SRIRAM BIOCARE
GSTIN : 33ADQFS9635L1Z9
98653 53738 | 86670 82159
Email : srirambiocare18@gmail.com

10.06.2020

To

The Principal,
JKK Munirajah Institute of Health Sciences College of Pharmacy,
T.N Palayam, Erode.

Subject: Request for Collaboration on Research Project – Reg.

Dear Sir,

I hope this letter finds you well. I am writing on behalf of Sriram BioCare Pvt. Ltd., a dedicated organization in the field of biotechnology and healthcare. We are committed to advancing scientific research and innovation in the pharmaceutical and biotech sectors.

We are highly impressed by the research capabilities and academic excellence of JKK Munirajah Institute of Health Sciences College of Pharmacy. Your institution's dedication to fostering cutting-edge research aligns with our goals and vision for the advancement of biotechnology.

With this in mind, we wish to propose a collaborative research project in the field of biotechnology, specifically focused on the "Production of Pectin Lyase from Agro waste." This project holds great potential for contributing valuable insights to our industry.

To ensure the successful execution of this project, we kindly request your esteemed institution's collaboration and support. We are prepared to provide the necessary financial resources, research personnel, and logistical assistance required for the project's seamless implementation.

We understand that every collaboration comes with its specific terms and conditions, and we are open to discussing and complying with any guidelines set forth by JKK Munirajah Institute of Health Sciences College of Pharmacy.

We firmly believe that a collaboration between Sriram BioCare Pvt. Ltd. and your esteemed institution will yield groundbreaking results and make a significant contribution to the field of biotechnology. In order to proceed, we kindly request that you provide us Budget and Faculty details.

Thank you

Yours sincerely,

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

SRIRAM BIOCARE
No.4/1181, KTS Nagar,
J.J. Nagar, Karattur,
Gobichettipalayam - 638 476,
Erode

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES
COLLEGE OF PHARMACY

(Approved by Tamil Nadu Govt. & Pharmacy Council of India - New Delhi, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai)
Thookanaickenpalayam, Gobichettipalayam (TK), Erode (DT) - 638506, Tamil Nadu.

DR. P. PERUMAL M.Pharm., Ph.D., FIC.,
Professor & Principal

16.06.2020

To

Sriram BioCare Pvt. Ltd.,
No. 4/537, N.N. Arcade, Pariyur Main Road,
Murugan Puthur, Gobichettipalayam - 638 476.

Subject: Response to Proposal for Research Collaboration - Production of Pectin Lyase by fungi isolated from Agro waste.

Dear Sir,

I hope this letter finds you well. We greatly appreciate your interest in collaborating with JKK Munirajah Institute of Health Sciences College of Pharmacy for the research project titled **"Production of Pectin Lyase by fungi isolated from Agro waste."**

First and foremost, we are honoured and excited about the possibility of working with **Sriram Bio Care Private Limited** on this significant research endeavour. Your organization's dedication to advancing pharmaceutical research resonates with our mission to contribute to the field of pharmacology and improve healthcare outcomes.

We have carefully reviewed your proposal, and we are enthusiastic about the potential impact of this collaboration. The research project aligns perfectly with our expertise and ongoing efforts in the area of natural extracts and their therapeutic applications. We believe that this partnership will not only enhance our research capabilities but also foster valuable contributions to the scientific community.

We would like to express our gratitude for your willingness to provide financial support and logistical assistance for this project. We are confident that this collaboration will yield substantial results and advancements in the Production of Pectin Lyase.

To move forward, we propose scheduling a meeting to discuss the specific details of the collaboration, including project timelines, budget considerations, and other essential aspects. Our team is excited to engage in this research endeavour and is committed to ensuring the successful completion of the project. Please let us know your availability, and we will coordinate a meeting that accommodates your schedule. You can reach me at **principal@jkkmihscp.org** to coordinate further.

JKK Munirajah Institute of Health Sciences College of Pharmacy T.N.Palayam Erode Dt.

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES
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(Approved by Tamil Nadu Govt. & Pharmacy Council of India - New Delhi, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai)
Thookanaickenpalayam, Gobichettipalayam (TK), Erode (DT) - 638506, Tamil Nadu.

DR. P. PERUMAL M.Pharm., Ph.D., FIC.,
Professor & Principal

We look forward to a productive partnership and the opportunity to contribute meaningfully to the advancement of pharmaceutical research.

With reference to the letter dated 10/06/2020, JKKMIHSCP is permitting the following faculty members to do collaborative research with Sriram Bio Care Private Limited, and a proposal on the mentioned title "Production of Pectin Lyase by fungi isolated from Agro waste" is submitted along with this letter. The faculty members were assigned to do research work with Sriram Bio Care Private Limited, Gobi.

1. Mr. S. KANNAN, Associate Professor, Department of Pharmaceutics.
2. Mr. K. BALASUBRAMANIAN, Associate Professor, Department of Pharmaceutics.
3. Ms. A. R. GOMATHI, Assistant Professor, Department of Pharmaceutics.

Kindly permit the above faculty members to execute the above research work. We are expecting a positive reply from your end.

Thanking you,

Principal Investigator

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt.

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES
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Thookanaickenpalayam, Gobichettipalayam (TK), Erode (DT) - 638506, Tamil Nadu.

DR. P. PERUMAL M.Pharm., Ph.D., FIC.,
Professor & Principal

BUDGET AND FACULTY DETAILS

Project Title: Production of Pectin Lyase by Fungi Isolated from Agro Waste

Project Duration: 6 months

Project Budget:

S. No	*Budget category*	*Amount in lakhs*
1.	Equipment and Maintenance	0.30
2.	Raw Materials and (Research Samples)	0.40
3.	Laboratory Consumables (Glassware, Solvents)	0.20
4.	Personnel Costs	0.15
5.	Miscellaneous Expenses (Publication)	0.20
	Total Budget	***1.25 lakhs***

Project Team:

Principal Investigator (PI):	Mr. S. KANNAN, Associate Professor, Department of Pharmaceutics, JKKMIHSCP.
Co-Investigators:	Mr. K. BALASUBRAMANIAN, Associate Professor, Department of Pharmaceutics, JKKMIHSCP. Ms. A. R. GOMATHI, Assistant Professor, Department of Pharmaceutics, JKKMIHSCP.

We kindly request an opportunity to discuss this funding application further. Your support will contribute significantly to the success of our project.

Thank you

Principal
KK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Yours Sincerely,

JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

SRIRAM BIOCARE
GSTIN : 33ADQFS9635L1Z9
98653 53738 | 86670 82159
Email : srirambiocare18@gmail.com

30.06.2020

To

The Principal,
JKK Munirajah Institute of Health Sciences College of Pharmacy,
TN Palayam.

Subject: Sanction Order for Research Project – Reg.

Dear Sir,

We are pleased to inform you that, following a thorough review and evaluation of the research project proposal titled **"Production of Pectin Lyase by fungi isolated from Agro waste,"** we are granting the necessary financial assistance for the successful completion of this research endeavor.

This project aligns with our organization's commitment to promoting scientific research and innovation. We believe that the outcomes of this research will contribute valuable insights to the field and benefit society as a whole.

The sanctioned budget for this project is as follows:

Project Title: Production of Pectin Lyase by fungi isolated from Agro waste
Principal Investigator: Mr. S. Kannan, M. Pharm.,
Project Budget: ₹1.25 Lakhs

S.No	Detail of Expenditure	Amount in lakhs
1.	Equipment and Maintenance	₹0.30
2.	Raw Materials and (Research Samples)	₹0.40
3.	Laboratory Consumables (Glassware, Solvents)	₹0.20
4.	Personnel Costs	₹0.15
5.	Miscellaneous Expenses (Publication)	₹0.20
	Total Budget	**₹1.25 lakhs**

We are excited about the research outcomes that will emerge from this collaboration and the potential impact they may have on the field of biotechnology.

Sd/-

JKK Munirajah Institutes of Health Sciences College of Pharmacy T.N.Palayam Erode Dt

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

SRIRAM BIOCARE
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Furthermore, the project is expected to be completed within 6 months, and upon completion, you are required to submit the certified soft copy of the final project report, Statement of Expenditure, and Utilization Certificate. The Utilization Certificate must be counter-signed by the Head of the Institution for the release of the grant.

We believe that this research project has the potential to contribute significantly to our academic and research goals. We wish you and your team the very best in successfully executing the project. Please feel free to reach out to us for any clarifications or assistance required during the course of the project.

Thank you

Best regards,

SRIRAM BIOCARE
No.4/1181, KTS Nagar,
J.J. Nagar, Karattur,
Gobichettipalayam - 638 476,
Erode

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES
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Thookanaickenpalayam, Gobichettipalayam (TK), Erode (DT) - 638506, Tamil Nadu.

DR. P. PERUMAL M.Pharm., Ph.D., FIC.,
Professor & Principal

PROJECT COMPLETION REPORT

Title of the Project: Production of Pectin Lyase by Fungi Isolated from Agro Waste

Category of the Project: Research Project

Date of approval of competent authority : 30/06/2020

Total cost of the Project : Rs: 1,25,000/-

S. NO.	*ITEMS*	*AMOUNT (₹)*
1.	*Equipment and Maintenance*	*30000*
2.	*Raw Materials and (Research Samples)*	*40000*
3.	*Laboratory Consumables (Glassware, Solvents)*	*20000*
4.	*Personnel Costs*	*15000*
5.	*Miscellaneous Expenses (Publication)*	*20000*
	Total	***1,25,000***

Date of start of the Project : 30/06/2020

Date of completion of Project : 28/12/2020

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt

Name and Signature of Principal Investigator

(S. KANNAN)

Principal
KK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Name and Signature of Co-investigator

SRIRAM BIOCARE
GSTIN : 33ADQFS9635L1Z9
98653 53738 | 86670 82159
Email : srirambiocare18@gmail.com

Date: 07.01.2021

To

The Principal,
JKK Munirajah Institute of Health Sciences College of Pharmacy,
T.N. Palayam.

LETTER OF APPRECIATION

Dear Sir,

We would like to take opportunity to thank you for your time spending and valuable Research work to our organization. Once again, we extend our sincere gratitude to you and your team for your efforts in making the "Production of Pectin Lyase by fungi isolated from Agro waste" a resounding success.

We look forward to the continuation of our successful partnership and to exploring new opportunities for collaboration.

Thank you

Sincerely,

SRIRAM BIOCARE
No.4/1181, KTS Nagar,
J.J. Nagar, Karattur,
Gobichettipalayam - 638 476,
Erode

Copy to:

Mr. S. Kannan, M. Pharm.,
Associate Professor,
JKK Munirajah Institute of Health Sciences College of Pharmacy,
T. N. Palayam.

JKK Munirajah Institutes of Health Sciences College of Pharmacy
TN.Palayam
Erode Dt

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES
COLLEGE OF PHARMACY

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Thookanaickenpalayam, Gobichettipalayam (TK), Erode (DT) - 638506, Tamil Nadu.

DR. P. PERUMAL M.Pharm., Ph.D., FIC.,
Professor & Principal

UTILIZATION CERTIFICATE

Certified that out of Rs. ...1,25,000..... sanctioned by **Sriram Bio Care Pvt. Ltd.** towards financial assistance for the student project titled "..PRODUCTION OF PECTIN LYASE BY ...FUNGI ISOLATED FROM AGRO WASTE .....", an amount of Rs. ...1,25,000..... was utilized for the purpose for which it was sanctioned, leaving a balance of Rs. ...-NIL-..... at the close of ...28/12/2020.... As shown in the Statement of Expenditure annexed.

Institutes of Health Sciences College of Pharmacy T.N.Palayam Erode Dt.

J. Thiru (CS. KANNAN)

Name & Signature
of the Principal Investigator

Name & Signature
of Head of Institution

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Principal
KK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

"PRODUCTION OF PECTIN LYASE BY FUNGI ISOLATED FROM AGRO WASTE PRINCIPAL INVESTIGATOR"

PRINCIPAL INVESTIGATOR

Mr. S. KANNAN, M. Pharm.,

Associate Professor,

Department of Pharmaceutics

CO-INVESTIGATORS

Mr. K. BALASUBRAMANIAN, M. Pharm.,

Associate Professor,

Department of Pharmaceutics,

Ms. A. R. GOMATHI, M. Pharm.,

Assistant Professor,

Department of Pharmaceutics,

JKK Munirajah Institutes of Health Sciences College of Pharmacy T.N.Palayam Erode Dt.

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES COLLEGE OF PHARMACY T.N.PALAYAM - 638 506 COUNCIL CARE CURE

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

DECEMBER-2020

JKKMUNIRAJAHINSTITUTEOFHEALTHSCIENCES

COLLEGEOFPHARMACY,

T.N-PALAYAM-638506,

GOBI (TK), ERODE (DT), TAMILNADU.

CERTIFICATE

This is to certify that the Research entitled **"PRODUCTION OF PECTIN LYSAE BY FUNGI ISOLATED FROM AGRO WASTE"** submitted to the **Sriram BioCare Pvt. Ltd., Gobichettipalayam,** is the bonafide project work carried out in the Department of Pharmaceutics, JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi, Erode, Under the guidance of **Mr. S. KANNAN, M. Pharm., Associate Professor, Department of Pharmaceutics,** JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N Palayam, Gobi, Erode. During the academic year 2020-2021.

Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt.

Place: T.N-Palayam

Date: 28/12/2020

Dr. P. Perumal, M.Pharm., Ph.D., FIC

PRINCIPAL

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

DECLARATION

This is to certify that the Research entitled **"PRODUCTION OF PECTIN LYSAE BY FUNGI ISOLATED FROM AGRO WASTE"** submitted to the **Sriram BioCare Pvt. Ltd., Gobichettipalayam,** is the bonafide project work carried out in the Department of Pharmaceutics, JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi, Erode, Under the guidance of **Mr. S. KANNAN, M. Pharm., Associate Professor, Department of Pharmaceutics,** JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N Palayam, Gobi, Erode. During the academic year 2020-2021.

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt.

Place: T.N-Palayam

Date: 28/12/2020

Mr. S. KANNAN, M. Pharm.,
Principal Investigator

Mr. K. BALASUBRAMANIAN, M. Pharm.,
Co-Investigator

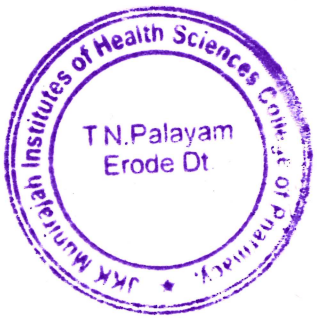
Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Ms. A. R. GOMATHI, M. Pharm.,
Co-Investigator

DECLARATION

We carried out the research work emboided in this work entitled "Production of Pectin Lysae" by Fungi Isolated from Agro Waste under the direct supervision of Mr. S. KANNAN, M. Pharm., Associate Professor, Department of Pharmaceutics, JKK Munirajah Institute of Health SciencesCollege of Pharmacy, T.N Palayam, Gobi.

The Project submitted to the **Sriram BioCare Pvt. Ltd., Gobichettipalayam,** during the academic year 2020-2021.



Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

ACKNOWLEDGEMENT

First and for most we express our heartful sense of gratitude and faithfulnessto God 'grace and our family members, which has enabled us to finish our project work successfully.

With the blessing of our Founder chairman Dr. J.K.K Munirajah, M.Tech, (Bolton). D.Litt., and Secretary Mrs. Kasthuripriya Kirupakarmurali, M.B.A.,

J.K.K Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi, Erode for providing all the facilities to carry out this work.

Our sincere gratitude to our beloved sir, Dr. P.Perumal, M.Pharm, Ph.D,FIC., Principal and Head of the Department of Pharmaceutical Chemistry, J.K.K Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi, Erode for his kindly support for our project work and for his encouragement and also providing all facilities in this Institute to the fullest possible extent enabling us to complete this work.

With the immense pleasure and pride, we would to take opportunity in expressing our deep sense of gratitude to our beloved guide Mr. S. KANNAN, M. Pharm., Associate Professor, Department of Pharmaceutics J.K.K Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi, Erode under whose active guidance, innovate ideas, constant inspiration and encouragement of the work entitled "Production of Pectin Lysae By Fungi Isolated from Agro Waste" has been carried out.

We also express our grateful thanks to all the teaching and non-teaching staff members of J.K.K Munirajah Institute of Health Sciences College of Pharmacy for their valuable advice and cooperation.

We express our heartful gratitude to the algmighty, for giving us the right way to achieve the best of our project.

We would like to give sincere thanks to our classmates for their timely help and co-operation.

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt

We also extend our thanks to all staff members of Department of Pharmaceutical Biotechnology, Pharmaceutical Chemistry, Pharmacognosy, Pharmaceutics and Pharmacology for their co-operation.

We would like to Thank to Sriram BioCare Pvt. Ltd., Gobichettipalayam give a Financial and moral support to completion of the project being a successful manner on the duration of 2020-2021.

Last but not least, great thanks from the heart to our beloved MOTHER and FATHER. They are our living god, as who guided us in the rightful way to achieve all our activities. They gave the incredible effort to become a successful for bright future in this world. Thanks a lot, to my parents.

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T N.Palayam
Erode Dt

ABSTRACT:

Abundant amount of waste materials are generated by agricultural and fruit processing industries, which possess considerable disposable problems and ultimately leads to pollution. Vast variety of microorganisms present in the environment, which can be exploited for the utilization of renewable resources, particularly agricultural and forest residues. Microorganisms have various advantages and can be used for enzymes production at higher level. Pectinolytic enzymes have great biotechnological potential and can be employed in many important industrial processes. Pectinases are commonly employed in juice, textile, paper and pulp industries. These enzymes catalyzed the conversion of complex polysaccharides into simpler molecules like galacturonic acids. These have wide industrial applications like oil extraction, tea extraction, juice clarification and waste water treatments.

In the present work, the enzyme producing fungi was isolated from waste soil samples of Erode district and it's used to produce pectin lyase enzyme by using solid state fermentation process.

With these was background information, the following objectives were made to

- Isolate fungi from dead organic matter, fruit waste and vegetable waste soil samplesof Erode district.
- Screen potential microfungi with reference to pectin lyase enzyme from PSAM (Pectin Screening Agar Medium).
- Assay of pectin lyase enzyme from isolated fungal species.
- Production of pectin lyase by using liquid state fermentation process.
- Optimization of pectin lyase enzyme production by fungi
- Characterization of Pectin lyase enzyme activity

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
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JKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt

Results:

A.niger and P.citrinum were found to utilize agricultural waste and byproducts for enzyme production and also A.niger potential fungus had the ability to produce high level of pectin lyase and protein with specified parameters and compared with other fungus P.citrinum.

Conclusions:

The overall investigation concluded that the *A.niger* and *P.citrinum* were found to utilize agricultural waste and byproducts for enzyme production and also *A.niger* potential fungus had the ability to produce high level of pectin lyase and protein with specified parameters and compared with other fungus *P.citrinum*. This study revealed that the potential of utilizing agricultural wastes provided cost effective and eco-friendly methodfor pectinlyase production on large scale.

Keywords:

Fermentation, colonization, pectinolytic enzymes, dialysis

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt

INTRODUCTION

Annually a more than 170 million tons of agricultural wastes are stacked in Tamil Nadu, annually being the main agro industrial residues piled up in the country. Nevertheless, biotechnology could offer opportunities to modify the chemical structure of these substrates to improve their digestion (decomposition) .

Agro waste

Abundant amount of waste materials are generated by agricultural and fruit processing industries, which possess considerable disposable problems and ultimately leads to pollution. Vast variety of microorganisms present in the environment which can be exploited for the utilization of renewable resources, particularly agricultural and forest residues. The major components are cellulose, starch, lignin, xylan and pectin.

Agro industrial residues are generally considered the best substrates for the solid state fermentation process and use of SSF for the production of enzymes is no expection to that. A number of such substrates have been employed for the cultivation of microorganisms to produce host of enzymes.

Some of the substrates formed on using include sugar cane bagasse, wheat bran, ricebran, maize bran, gram bran, wheat straw, rice straw, rice husk, soyhull, sagohampas. Grape wine trimmings dust, saw dust, corn cobs, coconut coir pith, banana waste, tea waste, cassava waste, palm oil mill waste, aspen pulls, sugar beet pulb, sweet sorghum pulb, apple pomace, peanut meal, rape seed cake, coconut oil cake, mustard oil cake, cassava flour, wheat flour, corn flour, steamed rice, steam pretreated willow starch etc.

Agro industrial residue and wastes such as wheat bran, rice bran, sugar cane bagasses,core cobs, citrus wastes, apple pomace and a variety of such by products are potentially goodsubstrates for SSF. Nagpur a Mandarin orange (*Citrus reticulate* Blanco) growing area has several orange processing factories in which considerable quantity of fruit peel is dumpedinto the environment.

Agricultural wastes are rich in sugar which can be easily assimilated by microorganisms. It means that residue like pineapple skin, jack fruit peel wastes, must melon skin, banana waste, apple pomace, orange peel, cereals straw. Tea waste, sugar beet pulp, wheat bran, rice bran, sugar cane

begasses can be used as carbon source in SSF to obtain industrially useful products.

Generally agroindustrial wastes are employed for pectinase production. The main source of microorganisms that produce pectinolytic enzymes are yeasts, bacteria and a large varieties of fungi and particularly *Aspergillus* sp. Fungi can produced diverse extracellular enzymes and they are used to break down complex polysaccharides into simple sugar to be assimilated and used for growth and reproduction.

Fungal colonization on substrates

Many enzyme preparation from microbial origin were reported to have macerating activity against plant tissues partially or down to individual cells i.e *Aspergillus alliaceus, A.awamori, Colletotrichum gloeosporioides, C.lindermauthianum, C.magna, Fusarium solani* and *Pythium splendens* .These enzyme preparations typically contain pectinases, cellulases, hemicellulase, and other carbohydrate enzyme activities.

Fungal organisms were isolated by different workers *Aspergillus* , *Penicillium Rhizopus stolonifer* .

Enzymes are the most important products obtained for human needs through microbial sources. A large number of industrial processes are in the area of industrial, environmental and food biotechnology.

Fermentation

Fermentation is the technique of biological conversion of complex substrates into simple compounds by various microorganisms such as bacteria and fungi. In the course of this metabolic breakdown, they also release several additional compounds apart from the usual products of fermentation, such as carbon dioxide and alcohol. These additional compounds are called secondary metabolites. Secondary metabolites are ranges from several antibiotics to peptides, enzymes and growth factors.

Solid-State Fermentation (SSF)

SSF utilizes solid substrates, like bran, bagasse, and paper pulp. The main advantage of using these substrates is that nutrient-rich waste materials can be easily recycled as substrates.

In this fermentation technique, the substrates are utilized very slowly and steadily, so the same substrate can be used for long fermentation periods. Hence, this technique supports controlled release of nutrients. SSF is best suited for fermentation techniques involving fungi and microorganisms that require less moisture content.

Some of the common substrates used in solid state fermentation are wheat bran, rice and rice straw, hay, fruit and vegetable waste, paper pulp, bagasse, coconut coir, and synthetic media. Some common substrates used in submerged fermentation are soluble sugars, molasses, liquid media, fruit and vegetable juices, and sewage or waste water.

The outcome of fermentation highly varies for each substrate; hence, it is extremely important to choose the right substrate. Fermentation techniques have to be optimized for each substrate. This is primarily due to the reason that an organism reacts differently to each substrate. The rates of utilization of various nutrients differ in each substrate and respective productivity.

Solid State Fermentation (SSF) is an attractive method for fungal enzymes production because it simulates the natural growth of microorganisms on a moist insoluble substrate in the absence or near absence of free liquid. This cultivation technique is acquiring a special relevance in the field of the biotechnological processes, as an alternative to the traditional submerged fermentation, because of lower energy requirements it produces less wastewater, gives high product concentrations, avoids the foaming and has lower risks of contamination.

Advantages of solid state fermentation

SSF offers numerous advantages for the production of bulk chemicals and enzymes. This process is known from ancient times and different fungi have been cultivated in SSF for the production of food. Typical examples of the fermentation of rice by *Aspergillus oryzae* to initiate the koji process and *Penicillium roquefortii* used for cheese production. In China, SSF has been used extensively to produce brewed foods (such as Chinese wine, soysauce and vinegar) since ancient time. Also, in Japan SSF is used commercially to produce industrial enzymes.

Thus, the production of bulk chemicals and value- added fine products such as ethanol, single-cell protein (SCP), mushrooms, enzymes, organic acids, amino acids, biologically active secondary metabolites, etc .

In recent years, SSF has received more and more interest from researchers, since several studies for enzymes, flavours , colourants and other substances of interestto the food industry have shown that SSF can give higher yields or better product characteristics than submerged fermentation (SmF). In addition, costs are much lower due to the efficient utilization and value-addition of waste, have performed a detail economic analysis of the production of *Penicillium restrictum* lipase in both SmF and SSF. The great advantage of SSF processes is the extremely cheap raw material used as main substrate. Therefore, SSF is certainly a good way of utilizing nutrient rich solid wastes as a substrate. Both food and agricultural wastes are produced in huge amounts. Since they are rich in carbohydrates and other nutrients, they can serve as a substrate for the production of bulk chemicals and enzymes using SSF technique.

Enzymes that hydrolyze pectin substances, which contribute to the structure of plant cells, are known as pectinolytic enzymes or pectinases. Based on their mode of actions, these include polygalacturonase, pectin esterase, pectin lyase and pectate lyase (PL) . PL (EC4.2.2.2.) hydrolyzes the -1,4-glycosidic bond of polygalacturonate and releases unsaturated soluble oligogalacturonates . It has been reported that products is produced from a wide variety of microbialsourcessuch as fungi, actinomycetesandbacteria.

Pectinolytic enzymes

Microorganisms have various advantages and can be used for enzymes production at higher level. Pectinolytic enzymes have great biotechnological potential and can be employed in many important industrial processes. *Aspergillus niger* belongs to Ascomycota group of fungi, genus *Aspergillus*. It is an opportunistically infectious microbe to human being and well adapted to environmental changes. The optimization of production of pectin lyase by *A. niger* and then its characterization was done only after partial purification.

Pectinolytic enzymes can be produced in large amount by microorganisms, using citrus peel as a substrate because it contains considerable quantity of pectin. It works as inducer for the synthesis of pectinolytic enzymes by microbial systems. These enzymes have the ability to degrade and chemically modify pectin .

Pectinases are commonly employed in juice, textile, paper and pulp industries. These enzymes catalyzed the conversion of complex polysaccharides into simpler molecules like galacturonic acids.

These have wide industrial applications like oil extraction, tea extraction, juice clarification and waste water treatments .

Pectin lyase assay

The activity of pectin lyase is assayed by measuring the optical density at 235 nm due to formation of 4,5-unsaturated oligogalactouranotes by -elimination mechanism and molar extinction coefficient for the product being 5.5 - 103 M^{-1} cm^{-1} at l = 235 nm (55).The activity is defined as the amount of enzyme that releases 1 mmole of unsaturated product per minute under standard assay conditions. Reducing group methods are also useful in determining the lyase activity. Viscosity reduction method in conjunction with a reducing group method or along with intermediate product analysis by HPLC or GC can be used to distinguish between endo and exo splitting enzymes. For the detection of this unsaturated compound, thiobarbituric acid (TBA) is claimed to be the colorimetric test specific for the quantification of the PNL activity.

Purification and characterization of pectin lyase

Pectin lyase have been obtained from commercial preparations of pectic enzymes and culture filtrates of fungi, bacteria and yeast. The first pectin lyase was purified from pectinol R-10 by simple purification procedure including column chromatography on DEAE-cellulose and sephadex G-75 and G-50. To purify pectin lyase from *Penicillium italicum*, DEAE cellulose chromatography, CM-cellulose chromatography, phenyl sepharose CL-4B chromatography, Mono P- chromatography followed by second phenyl sepharose chromatography was employed. A pectin lyase was purified homogencity by ammonium sulphate fractionation, CM-sepharose column chromatography and preparative electrofocussing.

Applications of pectin lyase

Pectin lyase are known as pectinases capable of degrading highly esterified pectins (like those found in fruits) into small molecules via b-elimination mechanism without producing methanol, in contrast with the combination of PG and PE, which are normally found in commercial products. This is important because methanol is toxic and may leads to health hazards. Methanol may be lost in vapour during juice concentration, but at different conditions occurs in viscous materials (Purees, baby foods, etc.) or non- concentrated juices where methanol is only partially released during pasteurization.

In addition, the presence of undesirable enzymatic activity in commercial pectinases may be detrimental to aroma because they are responsible for producing unpleasant volatile off flavour. The alkaline pectinase are in appropriate to be used in the food industries due to acidic pH of fruit juices. However, they have a very high demand in the textile industries. They are used for retting of plant fibers such as ramie, sunn hemp, jute, flax and hemp. It is noteworthy to mention that on retting of sunn hemp (*Crotalaria juncea*) by pectin lyase produced by *A. flavus* - MTCC 7589 but this aspect of pectin lyase need to be extensively investigated further.

Structural aspects of pectin lyase

Crystal structures of pectin lyase A (PNLA) from two strains of *Aspergillus niger*, N400 and 4M-147 reveal that PNLA folds into a parallel sheet and shares many of the structural features of pectate lyase despite not more than 17% sequence identity after pairwise structure-based alignment. These shared structural features include amino acid stacks and the asparagine ladder. The substrate-binding clefts of these two PNLs are dominated by aromatic residues and are enveloped by negative electrostatic potential. The major difference between these two PNLA structures is in the conformation of the loop formed by residues ranges from 182–187.

These observed differences are due to the different pH values of crystallization. The three-dimensional structure of pectin lyase B (PNLB) from *Aspergillus niger* has also been determined by crystallographic techniques at a resolution of 1.7AM. Therefore, the main aim of the present study was carried out for the production and characterization of pectin lyase from agricultural wastes using vapour during juice concentration, but at different conditions occurs in viscous materials (Purees, baby foods, etc.) or non-concentrated juices where methanol is only partially released during pasteurization.

In addition, the presence of undesirable enzymatic activity in commercial pectinases may be detrimental to aroma because they are responsible for producing unpleasant volatile off flavour. The alkaline pectinase are in appropriate to be used in the food industries due to acidic pH of fruit juices. However, they have a very high demand in the textile industries. They are used for retting of plant fibers such as ramie, sunn hemp, jute, flax and hemp. It is noteworthy to mention that on retting of sunn hemp (*Crotalaria juncea*) by pectin lyase produced by *A. flavus* - MTCC 7589 but this aspect of pectin lyase need to be extensively investigation.

MATERIALS AND METHODS

Sample collection

The samples were collected from agro waste soil samples in Erode District. Three

different soil samples viz., vegetable waste soil sample (VS) from Gobichettipalayam Vegetable market, fruit degrading soil sample (FS) from Gobichettipalayam fruits market and dead organic matter sample (DOS) from Household material were randomly collected, just 1cm below the soil surface and digested plant litters were aseptically transferred to sterile polythene bags, transported to the laboratory and stored in refrigerator till processing for analysis.

Isolation of fungi from soil

Ten gram of the soil sample was taken in a 250 ml conical flask containing 100ml sterile distilled water. The flask was shaken on an electric shaker to get a homogenous suspension and different dilution of the soil sample viz., 10-1, 10-2 and 10-3 were prepared by transferring serially about 10 ml of the soil suspension to 90 ml of sterile distilled water. One ml of 10-3 dilutions was plated in Petri dishes containing potato dextrose agar medium. The pH of the medium was adjusted to 5.6 and streptomycin sulphate (100 mg/L-2) was added to the media to prevent the bacterial growth. The plates were incubated at 25±2°C for five days and the fungal colonies appearing on the potato dextrose agar media were picked and isolated. Purified strains were obtained by streaking repeated in PDA medium and observed under compound microscopy. The cultures were characterized to genus level on the basis of macroscopic (colonial morphology, colour and appearance, shape) and microscopic characters (septation of mycelium, shape, diameter and texture of conidia) . Population of fungi was calculated by using following standard formula:

Percentage of contribution = Mean.no.of propagules In dilution plate

Weight of the dry soil

Identification of fungi

The fungi were identified by using standard manual, such as Manual of Soil Fungi , Dematiaceous Hyphomycetes , More Dematiaceous Hyphomycetes , Hyphomycetes . The fungi on PDA plates were identified based on characteristic features of colony morphology and reproductive structural characteristics like sporangiospore position, columella and spore shape . Freshly grown mycelia with small amount of

medium was stained with LCB and examined under stereobinocular microscope. Identified fungal cultures were maintained on the PDA medium in the laboratory by using conventional methods and sub cultured at regular intervals.

Screening for the Pectin lyase Enzyme Production

Pectin lyase Screening Agar Medium (PSAM) is used as selective medium for the growth of microbes which release pectin. After the preparation and sterilization of the media, block of fungal culture were made using cork borer from the master plate and transferred to Petridish with PSAM medium. The plates were stored at room temperature in inverted position for proper microbial growth. After incubation, the plates were screened for the identification of zones of hydrolysis which indicating the positive or negative results for pectin lyase production

Pectin lyase enzyme production by liquid state fermentation

Fungal isolates were placed in a basal medium used for pectinase production, the medium consist of 0.3% (NH4)2 HPO4; 0.2% KH2PO4; 0.01% K2HPO4; 0.01%-MgSO4;

2.5%- Pectin. The type of fermentation used submerged liquid state fermentation. The culture was incubated for 10-12 days at 25°C (49).

Pectin lyase enzyme assay

Assay of pectin lyase was performed by the method described by Preiss and Ashwell. 0.5 mL of enzyme was incubated for 1 h with 0.5 mL of 0.5% pectin and 1 mL of 50 mM Tris HCl buffer (pH 8) and 1 mL of 0.2 mM CaCl2. After 1 h, absorbance was measured at 548 nm against blank. One unit of pectin lyase activity was defined as "the amount of enzyme present in 1 mL of original enzyme solution which released 1 M of galacturonic acid in 1 min (84). Enzyme activity was calculated by using the following formula:

Enzyme activity (IU/ml) = Absorbance of enzyme solution× standard factor

Time of incubation

Whereas, Standard = Concentration (μ M/ml) of standard Absorbance Optimization of pectin lyase enzyme production by fungi Optimization of pectin lyase enzyme production by fungi was performed in Erlenmeyer flask method to determine the effect of pH, temperature, incubation period and nutrient sources on pectin lyase enzyme production was assayed.

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For optimization studies various parameters that influence the pectin lyase enzyme production, eight days of fungal spores of Aspergillus niger and Penicillium citrinum were inoculated in to 100 ml of selective medium in 250 ml Erlenmeyer flask.

Effect of pH

Fungi inoculated in culture medium were incubated at different pH (5.0, 6.0, 7.0, 8.0 and 9.0). The pH of the solution was altered using 0.1N HCl and 0.1N NaOH solution. After incubation period, pectin lyase enzyme production was determined .

Effect of temperature

Aspergillus niger and Penicillium citrinum were used to determine the effectiveness of different temperatures such as 15, 30 and 45°C. Fungi inoculated culture medium was incubated at temperature of 15, 30 and 45°C for one week. After incubation period, the culture medium was filtered and analysed for pectin lyase enzyme production .

Effect of incubation period

The optimum incubation period required by A.niger and P.citrinum was determined for the period of 3, 6 and 9 days at room temperature. After each end point of 3, 6, 9 and 12 days, the culture medium was filtered and analyzed to determining the pectin lyase enzyme production .

Characterization of Pectin lyase enzyme activity

Ammonium sulphate fractionation

Ammonium sulphate was added to the extract with stirring to bring the saturation 30% and after standing it for 4 hours at 40C, the precipitate and supernatant was determined. Additional ammonium sulphate was added to the supernatant to bring the saturation to 60% and the mixture was left overnight. The supernatant was further subjected to saturation 90% the precipitates were collected, dissolved in distilled water and the solution was dialyzed against water for 48 hours using dialysis bag. The dialyzed enzymatic fractions were subjected to protein and Pectin lyase activity was determined .

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Dialysis

The dialysis bag was sterilized. The bag was cut into pieces of convenient length (10-20cm) and boiled for 10 minutes in a large volume of 2% (w/v) sodium bicarbonate solution, which removes salt from pores. On rinsing the bag thoroughly with distilled water and boiled for 10 minutes of followed by boiling in 1mm EDTA pH 8.0 which removes heavy metal ions form pores again boiled at 1000C for 10 minutes. Bag was allowed to cool and used for dialysis. Filled the bag with enzyme, tied tightly with band on both the sides, and kept immersed in buffer solution (Tris Hcl buffer pH 7) for 5 hours.

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RESULTS AND DISCUSSION

Isolation of fungi from dead organic matter

In dead organic matter sample, totally 33 fungal colonies were recorded, 25 in fruit sample and 20 in vegetable sample. Totally 14 species belonging to 6 genera were observed in dead organic matter sample, 9 species belonging to 5 genera were observed in fruit sample and 9 species belonging to 3 genera were observed in vegetable sample. Maximum number of *A.niger* were observed in DOS and fruit sample. 13 colonies of *A.niger* in dead organic matter sample and 8 colonies in fruit sample. Maximum number of *P.citrinum* (17 colonies) were recorded in vegetable sample. The minimum number of *A.terreus* in dead organic matter samples; *Chetomium globosum* in fruit samples and *A.niger* in vegetable samples were recorded.(Table-1 and Figure-1).

Screening of pectinolytic enzyme from fungal isolates

In the primary screening studies of pectinolytic enzyme, totally 25 fungal isolates were oberseved and selected. Among that 21 fungal isolates had the ability to produce pectinolytic enzymes but 4 fungal isolates gave negative results. Belonging to the 21 fungal isolates *A.niger* and *P.citrinum* were showed excellent activities for pectin lyase enzyme production purpose (Table-2 and Figure-2).

Quantification assay of pectinolytic enzymes from fungi

Secondary screening quantification assay was done for 25 fungal isolates. Maximum amount of enzyme activity was recorded in *A.niger* (5.2 IU/ml) and 4.8 IU/ml recorded in *P.citrinum*. Minimum enzyme production was observed in *A.sydowi* (0.1 IU/ml). Remaining*Aspergillus* sp. produced only a moderate amount of pectin lyase enzyme (Table-3).

Effect of pH and temperature optimized for pectin lyase enzymes by potentialfungi

pH and temperature were optimized for pectin lyase enzyme production of *A.niger*

1.67 IU/ml of enzyme produced in pH 6, minimum amount (0.2 IU/ml) was observed in pH

In temperature optimization, 30°C was suitable for maximum enzyme production (1.78 IU/ml). But 20°C was not suitable for enzyme production, because enzyme production was very low (1.4 IU/ml) in this temperature.

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P.citrinum produced 0.76 IU/ml of enzyme in pH 6, which was the maximum and minimum amount (0.1 IU/ml) was recorded in pH 5. 1.0 IU/ml of enzyme was recorded at 35 ºC, and minimum (0.16 IU/ml) was recorded at 20ºC (Table-4; Figure-3 and 4).

Effect of incubation period on production of pectin lyase activity by potential fungi

Various incubation periods were tested for pectin lyase activity by *A.niger* and *P.citrinum.* 6.1 IU/ml pectin lyase activity was recorded after 9 days of incubation by *A.niger,* whereas 6.9 IU/ml was recorded after 9 days of incubation by *P.citrinum.* Minimum activity was recorded after 3 days of incubation *A.niger* (2.6 IU/ml) and *P.citrinum* (2.7 IU/ml). Maximum amount of protein was recorded after 9 days of incubation by both species. 10.3 IU/ml protein was recorded by *P. citrinum* and 8.5 IU/ml protein by *A.niger* after 9 days of incubation. After 3 days of incubation period, protein production was very low (Table-5).

Purification of Pectin lyase enzyme from *A.niger*

Partial purification of Pectin lyase enzyme was carried out by dialysis followed by ammonium sulphate precipitation method. The specific activity of the crude sample was found to be 49.0 IU/mg of dialyzed sample and ammonium sulphate precipitated sample was found to be 61.09 IU/mg and 78.0 IU/mg respectively(Table-6).

Purification of Pectin lyase enzyme from *P.citrinum*

Partial purification of Pectin lyase enzyme was carried out by dialysis followed by ammonium sulphate precipitation method. The specific activity of the crude sample was found to be 56.7 IU/mg of dialysed sample and ammonium sulphate precipitated sample was found to be 65.0 IU/mg and 42.8 IU/mg respectively (Table-7).

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Table 1: Isolation of fungi from different soil samples dead organic matter

S. No	Name of the fungi	Total no. of colonies		
		DOS	FS	VS
1	*Aspergillus awamori*	-	-	2
2	*A.candidus*	-	-	1
3	*A.clavatus*	-	-	2
4	*A.flavus*	-	-	2
5	*A.fumigatus*	-	4	-
6	*A.luchensis*	-	-	2
7	*A. niger*	-	-	1
8	*A.nidulans*	3	4	4
9	*A.sulphureus*	-	-	1
10	*A.sydowi*	1	-	-
11	*A.terreus*	1	1	-
12	*A.terricola*	1	3	-
13	*A.variecolor*	2	-	-
14	*A.versicolor*	2	1	-
15	*Bipolaris oryzae*	-	2	-
16	*Collectotrichum falcatum*	-	2	-

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17	*Chaetomium globosum*	-	2	-
18	*Curvularia lunata*	2	-	-
19	*Fusarium chlamydosporium*	3	-	-
20	*F.oxysporum*	3	-	-
21	*Penicillium chrysogenum*	4	2	-
22	*P.citrinum*	4	4	5
23	*Rhizoctonia solani*	2	-	-
24	*Trichoderma harzianum*	3	-	-
25	*T.viride*	2	-	-
	Total	33	25	20

DOS- Dead organic sample, FS-Fruit sample, VS- Vegetable sample, (-) Absent

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Table 2: Screening of pectin lyase enzyme from fungal isolates

S.No	Name of the fungi	Pectin lyase activity
1	*Aspergillus awamori*	++
2	*A. candidus*	++
3	*A. clavatus*	++
4	*A. flavus*	++
5	*A. fumigates*	++
6	*A. luchuensis*	++
7	*A. nidulans*	+
8	*A. niger*	+++
9	*A. sydowi*	+
10	*A. sulphureus*	+
11	*A. versicolor*	++
12	*A. terreus*	++
13	*A. terricola*	++
14	*A. variecolor*	+
15	*Bipolaris oryzae*	++
16	*Colletotrichum falcatum*	++
17	*Curvularia lunata*	++

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18	*Chaetomium globosum*	++
19	*Fusarium chlamydosporum*	++
20	*F.oxysporum*	++
21	*Penicillium citrinum*	+++
22	*P.chrysogenum*	++
23	*Rhizoctonia solani*	++
24	*Trichoderma harzianum*	++
25	*T.viride*	++

(+) low, (++) moderate, (+++) highly recommended

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Table 3: Quantification assay of pectin lyase enzyme isolated fromfungi

S.No	Name of the fungi	Activity (IU/ml)
1	*Aspergillus awamori*	1.7
2	*A. candidus*	0.9
3	*A. clavatus*	1.1
4	*A. flavus*	0.6
5	*A. fumigates*	0.7
6	*A. luchuensis*	0.5
7	*A. nidulans*	0.4
8	*A. niger*	5.2
9	*A. sydowi*	0.1
10	*A. sulphureus*	0.3
11	*A. versicolor*	1.1
12	*A. terreus*	0.8
13	*A. terricola*	1.3
14	*A. variecolor*	1.6
15	*Bipolaris oryzae*	1.2
16	*Colletotrichum falcatum*	1.0
17	*Curvularia lunata*	0.4

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18	*Chaetomium globosum*	0.6
19	*Fusarium chlamydosporum*	1.4
20	*F.oxysporum*	1.5
21	*Penicillium citrinum*	4.8
22	*P.chrysogenum*	1.4
23	*Rhizoctonia solani*	0.4
24	*Trichoderma harzianum*	0.6
25	*T.viride*	0.9

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Table 4: Effect of pH and temperature optimized for pectin lyase enzyme by potentialfungi

S.No	Parameters	Concentrations	*A.niger* (IU/ml)	*P.citrinum* (IU/ml)
1	pH	5	0.2	0.1
		6	1.67	0.76
		7	1.61	0.72
		8	1.16	0.13
		9	0.9	0.8
2	Temperature (^{0}C)	15	1.1	0.9
		20	1.5	0.16
		25	1.74	0.6
		30	1.78	0.74
		35	1.49	1.0

Table 5: Effect of incubation period on production of pectin lyase activity by potentialfungi

S.No	Name of the fungi	Incubation period (Days)	Pectin lyase activity (IU/ml)	Protein (IU/ml)
		3	2.6	3.9

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1	*A.niger*	6	3.7	5.1
		9	6.1	8.3
		12	5.1	6.2
2	*P.citrinum*	3	2.7	3.8
		6	3.5	5.6
		9	6.9	10.3
		12	5.4	7.3

Table 6: Partial purification of pectin lyase enzyme from *A.niger*

S.No	Purification level	Volume (ml)	Activity (IU/ml)	Specific activity (IU/mg)	Protein	Yield	Purification fold
1.	Crude enzyme extract	100	43.26	49.0	87.19	100	1
2.	Ammonium sulphate fractionation	74	42.05	61.09	38.37	97	2.2
3.	Dialysis	25	30	78.0	21.71	69	2.8

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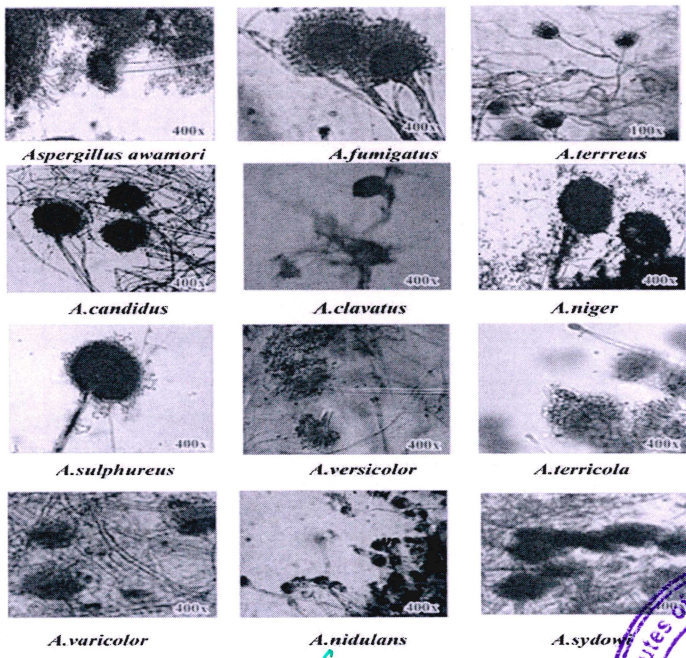
Table 7: Partial purification of pectin lyase enzyme from *P.citrinum*

S.No	Purification level	Volume(ml)	Activity (IU/ml)	Specific activity (IU/mg)	Protein	Yield	Purification fold
1.	Crude enzyme extract	100	34.8	56.7	76.8	100	1
2.	Ammonium sulphate fractionation	40	32.0	65.0	68.7	91	1.9
3.	Dialysis	25	20.0	42.8	22.5	57	1.9

Figure 1: Isolation of fungi from different soil samples



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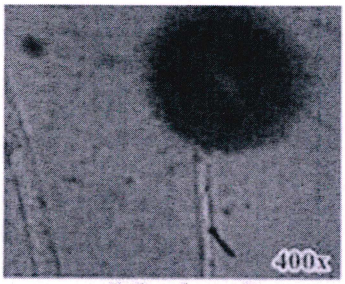
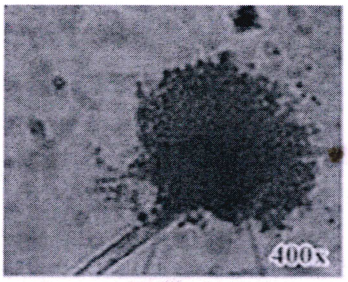
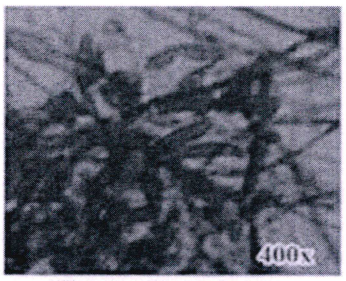


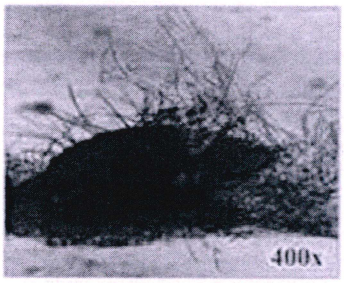
A.luchensis



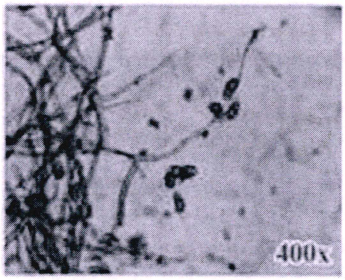
A.flavus



Bipolaris oryzae

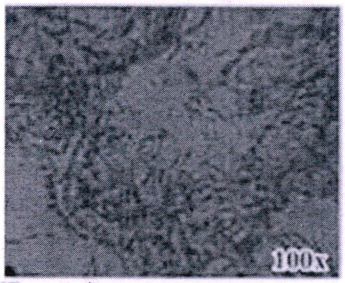


Chaetomium globosum

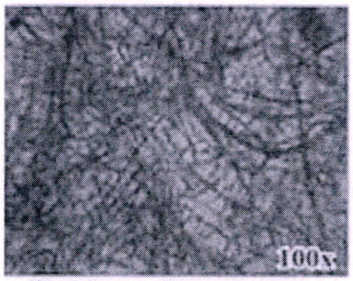


Curvularia lunata

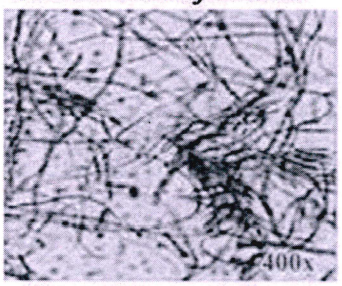
Colletotrichum falcatum



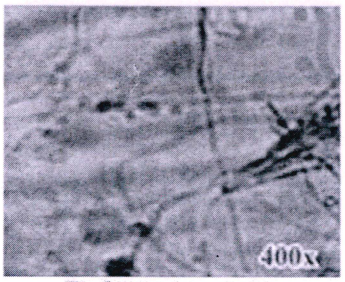
Fusarium oxysporum



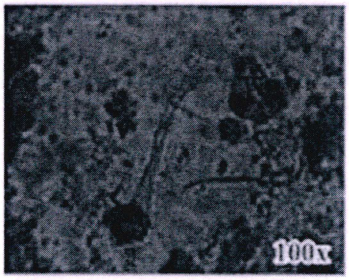
F.chlamydosporum



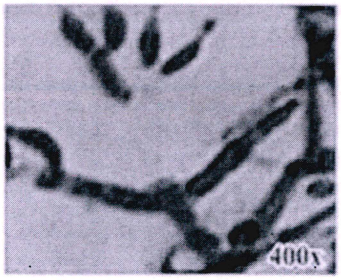
Penicillium citrinum



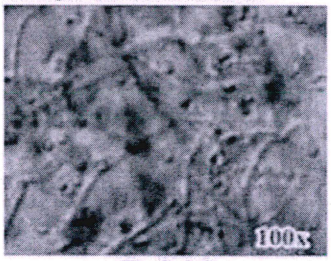
P.chrysogenum



Rhizoctonia solani



Trichoderma harzianum



T.viride

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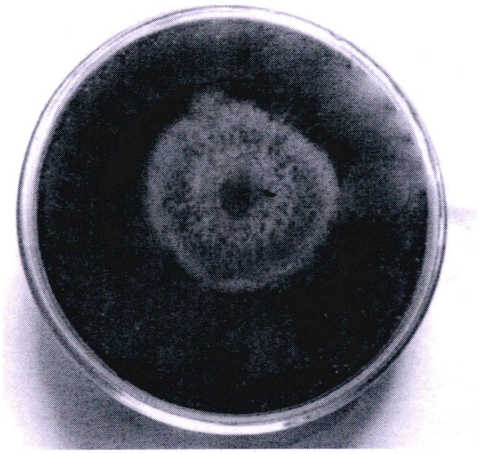
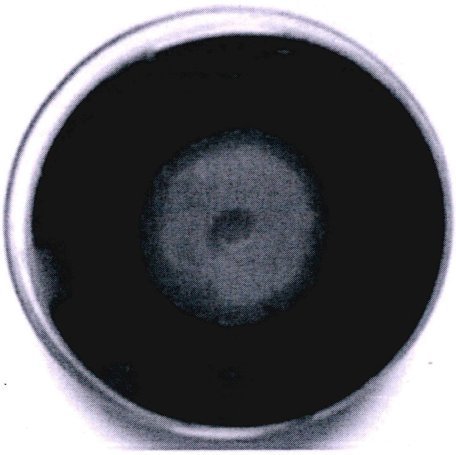
Figure 2: Screening of pectin lyase enzyme producing fungi

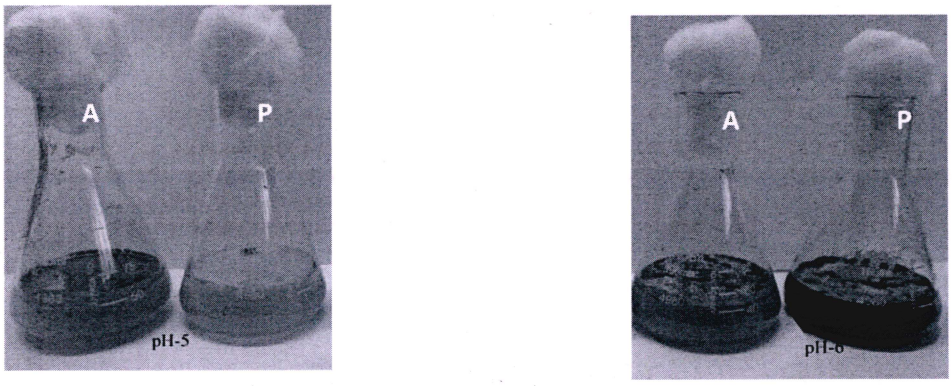


Aspergillus niger



Penicillium citrinum

Figure 3: Effect of pH on the growth of *A.niger* and *P.citrinum* after 7 days of incubation



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DISCUSSION

Fungal diversity

Pectinolytic enzyme can be produced in large amount by microorganisms. Pectinolytic enzymes have great biotechnological potential and can be employed in many industrial process. Among all pectinases (poly galacturonase, pectin esterase, pectate lyase and pectin lyase), only pectin lyase can degrade pectin -elimination mechanism without complementary actioβ of other enzymes .

In the present investigation totally 25 fungal species alone were isolated during the study period from three different samples such as dead organic, fruit and vegetable soil samples.

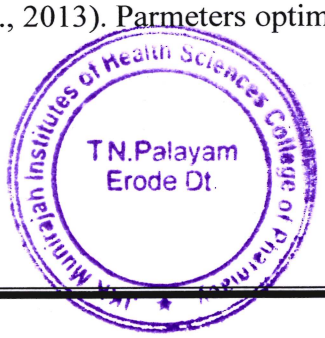
The fungal species were *Aspergillus awamori, A.candidus, A.clavatus, A.flavus, A.fumigatus, A.luchensis, A. alternate, A. niger, A.nidulans, A.sulphureus, A.sydowi, A.terreus, A.terricola, A.vericolor, A.versicolor, Bipolaris oryzae, Colletotrichum falcatum, Chaetomium globosum, Curvularia lunata, Fusarium chlamydosporium, F.oxysporum, Penicillium chrysogenum, P.citrinum, Rhizoctonia solani, Trichoderma harzianum* and *T.viride.* The fungal cultures were identified by standard manual and kept on PDA medium.

Screening and assay of pectin lyase enzymes by fungal species

The present data demonstrate that maximum enzyme activity was exhibited in *A.niger* (5.2 IU/ml) and *P.citrinum* (4.8 IU/ml) in both plate assay and quantification assay. The minimum enzyme activity expressed as *Curvularia lunata, Rhizoctonia solani; Aspergillus flavus*, *A.lunchensis* and *A.nidulans.*

Optimization studies

Pectin lyase production was increased by optimizing various fermentation parameters. Classical method was adopted for optimization of parameters by varying one parameter in an experiment and to incorporate it at a standardized level before, optimizing the next parameters (Sidra Batool *et al.*, 2013). Parmeters optimizing during the current study are as follows:



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pH

In the present investigation the enzyme activity was assayed over a pH range of 5.0 to 9.0 showed in the table 5. *A.niger* and *P.citrinum* produced maximum amount of pectin lyase enzyme in the pH 6. *A.niger* produced 1.67 IU/ml and *P.citrinum* produced 0.76 IU/ml enzyme.

Temperature

In the present investigation the enzyme activity was assayed at different temperature (15-35°C). *A.niger* produces maximum enzymes at 30±2°C temperature (1.78 IU/ml) and at 20±2°C enzyme production (1.4 IU/ml) was very low. Maximum enzyme production (1.0 IU/ml) by *P.citrinum* was recorded at 35±2°C, and minimum amount of enzyme (0.16 IU/ml) was recorded at 20±2°C.

Incubation time

This was agreed with present findings i.e., 6.1 IU/ml of pectin lyase activity wasrecorded after 9 days of incubation by *A.niger* and *P.citrinum* was 6.9 IU/ml maximum amount of protein was also recorded after 9 days of incubation.

Partial purification of pectin lyase enzymes

In the present study, partial purification of Pectin lyase enzyme from *A.niger* was carried out by dialysis followed by ammonium sulphate precipitation method.

The specific activity of the crude sample was found to be 49.0 IU/mg of dialysed sample and ammonium sulphate precipitated sample was found to be 61.09 IU/mg and 78.0 IU/mg respectively.

In the present study, partial purification of Pectin lyase enzyme from *P.citrinum* was also carried out by dialysis followed by ammonium sulphate precipitation method. The specific activity of the crude sample was found to be 56.7 IU/mg of dialysed sample and ammonium sulphate precipitated sample was found to be 65.0 IU/mg and 42.8 IU/mg respectively.

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CONCLUSION

The thesis entitled "Production of Pectin lyase by fungi isolated from agro waste" deals with isolation and identification of fungi from different agro waste soil samples. Production of pectin lyase by using liquid state fermentation, optimization studies like pH, temperature and incubation period and partial purification of pectin lyase by using Ammonium sulphate and Dialysis technique was performed.

Fungi were isolated from 3 different types of soil samples likewise dead organic samples, fruit sample and vegetable samples. 33 colonies were recorded in dead organic sample, 25 colonies were in fruit sample and 20 colonies in vegetable sample.

Totally 25 fungal species belonging to 10 genera were identified from 3 different types of soil samples. Maximum number of *Aspergillus* sp. was isolated during the study period. Totally 13 species of *Aspergillus* were recorded. All are deuteromycetes in which 14 species are belonging to 6 genera from dead organic sample, 9 species belonging to 5 genera from fruit sample and 9 species belonging to 3 genera from vegetable waste soil sample.

Out of 25 fungal species 24 had ability to produce pectinolytic enzymes. Deuteromycetes such as *Aspergillus awamori, A. candidus, A. clavatus, A. flavus, A.fumigatus, A. luchuensis, A.niger, A. versicolor, A. terreus, A. terricola, A.variecolor, A.sulphureus, A.nidulans, Bipolaris oryzae, Colletotrichum falcatum, Curvularia lunata, Chaetomium globosum, Fusarium chlamydosporum, F.oxysporum, Penicillium citrinum, P.chrysogenum,*

Rhizoctonia solani, Trichoderma harzianum and *T.viride*. Aspergillus awamori produced 1.7 IU/ml, A. candidus 0.9 IU/ml, A. clavatus 1.1 IU/ml, A. flavus 0.6 IU/ml, A. fumigatus 0.7 IU/ml, A. luchuensis 0.5 IU/ml, A. niger

5.2 IU/ml, A. versicolor 1.1 IU/ml, A. terricola1.1 3U/ml, *Bipolaris oryzae* 1.2 IU/ml, *Colletotrichum falcatum* 1.0 IU/ml, *Curvularia lunata* 0.4 IU/ml, *Chaetomium globosum* 0.6 IU/ml, *Fusarium chlamydosporum* 1.4 IU/ml, *F.oxysporum* 1.4 IU/ml, *Penicillium citrinum* 4.8 IU/ml, *P.chrysogenum* 1.4 IU/ml, *Rhizoctonia solani* 0.4 IU/ml, *Trichoderma harzianum* 0.6 IU/ml and *T.viride* 0.9 IU/ml of enzyme.

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Among that *A.niger* and *P.citrinum* are highly recommended for pectin lyase enzyme production. In enzyme assay maximum enzyme production was noticed in *A.niger* (5.2 IU/ml) and *P.citrinum* (4.8 IU/ml), minimum level in *A.sydowi* (0.1 IU/ml).

In the pH 5, *A.niger* produced 0.2 IU/ml, pH 6 - 1.67 IU/ml, pH 7 - 1.62 IU/ml, pH8 - 1.16 IU/ml and pH 9 produced 0.9 IU/ml pectin lyase.In the pH 5, *P.citrinum* produced IU/ml, pH 6 - 0.76 IU/ml, pH 7- 0.72 IU/ml, pH 8 - 0.13 IU/ml, and pH 9 produced 0.8 IU/ml pectin lyase.

A.niger produced 1.5 IU/ml pectin lyase at 20°C, it produced 1.74 IU/ml pectin lyase at 25°C, 1.78 IU/ml at 30°C and 1.49 IU/ml pectin lyase at 35°C.*P.citrinum* produced 0.16 IU/ml pectin lyase at 20°C, 0.6 IU/ml at 25°C, 0.74 IU/ml pectin lyase at 30°C and 1.0 IU/mlpectin lyase at 35°C.

Effects of incubation period on production of pectin lyase activity by

potential fungiwere screened. The obtained results revealed that *A.niger*

produced 3.9 IU/ml protein after3 days, 5.1 IU/ml protein after 6 days of

incubation, 8.3 IU/ml protein after 9 days and 6.2 IU/ml protein after 12 days of incubation. *P.citrinum* produced 3.8 IU/ml protein after 3 days of incubation, 5.6 IU/ml protein after 6 days, 10.1 IU/ml protein after 9 days and 7.3 IU/ml protein after 12 days of incubation.

Partial purification of Pectin lyase enzyme was carried out by dialysis followed by ammonium sulphate precipitation method. The specific activity of the crude sample was found to be 49.0 IU/mg of dialysed sample and ammonium sulphate precipitated sample wasfound to be 61.09 IU/mg and 78.0 IU/mg respectively in *A.niger*. The specific activity of the crude sample was found to be 56.7 IU/mg of dialyzed sample and ammonium sulphate precipitated sample was found to be 65.0 IU/mg and 42.8 IU/mg respectively in *P.citrinum*.

The overall investigation concluded that the *A.niger* and *P.citrinum* were found to utilize agricultural waste and byproducts for enzyme production and also *A.niger* potential fungus had the ability to produce high level of pectin lyase and protein with specified parameters and compared with other fungus *P.citrinum*. This study revealed that the potential of utilizing agricultural wastes provided cost effective and eco-friendly methodfor pectinlyase production on large scale.

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