5	211301011	AMEYA BAKSHI	21	14	35	E
6	211301012	PRACHI SINHA	46	19	65	C
7	211301014	NEHAL AGARWAL	47	22	69	C
8	211301015	PARAS SINGH RAO	48	31	79	B
9	211301016	GRACY SINGH GEHLOT	53	34	87	B
10	211301017	SYED MOHAMMAD ASIM ABBAS	41	15	56	D
11	211301018	RIYA SINGH	59	36	95	A+
12	211301019	PRIYAL MALVIYA	45	33	78	B
13	211301020	NISHCHAY	22	15	37	E
14	211301021	KUSHAL SHARMA	15	0	15	F
15	211301022	KAMAYANI SHARMA	49	34	83	B
16	211301023	ALINA ALI	52	35	87	B
17	211301024	PRAYANSH GARHWAL	45	28	73	C
18	211301025	DHRUV PRATAP SINGH	11	2	13	F
19	211301026	UJJWAL SHARMA	56	0	56	F
20	211301027	PRANJAL LEGHA	52	33	85	B
21	211301028	SHUBHAM JHORAR	32	0	32	F
22	211301029	ROYAL RAJPUROHIT	50	25	75	C
23	211301030	HIMANSHI SHARMA	53	31	84	B
24	211301031	SATYENDRA SINGH SHEKHAWAT	33	15	48	D
25	211301032	PEARL SINGH	53	30	83	B
26	211301033	KHYATI RAGHAV	43	22	65	C

27	211301034	RAJNANDANI KHANGAROT	49	30	79	B
28	211301035	YASH CHATURVEDI	55	33	88	A
29	211301036	EISHA SURANA	57	36	93	A+
30	211301037	ANUREET KAUR	59	39	98	A+
31	211301038	NAVYA SHARMA	56	31	87	B
32	211301039	DEEPESH SHARMA	40	23	63	C
33	211301040	SHIVANSHI SHARMA	49	25	74	C
34	211301041	ANSHIKA GARG	57	36	93	A+
35	211301042	MANOJ KUMAWAT	50	19	69	C
36	211301043	DEWANG ARHA	44	16	60	C
37	211301044	HARSHITA	0	0	0	F
38	211301045	MUKUND MAHESHWARI	52	35	87	B
39	211301046	DEEPANSHU SINGH	45	26	71	C
40	211301047	SHASHWATI SOMYA	44	14	58	C
41	211301048	LAKSHIT KASWAN	22	15	37	E
42	211301050	MEGHA TANWAR	49	24	73	C
43	211301051	UNEZA KHAN	52	31	83	B
44	211301052	RISHIKA SWAMI	45	22	67	C
45	211301053	SHIVANSH SRIVASTAVA	34	15	49	D
46	211301055	AYUSHI MAHESHWARI	58	38	96	A+
47	211301056	YUVRAJ SINGH	45	18	63	C
48	211301057	NIKHIL BANA	41	11	52	F
49	211301058	VINEET MAHARSHI	50	20	70	C
50	211301060	AKSHITA PRADHAN	54	35	89	A
51	211301061	GAYATRI VIJAYKUMAR JOSHI	53	35	88	A
52	211301124	GAURVI PALIWAL	43	21	64	C
53	211301126	VARSHITA PALSANIA	53	36	89	A
54	211301127	CHAHAT AGGARWAL	54	34	88	A
55	211301128	KHUSHBOO MAHLA	42	23	65	C
56	211301129	MADHVI JANGIR	48	24	72	C

No of Student present : 51

No of Student absent : 4

FACULTY SIGNATURE

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Sony Kulshrestha (MUJ0351)

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Faculty of Art & Low (FOA&L)
External Marks Award List (LA3105)

ACADEMIC YEAR : 23-24 **ACADEMIC SESSION** : JUL-NOV 2023

PROGRAM CODE : INT-001 **BRANCH/SPECIALIZATION** : BA LLB (HONS)

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SECTION : B **SEMESTER** : V

FACULTY NAME : AYUSHI RAGHUWANSHI (MUJ1493)

S.No	Reg. No	Student Name	Internal Marks (Max - 60)	External Marks (Max - 40)	Total Marks (Max - 100)	Rev Grade
1	211301063	AJITA RATHOD	48	33	81	B
2	211301064	TEJASWARDHAN NARUKA	9	4	13	F
3	211301065	SHUBHAM CHOUDHARY	48	25	73	C
4	211301066	RIYA SINGH	18	16	34	F
5	211301067	PAAVNI CHADHA	52	36	88	A
6	211301069	SANYA MATHUR	53	37	90	A
7	211301070	SANJAY KUMAR	40	21	61	C
8	211301071	HARSHIT SAXENA	48	28	76	B
9	211301072	DHIRGHAYU SHARMA	54	37	91	A+
10	211301073	SHREYA SHARMA	46	16	62	C
11	211301074	VISHAKHA KANWAR	51	25	76	B
12	211301075	UDDHAVRAJ SINGH SHAKTAWAT	44	0	44	F
13	211301076	ABHISHEK SINGH NARUKA	48	7	55	F
14	211301077	KAUSHIK BAROLIYA	55	34	89	A
15	211301078	ANANT RAJ SINGH	53	34	87	B
16	211301080	DIVISHA YADAV	56	36	92	A+
17	211301084	BHARAT SOGARWAL	39	9	48	F
18	211301085	ANANYA DWIVEDI	55	37	92	A+
19	211301086	SHREE BANSAL	51	31	82	B
20	211301088	TANVI LAKHLAN	55	37	92	A+
21	211301089	MANAV DHABHAI	13	15	28	F
22	211301091	ASAD ALI KHAN	22	18	40	D
23	211301092	ABHISHEK SINGH SHEKHAWAT	37	14	51	D
24	211301093	ARYAN KUMAWAT	42	20	62	C
25	211301094	ANMOL BALI	55	36	91	A+
26	211301095	SRISHTI PARNAMI	53	35	88	A

27	211301096	ARCHI SARWGI	33	23	56	D
28	211301097	AYUSH RAO	30	18	48	D
29	211301098	ASHAY APURV	30	10	40	F
30	211301099	MEHWISH KHAN	49	0	49	F
31	211301100	AMAN PRAKASH	44	25	69	C
32	211301101	VIVEK RAJ SINGH	40	24	64	C
33	211301102	PUNJIKA SHEKHAWAT	47	22	69	C
34	211301103	SUNIL MEHRIYA	32	8	40	F
35	211301104	PRANAV MATHUR	53	36	89	A
36	211301105	TITHI GUPTA	53	36	89	A
37	211301106	PRATIBHA KARNOT	42	29	71	C
38	211301107	DEEPANKAR SINGH	56	32	88	A
39	211301109	MAHAK MAHAJAN	47	29	76	B
40	211301110	MANAN SHARMA	15	14	29	F
41	211301111	ABHAY SINGH SHEKHAWAT	19	16	35	E
42	211301112	LAKSHYA PAL MANDA	46	29	75	C
43	211301113	YUVRAJ SINGH RAJPUROHIT	29	17	46	D
44	211301114	MANASVINI TIWARI	48	0	48	F
45	211301115	SHRUTI MANTRI	36	15	51	D
46	211301116	AKSHITA CHOUDHARY	44	6	50	F
47	211301117	KHALID SHEIKH	12	8	20	F
48	211301118	SHIVANGI NANDWANA	56	33	89	A
49	211301119	DEEPAK CHOUDHARY	47	34	81	B
50	211301120	ASHUTOSH SINGH	55	37	92	A+
51	211301121	TANISHKA SINGH	40	17	57	D
52	211301122	RAKESH KUMAR	12	0	12	F
53	211301123	VINEET JAKHAR	15	14	29	F
54	211301125	TANMAY GOKULKA	23	0	23	F

No of Student present : 49

No of Student absent : 4

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Bioprospecting of novel ligninolytic bacteria for effective bioremediation of agricultural by-product and synthetic pollutant dyes

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ABSTRACT

Lignin is a significant renewable carbon source that needs to be exploited to manufacture bio-ethanol and chemical feedstocks. Lignin mimicking methylene blue (MB) dye is widely used in industries and causes water pollution. Using kraft lignin, methylene blue, and guaiacol as a full carbon source, 27 lignin-degrading bacteria (LDB) were isolated from 12 distinct traditional organic manures for the current investigation. The ligninolytic potential of 27 lignin-degrading bacteria was assessed by qualitative and quantitative assay. In a qualitative plate assay, the LDB-25 strain produced the largest zone, measuring 6.32 ± 0.297, on MSM-L-kraft lignin plates, while the LDB-23 strain produced the largest zone, measuring 3.44 ± 0.413, on MSM-L-Guaiacol plates. The LDB-9 strain in MSM-L-kraft lignin broth was able to decolorize lignin to a maximum of 38.327 ± 0.011% in a quantitative lignin degradation assay, which was later verified by FTIR assay. In contrast, LDB-20 produced the highest decolorization (49.633 ± 0.017%) in the MSM-L-Methylene blue broth. The highest manganese peroxidase enzyme activity, measuring 6322.314 ± 0.034 U L^{-1}, was found in the LDB-25 strain, while the highest laccase enzyme activity, measuring 1.5105 ± 0.017 U L^{-1}, was found in the LDB-23 strain. A preliminary examination into the biodegradation of rice straw using effective LDB was carried out, and efficient lignin-degrading bacteria were identified using 16SrDNA sequencing. SEM investigations also supported lignin degradation. LDB-8 strain had the highest percentage of lignin degradation (52.86%), followed by LDB-25, LDB-20, and LDB-9. These lignin-degrading bacteria have the ability to significantly reduce lignin and lignin-analog environmental contaminants, therefore they can be further researched for effective bio-waste management mediated breakdown.

1. Introduction

Plant biomass naturally decomposes over time and is mostly triggered by enzymatic activity of neighboring bacterial and fungal species (Janusz et al., 2017; da Costa et al., 2018; Jimenez et al., 2018; Lee et al., 2019; Riyadi et al., 2020). Numerous microorganisms, such as bacteria and fungi, have been the focus of the most thorough investigations on lignin alteration and breakdown (Lee et al., 2019; Atiwesh et al., 2022). When lignocellulosic organic wastes are processed for use in the production of bioethanol and the paper industry, respectively, powerful lignin-degrading microorganisms or their ligninolytic enzymes can be used successfully (Fang et al., 2018; Brink et al., 2019; Li et al., 2022). Plants' rigidity and tensile strength come from lignin, a complex, chemically heterogeneous polymer made up of 4-hydroxyl phenylpropanoid units (Hasanin et al., 2018). Biomass is essentially resistant because lignin acts as a physical barrier to stop cellulose from being hydrolyzed by biological or chemical processes (Wu et al., 2022).

For the production of biofuels, lignocellulosic biomass is highly

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desirable as a raw component (Malode et al., 2021). Lignin and cellulose make up the majority of 80% of crop residue/biomass (Chen et al., 2018). Large volumes of lignocellulosic waste are produced by forestry and agricultural activities, paper-pulp companies, wood industries etc. and burning is a common method of decreasing this waste, which otherwise may contribute to pollution (Haile et al., 2021). Therefore, enhancing lignin breakdown has enormous promise to save the environment and build new beneficial products (Gupta et al., 2022). Chemical contaminants like synthetic dyes are significant contributors to water pollution. Methylene blue (MB) dye is one of these and is frequently used in various industries such as dyeing, textile, tannery, and paper, etc. (Bhat et al., 2022; Pham et al., 2022). Using promising microbial isolates, a biological strategy giving a more affordable and sustainable alternative method has been used to remove MB. Microorganisms, which can degrade MB, come from a wide range of taxa, including *Acinetobacter, Aspergillus, Bacillus, Pseudomonas, Staphylococcus* etc. (Eslami et al., 2017; Karim et al., 2018; Bharti et al., 2019; Ogunlaja et al., 2020; Kishor et al., 2021b; Haque et al., 2021). To participate in central carbon metabolism, bacteria that degrade lignin and lignin-imitating dyes secrete extracellular enzymes that break down lignin into smaller components (Beckham et al., 2016). A range of extracellular oxidative enzymes, such as laccases, lignin peroxidases (LiPs), manganese peroxidases (MnPs), and multifunctional peroxidases, are released by the lignin-degrading organisms (Kumar and Chandra, 2020).

Several bacteria, in addition to wood-rot fungus, can degrade lignin (Haq et al., 2022). Pretreatments may become economically viable if lignin is removed selectively using lignin-degrading enzymes (Lee et al., 2019; Wu et al., 2022). A biological (or primarily biological) approach to removing lignin could overcome these limitations, enabling the generation of bio-fuel at a cheaper cost and with less impact on the environment. Microorganisms can produce metabolites and enzymes that hasten the breakdown of organic waste and raise the caliber of soil humus (Singh et al., 2017).

The breakdown of rice straw into smaller products so they can be digested by microorganisms occurs through a microbial process (Goodman, 2020). In the past, the majority of these lignin-degrading bacteria were found in soil and the intestines of insects that fed wood (Zhang et al., 2021). This research was focused on identifying and characterizing the microbial inoculants that causes rice straw to break down so quickly. In this study, we identify new lignin-degrading bacteria from a variety of organic sources, characterize them, and assess their potential for lignin degradation.

2. Material and methods

2.1. Isolation and screening of lignin-degrading bacteria

The isolation of LDB was carried out using the method reported by Rahman et al. (2013). The minimal salt media-Luria agar (MSM-L agar) medium supplemented with 1% Kraft lignin (KL) as the primary carbon and energy source was used for the isolation of ligninolytic bacteria from freshly prepared organic manures (Jain et al., 2021) (Table 1). The bacterial strains that showed proper growth were purified on fresh MSM-L agar plates and stored at 4 °C. Screening of LDB was carried out on MSM-L agar plate containing Kraft lignin (0.5%) (Chen et al., 2012b) and guaiacol (10%) (Atalla et al., 2010) and methylene blue indicator dye (50 mg/L) (Bandounas et al., 2011) and incubated at 30 ± 2^0 C for 4–5 days for the development of the de-colorization zone. After growth, the plates were taken out and flooded with 10 ml of a ferric chloride/potassium ferricyanide solution [1% (w/v)]. After 10 min, the solution was drained off, leaving the agar where the aromatic compounds were present stained blue or green. It was assumed that a yellowish zone where growth had been eradicated was proof of lignin breakdown (McCarthy and Broda, 1984). The degrading of indicator dye is considered a positive test for lignin degradation (Bandounas et al., 2011).

Table 1
Determination of lignolytic activities of lignin degrading bacteria isolated from traditional organic manures.

S. N.	Source	Strain Name	Growth and de-colorization of ligninolytic substrates		
			Kraft Lignin	Guaiacol	Methylene blue
1	JeevaAmrat	SBD-LDB-1	+	+	-
2	BhabhutAmritPani	SBD-LDB-2	+	+	+
3	PanchGavya	SBD-LDB-3	+	+	+
4	MatkaKhad	SBD-LDB-4	+	+	+
5	Vermiwash (Silica enriched)	SBD-LDB-5	+	+	-
6	BeejaAmrat	SBD-LDB-6	+	-	+
7	Vermi Wash	SBD-LDB-7	+	+	+
8	Silica enriched Compost tea	SBD-LDB-8	+	+	+
9	Compost Tea	SBD-LDB-9	+	+	+
10	BD500	SBD-LDB-10	+	+	-
11	BD500	SBD-LDB-11	+	+	+
12	JeevaAmrat	SBD-LDB-12	+	+	-
13	BhabhutAmritPani	SBD-LDB-14	+	+	+
14	PanchGavya	SBD-LDB-15	+	+	-
15	MatkaKhad	SBD-LDB-16	+	+	-
16	Silica enriched vermi wash	SBD-LDB-17	+	+	-
17	BeejaAmrat	SBD-LDB-18	+	-	-
18	Vermi Wash	SBD-LDB-19	+	+	+
19	Compost tea (Silica enriched)	SBD-LDB-20	+	+	+
20	Compost Tea	SBD-LDB-21	+	-	-
21	BD 501	SBD-LDB-22	+	+	+
22	BD 501	SBD-LDB-23	+	+	+
23	PanchGavya	SBD-LDB-24	+	+	+
24	MatkaKhad	SBD-LDB-25	+	+	+
25	Silica enriched vermi wash	SBD-LDB-26	+	+	+
26	BeejaAmrat	SBD-LDB-28	+	+	-
27	Vermi Wash	SBD-LDB-29	+	+	+

3. Ligninolytic enzyme activity assay

A 50 ml conical flask filled with MSM-L medium was used to inoculate the chosen positive isolates for screening assays. These inoculated flasks were incubated for 6 days per the procedures outlined by Rahman et al. (2013), during which time culture samples were collected for manganese peroxidase (MnP) and laccase activity were assessed using the oxidation of Guaiacol (2-methoxyphenol) and ABTS (2, 2′-azinobis-(3-ethylbenzethiazoline-6-sulphonate) method respectively (Rahman et al., 2013; Bourbonnais et al., 1995).

3.1. Decolorization activity of bacteria on liquid MSM containing MB dye and Kraft lignin

Decolorization of lignin-mimicking dyes i.e. Methylene Blue (MB) was assessed in test tubes as liquid-phase assays. For broth assays, the individual bacterial strains were grown in MSM containing methylene blue indicator dye (50 mg/L) at 30 °C with shaking at 200 rpm. Control without inoculation was also maintained. The samples were centrifuged and dye de-colorization was absorbance was measured at λ_{663} (Saratale et al., 2009).

All the test bacterial cultures were inoculated in 10 ml of MSM-L agar medium containing 0.5% lignin in 25 ml capacity screwed cap tubes with the maintenance of un-inoculated control for comparison. All the tubes were incubated at 30 ± 2^0 C at 120 rpm for 5 days in a shaker incubator and color change was measured by spectrophotometer on the 5th day at 465 nm. The decolorization percentage of 0.5% lignin by respective bacteria was calculated using the formula given by (Sani and Banerjee, 1999).

$$\% \text{ decolorization} = \frac{\text{Initial absorbance} - \text{observed absorbance}}{\text{Initial absorbance}} \times 100$$

Additionally, Fourier transform infrared (FTIR) spectroscopy study was done to verify that the isolates had degraded and depolymerized kraft lignin (Khan et al., 2022).

3.2. In vitro efficacy of ligninolytic bacteria for degradation of agro-waste residues

After being treated with 1% NaOH for 24 h at room temperature, deionized water was used to adjust the pH to 7, and then the rice straw was dried at 80 °C (Yu et al., 2009). The 3 g quantity of pretreated rice straw was immersed in 100 ml flasks in triplicate and steam sterilized and then 5 ml of selected lignin-degrading bacteria were inoculated in each flask incubated for 21 days at ambient temperature (room temperature) and assayed for lignin degradation and the observations of weight reduction and consistency were also monitored (Bakar et al., 2018). After the end of the incubation period, the acid detergent fiber (ADF) and acid detergent lignin (ADL) methods (Thimmaiah, 2009) were used to determine the amount of lignin in rice straw.

4. Effects of bio-pretreatment on the structure of the rice straw

Using a freeze drier, untreated and bio-pretreated rice straw was dehydrated. Using a scanning electron microscope (SEM), the various surface morphologies of the untreated and bio-pretreated rice stover were observed (Dong et al., 2019).

5. Sequencing and Phylogenetic Analysis of 16 S rDNA of Potent LDB Isolates

The five most effective LDB isolates' PCR-amplified 16 S rDNA region was sequenced using an automated DNA sequencer (ABI model 377, Applied Biosystems, USA) in accordance with the normal technique utilizing universal 16 S rDNA primers (27 F and 1492 R; amplicons size ≈ 1465 bp). Prior to BLAST, the 16 S rDNA sequences were modified using the Bio Edit software. Utilizing the nucleotide BLASTn programme, the sequences collected during the investigation were compared to previously submitted sequences in the nucleotide database GenBank at the National Center for Biotechnology (NCBI) (Altschul et al., 1990). The online programme CLUSTAL-W was used to align the 16 S rDNA consensus sequences (Thompson et al., 1994). Phylogenetic trees were constructed using this alignment and the greatest likelihood technique using MEGA 6.06 software (Tamura et al., 2013).

6. Statistical analyses

Using SPSS-20, the statistical analysis known as the standard deviation (SD) was performed on all the observed data. Additionally, correlation functional interaction between all chosen bacterial isolates' production of the enzymes that break down Kraft lignin and Methylene Blue was performed using Past3 software, and correlation heat maps were created using TB tools.

7. Result and discussion

7.1. Isolation and screening of lignin degrading bacteria

The isolation and screening of lignin-degrading bacteria were carried out on the MSM-L agar medium supplemented with Kraft lignin (0.5%), Guaiacol (10%), and Methylene blue (50 mg/L). In the present study, 27 lignin-degrading bacteria were isolated and purified from 12 different organic produces and are summarized in Table 1. All the strains were able to grow on MSM-L containing Kraft lignin (0.5%), whereas 24 strains and 18 strains were able to grow and utilize Guaiacol (10%) and Methylene blue (50 mg/L) respectively. The 16 strains along with the positive culture showed positive growth and de-colorization on all three different ligninolytic substrates. The decolorization zone was measured to determine the lignin degradation index (LDI), and the findings are shown in Table 2. This is the first report that we are aware of lignin-degrading microbial strains being isolated from conventional liquid organic manures.

The various lignin-degrading bacteria were identified in rotting oil palm, empty fruit bunches, rotten wood, textile effluent, and sludge etc. (Faisal et al., 2021; Kishor et al., 2021a). While Harith et al. (2014) isolated 8 strains with lignin degradation ability from agro-industrial waste, Sharifi-Yazdi et al. (2001) isolated 22 LDB strains from decayed plants, Falade et al. (2017) isolated 30 potential strains of ligninolytic bacteria from water and sediment samples, and Couger et al. (2020) isolated lignin-degrading bacteria from the termite gut. Nahrowi et al. (2018) previously reported a correlation between ligninolytic activities of bacterial isolates and the lignin degradation index (LDI), and they

Table 2
Determination of lignolytic activities of lignin degrading bacteria using plate assay by measuring decolourisation zone.

S.NO	Strain Name	Lignin degradation index by measuring decolourisation zone after 72 h	
		Kraft Lignin (0.5%)	Guaiacol (10%)
1	SBD-LDB-1	3.24 ± 0.035	2.86 ± 0.125
2	SBD-LDB-2	2.1 ± 0.10	2.21 ± 0.257
3	SBD-LDB-3	3.6 ± 0.200	2.28 ± 0.145
4	SBD-LDB-4	3.46 ± 0.351	1.31 ± 0.162
5	SBD-LDB-5	1.01 ± 0.11	1.83 ± 0.175
6	SBD-LDB-6	1.04 ± 0.12	ND
7	SBD-LDB-7	3.38 ± 0.325	1.63 ± 0.126
8	SBD-LDB-8	5.23 ± 0.321	3.01 ± 0.225
9	SBD-LDB-9	5.47 ± 0.240	3.21 ± 0.256
10	SBD-LDB-10	3.48 ± 0.141	1.65 ± 0.200
11	SBD-LDB-11	4.53 ± 0.251	3.02 ± 0.087
12	SBD-LDB-12	5.15 ± 0.160	2.21 ± 0.256
13	SBD-LDB-14	2.35 ± 0.100	2.01 ± 0.225
14	SBD-LDB-15	0.94 ± 0.18	1.78 ± 0.257
15	SBD-LDB-16	4.32 ± 0.192	2.26 ± 0.205
16	SBD-LDB-17	5.57 ± 0.222	2.41 ± 0.272
17	SBD-LDB-18	0.98 ± 0.08	ND
18	SBD-LDB-19	1.24 ± 0.250	1.52 ± 0.087
19	SBD-LDB-20	5.17 ± 0.210	3.30 ± 0.174
20	SBD-LDB-21	0.84 ± 0.14	ND
21	SBD-LDB-22	3.34 ± 0.262	2.36 ± 0.127
22	SBD-LDB-23	6.14 ± 0161	3.44 ± 0.413
23	SBD-LDB-24	6.31 ± 0.298	2.88 ± 0.247
24	SBD-LDB-25	6.32 ± 0.297	3.29 ± 0.187
25	SBD-LDB-26	3.61 ± 0.135	2.09 ± 0.085
26	SBD-LDB-28	1.04 ± 0.13	1.93 ± 0.081
27	SBD-LDB-29	3.42 ± 0.186	2.84 ± 0.177

*ND: Not Detected; Data (Mean of triplicate value ± SD)

demonstrated that lignin-degrading bacterial isolates had LDI values ranging from 2.6 to 1.22, validating the results of the Lignin degradation index by lignin-degrading bacterial strains. Falade et al. (2017) assessed the lignin-degrading activity of bacterial strains using guaiacol decolorization and found that only 5 strains out of 30 displayed decolorization zone, which validates our current findings.

8. Ligninolytic enzyme activity assay

The quantification of ligninolytic enzymes viz. Manganese peroxidase enzyme and Laccase enzyme in the lignin-degrading bacteria were conducted further to understand the mechanism of their ligninolytic activities. The results of the Manganese peroxidase enzyme and Laccase enzyme were summarized in Fig. 1.

Fig. 1 shows the correlation between the percentage of KL and MB elimination and the bacterial enzymes (MnP and laccase). The lines in the graph below indicate the ligninolytic bacteria, while the columns stand in for the enzymes. The level of enzymatic activity was indicated by the colour of the tiles. The bacterial strains SBD-LDB-9, SBD-LDB-8, SBD-LDB-20, SBD-LDB-23, and SBD-LDB-25 that significantly reduce KL and MB were determined to have the highest enzymatic activity, as depicted in Fig. 1.

The highest Manganese peroxidase enzyme activity of 6322.314 $\pm$ 0.034 U L^{-1} was observed in the strain SBD-LDB-25 whereas the minimum activity of 2630.854 $\pm$ 0.031 U L^{-1} was observed in SBD-LDB-3 strain. In the case of Laccase enzyme, the maximum degradation activity was observed in SBD-LDB-23 strain of 1.510 $\pm$ 0.017 U ml^{-1} whereas minimum activity was reported in SBD-LDB-18 strain of 0.825 $\pm$ 0.026 U ml^{-1}. Shamseldin and Abdelkhalek (2015) studied Manganese peroxidase enzyme activities and reported that MnP activity was found as 1720 U L^{-1} and 1750 U L^{-1} after the third day whereas the Laccase enzymes were found as 810 U L^{-1} and 915 U L^{-1}after six days in two examined LDB strains. The enzyme activities reported by Shamseldin, Abdelkhalek (2015) were lower compared to the results obtained in the present studies. The results observed by Chen et al. (2012a) and Yang et al. (2017) were in close agreement with the result obtained in the present study. Chen et al. (2012b) revealed the maximum Manganese peroxidase activity was 3229.8 U L^{-1} on the 4th day and the highest Laccase activity of 1275 U L^{-1} was recorded at the 5th day of the growth of the bacteria. Yang et al. (2017) reported that the Laccase activity of most LDB increased significantly from 24 to 48 h, with a maximum of 2250 U L^{-1} in the bacterial strain H2 at 60 h incubation. In the case of the Manganese peroxidase enzyme, the highest activity of 1119 U L^{-1} was observed in strain J12 at 24 h.

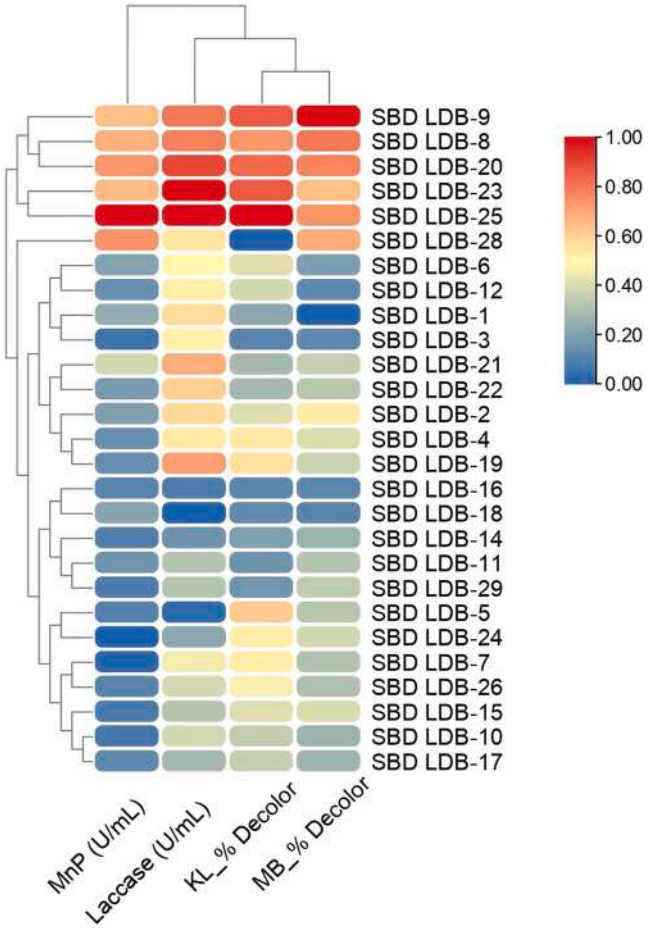

Fig. 1. Heatmap correlation analysis of the percentage of KL (Kraft Lignin) and MB (Methylene Blue) removed by several bacterial isolates producing the enzymes MnP and laccase.

8.1. Decolorization activity of bacteria on liquid MSM containing MB dye and Kraft lignin

The quantitative determination of the ligninolytic activities of the LDB was identified by measuring of percent decolorization of MSM-L broth medium supplemented with kraft lignin (0.5%) and Methylene blue indicator dye (50 mg/L) after 72hrs of incubation. All the strains showed significant activity and the results of the % degradation of Kraft lignin and methylene blue indicator dye were summarized in Table 3.

The data of the % decolorization is displayed in heat map graph format in Fig. 1. It is a method of condensing information, with the lines denoting the investigated ligninolytic bacteria and the columns denoting Kraft Lignin and Methylene Blue decolorization. The degree of the % decolorization is shown by the colored tiles. Fig. 1 demonstrates that the bacterial strains SBD-LDB-9, SBD-LDB-8, SBD-LDB-20, SBD-LDB-23, and SBD-LDB-25 achieved the highest % decolorization.

The maximum percent decolorization of 38.327 $\pm$ 0.011% was observed by the SBD-LDB-9 strain in MSM-L broth containing Kraft

Table 3
Quantitative determination of Ligninolytic activity of bacteria.

S.N.	Percent decolourisation		
	Strain Name	Kraft lignin (0.25%)	Methylene blue (50 mg/L)
1	SBD-LDB-1	19.566 $\pm$ 0.010	17.866 $\pm$ 0.013
2	SBD-LDB-2	21.025 $\pm$ 0.005	30.250 $\pm$ 0.016
3	SBD-LDB-3	23.587 $\pm$ 0.025	23.495 $\pm$ 0.018
4	SBD-LDB-4	19.771 $\pm$ 0.027	21.684 $\pm$ 0.012
5	SBD-LDB-5	19.809 $\pm$ 0.012	22.222 $\pm$ 0.017
6	SBD-LDB-6	24.429 $\pm$ 0.021	26.383 $\pm$ 0.015
7	SBD-LDB-7	23.419 $\pm$ 0.020	28.732 $\pm$ 0.012
8	SBD-LDB-8	37.298 $\pm$ 0.013	46.256 $\pm$ 0.020
9	SBD-LDB-9	38.327 $\pm$ 0.011	48.458 $\pm$ 0.015
10	SBD-LDB-10	24.317 $\pm$ 0.021	29.613 $\pm$ 0.007
11	SBD-LDB-11	20.819 $\pm$ 0.014	30.886 $\pm$ 0.016
12	SBD-LDB-12	30.134 $\pm$ 0.028	38.228 $\pm$ 0.017
13	SBD-LDB-14	22.409 $\pm$ 0.031	29.662 $\pm$ 0.019
14	SBD-LDB-15	19.0422 $\pm$ 0.012	21.390 $\pm$ 0.013
15	SBD-LDB-16	22.858 $\pm$ 0.011	23.201 $\pm$ 0.014
16	SBD-LDB-17	29.985 $\pm$ 0.012	44.542 $\pm$ 0.019
17	SBD-LDB-18	17.385 $\pm$ 0.015	22.663 $\pm$ 0.014
18	SBD-LDB-19	18.387 $\pm$ 0.011	30.299 $\pm$ 0.012
19	SBD-LDB-20	35.185 $\pm$ 0.030	49.633 $\pm$ 0.017
20	SBD-LDB-21	23.943 $\pm$ 0.016	29.075 $\pm$ 0.012
21	SBD-LDB-22	23.228 $\pm$ 0.007	32.795 $\pm$ 0.006
22	SBD-LDB-23	35.652 $\pm$ 0.005	46.941 $\pm$ 0.022
23	SBD-LDB-24	22.521 $\pm$ 0.019	31.082 $\pm$ 0.012
24	SBD-LDB-25	33.558 $\pm$ 0.017	47.969 $\pm$ 0.007
25	SBD-LDB-26	25.065 $\pm$ 0.009	39.452 $\pm$ 0.020
26	SBD-LDB-28	16.666 $\pm$ 0.009	26.258 $\pm$ 0.017
27	SBD-LDB-29	23.251 $\pm$ 0.014	30.299 $\pm$ 0.010

Data (Mean of triplicate value $\pm$ SD)

lignin whereas the minimum activity was observed in the SBD-LDB-28 strain with 16.666 ± 0.009% decolonization. A similar percent decolorization was observed in MSM-L broth containing Methylene blue dye and after 72 hrs incubation, the maximum percent decolorization (49.633 ± 0.017%) was observed in the SBD-LDB-20 strain whereas the minimum percent decolorization (17.866 ± 0.013%) was observed in SBD-LDB-1 strain. It has been speculated that the depolymerization of lignin polymers by bacterial ligninolytic systems, which consists of several enzymes secreted by these bacteria, is what causes the reduction in a color that results from lignin biodegradation. *Bacillus amyloliquefaciens* (SL-7) bacteria produced manganese peroxidase, lignin peroxidase, Laccase activity, and degraded 28.55% of tobacco straw lignin (Mei et al., 2020). Xiong et al. (2013) reported that *Panteoa spp.* strain Sd-1effectively reduced lignin color (52.4%) after 4 days of incubation. The ligninolytic bacterial strain, *Bacillus velezensis*, was used by Verma et al. (2020) and found that under ideal conditions, this strain had a maximum capacity for KL decolorization and degradation of 56.16% and 40.39%, respectively. These findings were in close agreement with those of the current study.

9. FTIR analysis

The lignin-degrading bacterial isolates were evaluated using FT-IR to determine how they had altered the structural and chemical properties of kraft lignin (Fig. 2). Lignin degradation was evident from the FTIR spectra of untreated and treated kraft lignin using lignin degrading bacteria depicted discrete changes, especially in the FTIR absorbance range from 1350 to 1715 cm^{-1} correlated to the stretching of C═C bonds in the aromatic skeleton of lignin when incubated in the broth for 5 days (Wang et al., 2021). The C═C bonds in the aromatic skeleton of lignin are the primary targets of the ligninolytic enzymes, resulting in lignin structural depolymerization (Zeng et al., 2014). Further, the decrease in absorbance around wave number 3500–3000 cm^{-1} corresponds to –OH bonds in alcohol and phenol in lignin in treated samples also indicating lignin degradation (Khan et al., 2022). Kraft lignin's FTIR spectrum underwent significant changesat 3210 cm^{-1} (OH stretching vibration), 2927 cm^{-1} (stretching vibration of C–H band in CH_2, CH_3, and CH_3O groups of the lignin structure), 2860 cm^{-1} (C–H stretching in aromatic methoxyl groups), 1715 cm^{-1} (C═O stretching), 1650 cm^{-1} (Absorbed O–H and conjugated C–O), 1635 cm^{-1} (C═C stretching vibration in benzene ring), 1580 cm^{-1}, 1511 cm^{-1} (attributed to the stretching vibration of aromatic rings), 1420 cm^{-1} (O-CH_3 stretching vibration), 1330 cm^{-1} (-CH stretching vibration), 1042 cm^{-1} (C–O vibrations) and 618 cm^{-1} (stretching vibrations of the C–S bond linked to the aromatic ring) (Kumar et al., 2015; Xu et al., 2018; Ma et al., 2021). The absorbance at 1335 cm^{-1} (S) significantly decreased during biodegradation, but the absorbance at 1275 cm^{-1} (G) barely changed. Significant differences between the FTIR spectra of the treated samples and the control samples showed that the lignin structure was largely destroyed by the different enzymes secreted by LDB used in the present study.

9.1. In vitro screening of lignin-degrading bacteria using rice straw biowaste as substrate

Furthermore, research was conducted to determine the optimal strain for in vitro degradation of agricultural waste. The consistency of rice straw altered when the lignin content in the straw was broken down by lignin-degrading bacteria, as shown in Fig. 3. The results of the decomposition of rice straw by using lignin degrading bacteria were summarized in Table 4. In rice straw that had been exposed to lignin-degrading bacteria, the amount of lignin was substantially reduced after 20 days after inoculation compared to the control rice straw. Among all LDB strains, the maximum percent lignin degradation were obtained in the SBD-LDB-8 strain (52.86%) followed by SBD-LDB-25 (52.69%), and SBD-LDB-20 (48.01%) and SBD-LDB-9 (45.99%) whereas the minimum percent lignin degradation was observed in SBD-LDB-17 strain (17.98%).

In a comparison of three bacterial isolates for lignin degradation on rice straw, Bakar et al. (2018) found that the rice straw treated with the AMB1 bacterial strain had significantly less lignin, at 4.97% compared to the lignin content of the control which was 8.89 ± 1.0. Similarly,

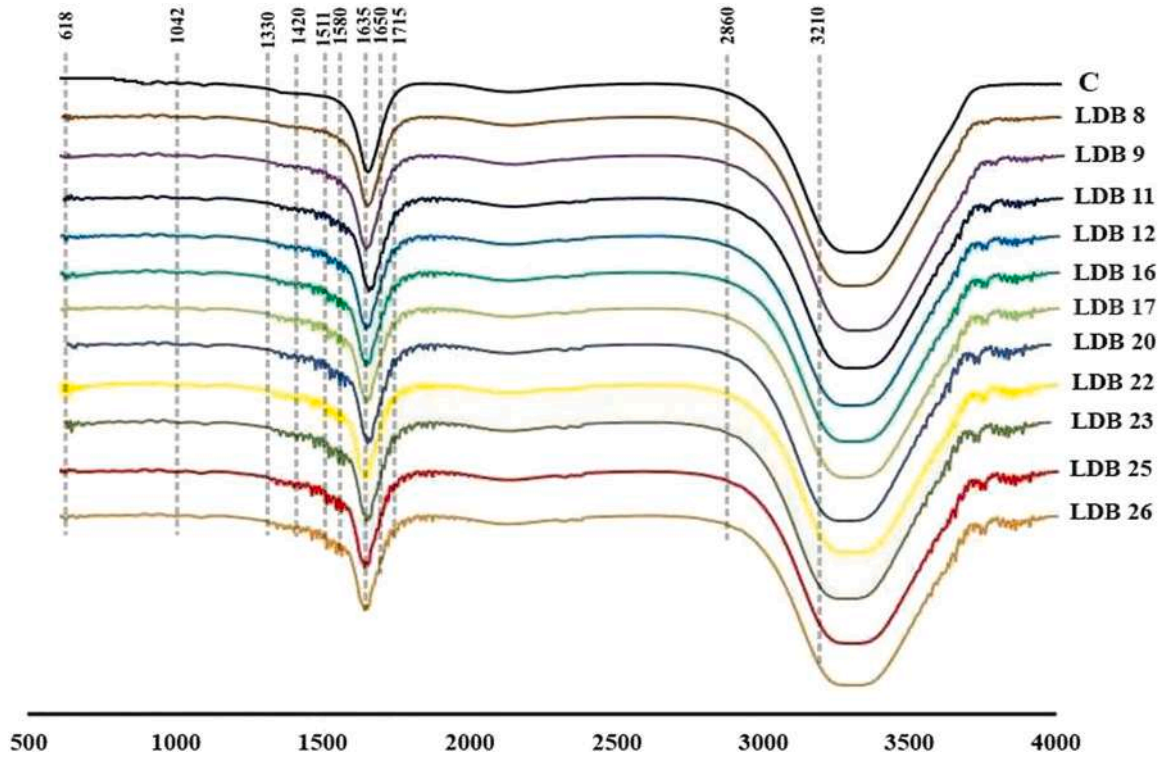

Fig. 2. FTIR spectrum of LDB treated and untreated kraft lignin.

Fig. 3. *In vitro* efficacy evaluation of lignin degrading bacteria for rice-straw degradation.

Table 4
Effect of effective lignin degrading bacteria pretreatment on the composition of rice straw.

Strain Name	ADF %*	ADL %* *	ASH %	Lignin%	% Lignin degradation
CONTROL	88.04	25.35	7	18.35 ± 0.014	100
SBD-LDB-8	68.43	13.98	5.3	8.65 ± 0.018	52.86
SBD-LDB-9	69.55	13.91	4	9.91 ± 0.016	45.99
SBD-LDB-11	76.03	18.73	4.6	14.06 ± 0.044	23.37
SBD-LDB-12	74.06	15.03	4	11.03 ± 0.027	39.89
SBD-LDB-16	75.44	16.55	4.6	11.08 ± 0.045	39.61
SBD-LDB-17	78.14	18.72	3.6	15.05 ± 0.023	17.98
SBD-LDB-20	72.46	13.87	4.3	9.54 ± 0.023	48.01
SBD-LDB-22	77.72	17.73	4.6	13.06 ± 0.015	28.82
SBD-LDB-23	69.42	13.57	3.6	9.91 ± 0.008	45.99
SBD-LDB-25	66.53	12.02	3.3	8.68 ± 0.038	52.69
SBD-LDB-26	76.16	16.78	4	12.78 ± 0.016	30.35

* *Acid Detergent Lignin: Lignin+Ash (Data recorded after 21 days pre-treatment)
* Acid Detergent Fiber (Cellulose+Hemi-cellulose+Lignin+Mineral);

Ochrobactrum oryzae BMP03 strain-treated rice straw's chemical makeup was investigated by Tsegaye et al. (2018) and reported that the BMP03 treatment degraded and mineralized 53.74% lignin after 14 days of pretreatment. According to Chandra et al. (2007), *Novosphingobium* sp. B-7 bacteria were responsible for 37% of the breakdown of Kraft lignin. Shi et al. (2017) found that after 7 days of pretreatment with the *Cupriavidus basilensis* B-8 bacterial strain, 41.5% of kraft lignin was eliminated, which was quite similar to the findings of the current study.

9.2. Effects of bio-pretreatment on the structure of the rice straw using SEM

The morphological modification of the rice straw that was treated by lignin-degrading bacterial isolates was analysed by scanning electron microscopy. The original rice straw displayed smooth, well-organized, and complete frameworks (Fig. 4). It was noticed that the surface was fairly smooth. It became fragmented and porous after being treated with lignin-degrading bacterial isolates. The rice straw developed some holes. After bacterial treatment, the rice straw's morphology showed that its linkages had been broken, and its lignin contents had been noticeably reduced. Various researchers observed the change in morphology of lignin using SEM which confirm the degradation of lignin visually (Xu et al., 2018; Ma et al., 2021). Dong et al. (2019) analyzes the lignin degradation by SEM technique and they observed that the microbial treated lignin sample became cracked and porous as compared to ordered and intact structures of untreated lignin samples which further supports the observation in the present study.

9.3. Sequencing and phylogenetic analysis of 16 S rDNA of potent LDB isolates

Based on in vitro rice straw degradation efficacy, the comprehensive sequences of the 16 S rDNA genes of the most effective lignin-degrading bacterial strains were sequenced and examined using the nucleotide BLASTn programme. These strains were identified as LDB-8: *Enterobacter ludwigii* (MW264070), LDB-25: *Klebsiella variicola* (MW265009), LDB-20: *Rahnella aquatilis* (MW264333), LDB-9: *Bacillus paramycoides* (MW264994), and LDB-23: *Bacillus paramycoides* (MW423733) as shown in Fig. 5. Table 5 lists the molecular details and NCBI GeneBank accession number associated with these strains. Similar approach was adopted by Upadhyay et al. (2009, 2011) for identification of salt tolerant rhizobacterial isolates. Rahman et al. (2013) characterized lignin-degrading bacteria using 16 SrRNA gene sequencing analysis and identified the bacterial strains as *Bacillus* sp., *Ochrobactrum* sp., and *Leucobacter*sp., with 99% sequence similarity to the strains from NCBI-Gene bank databases. El-Hanafy et al. (2008) isolated two lignin-degrading bacterial strains from Egyptian soils and identified them as *Bacillus sp.* (EU344809) and *Bacillus subtilis* based on the partial 16 S rRNA sequencing (EU344808). From a decomposing empty fruit bunch of an oil palm, Riyadi et al. (2020) isolated and described a lignin-degrading bacterial strain. Based on 16S rDNA sequencing, the isolated strain was identified as *Streptomyces* sp. S6.

10. Conclusion

The current investigation was based on the isolation of 27 lignin-degrading bacteria from 12 conventional manures. These strains were strong in ligninolytic enzymes such laccase and MnP, which can degrade KL and MB. SBD-LDB-8, SBD-LDB-25, SBD-LDB-20, SBD-LDB-9, and SBD-LDB-23 were shown to be the best LDB isolates based on their in vitro lignin degradation capacity using rice straw as the substrate. SEM images indicating changes in surface morphological properties linked with lignin breakdown backed up this claim. Despite tremendous progress in the isolation and characterization of lignin-degrading microbes to date, appropriate and effective formulations for waste breakdown and biodegradation of synthetic dyes like MB must be developed. According to these results, the isolated LDB strains from the current study would make a good choice for lignin valorization. Therefore, more thorough research is needed to establish their capacity to degrade waste biomass on the ground and in certain environmental conditions.

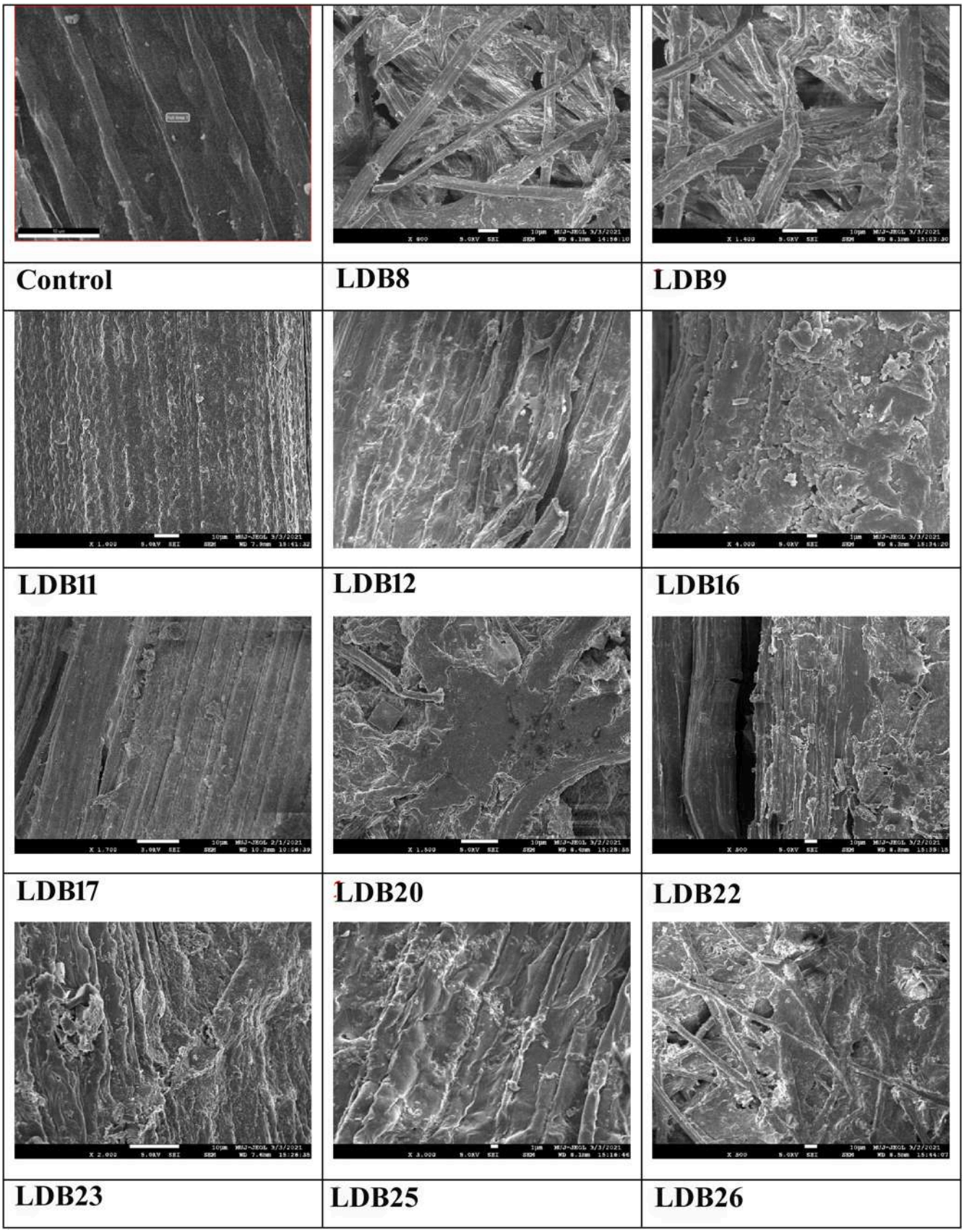

Fig. 4. Scanning electron micrographs of the control and LDB treated rice straw for 21 days.

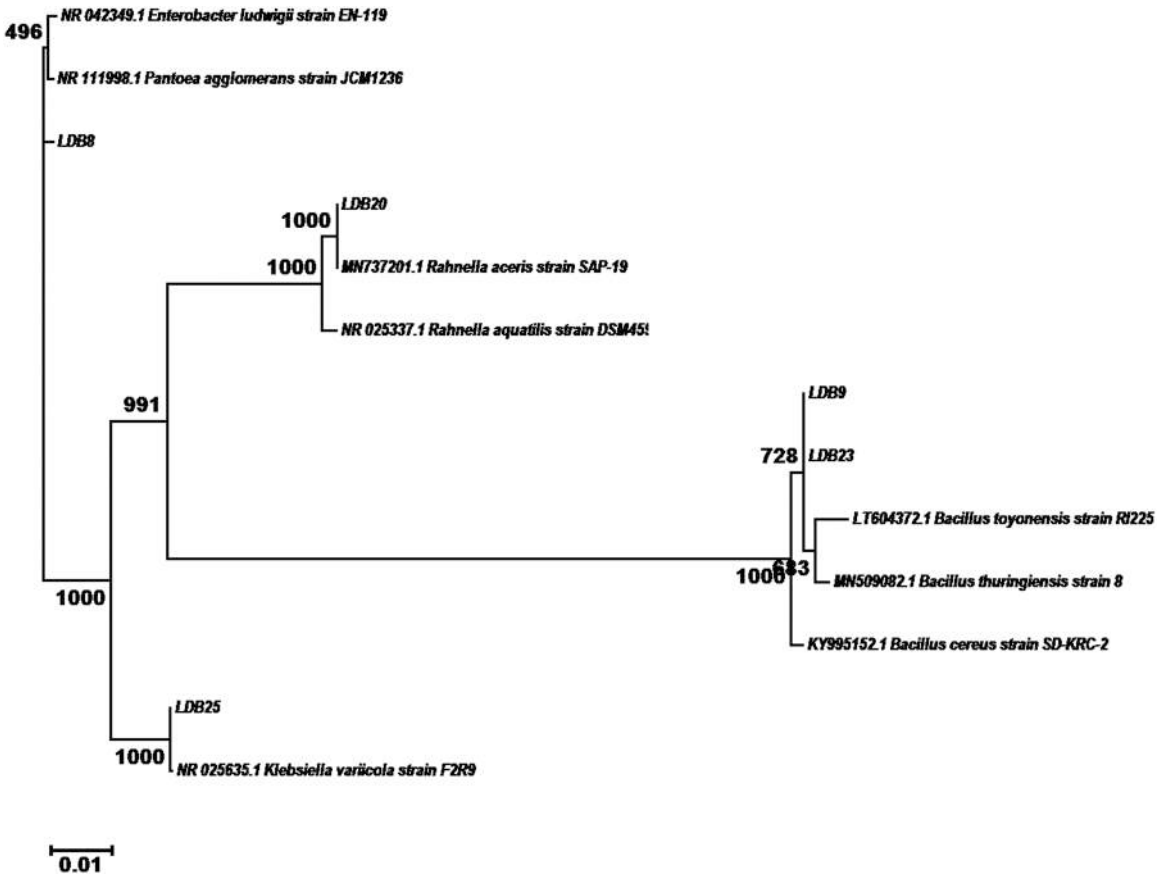

Fig. 5. Phyogenetic analysis of potent lignin degrading bacteria.

Table 5
Molecular identification of potent Lignin Degrading Bacteria.

Strains	Identification/ Accession number	Closest type strain			
		Molecular identity	Strain	Accession number	% Similarity/ Query Coverage
LBD-8	*Enterobacter ludwigii* / MW264070	*Enterobacter ludwigii*	EN-119	NR_042349.1	99.71/100
LDB-9	*Bacillus paramycoides* / MW264994	*Bacillus paramycoides*	MCCC 1A04098	NR_157734.1	99.00/99.09
LBD-20	*Rahnella aquatilis* / MW264333	*Rahnella aquatilis*	DSM 4594	NR_025337.1	99.57/100
LDB-23	*Bacillus paramycoides* / MW423733	*Bacillus paramycoides*	MCCC 1A04098	NR_157734.1	99.00/99.09
LBD-25	*Klebsiella variicola* / MW265009	*Klebsiella variicola*	F2R9	NR_025635.1	99/100

CRediT authorship contribution statement

DJ conceived and designed the experiments; JKN, AAB performed laboratory experiments; AS performed FTIR and SEM analysis; DJ, AAB, SKU, and SRM wrote the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgments

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Disclosure statement

No potential conflict of interest was reported by the authors.

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MANIPAL UNIVERSITY JAIPUR

VENDOR NAME & ADDRESS	PURCHASE ORDER
JUNGLE D-BLOCK, MANSI MARKET, BHORKI ROAD, GUDHAGORJI, JHUNJHUNU # 9649444411 E-Mail: jungle.lalbagh@gmail.com **GST No. : 08HPAPB7157H1ZU**	**ORDER NO. : MUJ/24-25/0282** **DEPARTMENT: General Services & Administration - Horticulture, Plants** **DATE OF ORDER : 24/07/2024**

BILL TO/SHIP TO -

REGISTRAR,

Manipal University Jaipur
VPO - Dehmi Kalan, Near GVK Toll Plaza,Jaipur-Ajmer Expressway, Jaipur, Rajasthan
Jaipur-303007,RAJ
PH. 0141 3999100
MUJ GST No. : 08AAAJM1881F1Z6

Kindly supply the following items

S.NO.	ITEM / DESCRIPTION	CGST		SGST		IGST		UOM	QTY	RATE (Rs.) / UNIT	AMOUNT (Rs.)
1	Estate & Grounds NEEM PLANTS, 5-6 FT	0%	-	0%	-	0%	-	NOS	100	425.00	42,500.00
2	Estate & Grounds MILLE PLANTS,1FT	0%	-	0%	-	0%	-	NOS	3700	25.00	92,500.00
3	Estate & Grounds INRME PLANTS,1FT	0%	-	0%	-	0%	-	NOS	100	6.00	600.00
4	Estate & Grounds NERIUM OLEANDER YELLOW,1-2FT	0%	-	0%	-	0%	-	NOS	350	18.00	6,300.00
5	Estate & Grounds YELLOW ALLAMANDA PLANTS,1FT	0%	-	0%	-	0%	-	NOS	100	60.00	6,000.00
6	Estate & Grounds LAGERSTROEMIA FLOS REGINEE PLANTS,10-12FT	0%	-	0%	-	0%	-	NOS	20	1,000.00	20,000.00
7	Estate & Grounds BOGAMBILYA RAD,2-3FT	0%	-	0%	-	0%	-	NOS	100	50.00	5,000.00
TOTAL										**Rs.**	**172,900.00**
NET TOTAL										**Rs.**	**172,900.00**
GRAND TOTAL										**Rs.**	**172,900.00**

Total : ONE LAKH SEVENTY TWO THOUSAND NINE HUNDRED RUPEES AND ZERO PAISA ONLY

Terms & Conditions:

1. GST : NA
2. Packing and Forwarding : No
3. Freight : No
4. Delivery : 05 Days
5. Payment : Within 30 days from the date of delivery
6. Contact Person : For further co-ordination, contact with Mr. Rajendra @ 8963879397
7. Other Terms : Delay in supply / execution of order with more than 7 days will attract penalty clause of 2% per week. Order will be consider as cancelled due to delay by more than 10 days without giving any reason thereof.
8. Dispute : Any dispute related to this order shall be subject to Courts of Jaipur juridiction only. This order shall be governed in accordance with Law of India.

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4. The supplier/manufacturer should grant access to their facility to MUJ to conduct second party audit wherever it is recognized as critical to the environment.
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MANIPAL UNIVERSITY
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MANIPAL UNIVERSITY
JAIPUR

VENDOR NAME & ADDRESS	PURCHASE ORDER
HOME GARDENS NR CHANKYA GARDENTAGORE NAGAR AJMER ROAD JAIPURJAIPUR # 9887259220 E-Mail: homegarden.nursery@gmail.com GST No. : 08AFHPC7553E1ZK	ORDER NO. : MUJ/23-24/0392 DEPARTMENT: General Services & Administration - Horticulture, Plants DATE OF ORDER : 08/11/2023

BILL TO/SHIP TO -

REGISTRAR,

Manipal University Jaipur
VPO - Dehmi Kalan, Near GVK Toll Plaza,Jaipur-Ajmer Expressway, Jaipur, Rajasthan
Jaipur-303007,RAJ
PH. 0141 3999100
MUJ GST No. : 08AAAJM1881F1Z6

Kindly arrange the supply of following items

S.NO.	ITEM / DESCRIPTION	CGST		SGST		IGST		UOM	QTY	RATE (Rs.) / UNIT	AMOUNT (Rs.)
1	Estate & Grounds Petunia	0%	-	0%	-	0%	-	PCS	3000	22.00	66,000.00
2	Estate & Grounds Gazania	0%	-	0%	-	0%	-	PCS	300	22.00	6,600.00
3	Estate & Grounds Salvia	0%	-	0%	-	0%	-	PCS	150	22.00	3,300.00
4	Estate & Grounds Dehlia	0%	-	0%	-	0%	-	PCS	300	25.00	7,500.00
5	Estate & Grounds Impetion	0%	-	0%	-	0%	-	PCS	400	25.00	10,000.00
TOTAL										Rs.	93,400.00
NET TOTAL										Rs.	93,400.00
GRAND TOTAL										Rs.	93,400.00
Total : NINETY THREE THOUSAND FOUR HUNDRED RUPEES AND ZERO PAISA ONLY											

Terms & Conditions:

1. GST : NA
2. Freight : Through MUJ Pickup
3. Delivery : Within 10 Days
4. Payment : Within 20 days from the date of delivery
5. Contact Person : For further co-ordination, contact with Mr. Rajendra Kumar#8963879397

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2. You are requested to deliver material on working days, 9:00 AM to 5:00 PM only. Please confirm for working day of MUJ, before sending the material
3. Any special disposal instruction at the end of life cycle of the product may be intimated, keeping in mind the environmental hazardous requirements,if any.
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