Thesis title:

"Cloning, expression, purification, biochemical, functional and structure characterization of pectin methylesterase (*Ct*PME) from family 8 Carbohydrate Esterase (CE8) from *Clostridium thermocellum* ATCC 27405 and its applications in textile and food industry".

Abstract of the thesis:

Pectin is a hetero polysaccharide, present in middle lamella of plant cell wall. Pectin degrading enzymes are pectinases, pectin methylesterase (PME), polysaccharide lyases (PL) and polygalacturonase (PG). Pectinases are widely used in food, beverage, juice clarification and textile industry processing applications. PME hydrolyzes the ester decorations from pectin and releases pectate and methanol. Further, pectate can be degraded by PL and PG to produce pectic oligosaccharides. Pectin methylesterase (*Ct*PME) from *Clostridium thermocellum* ATCC 27405 was cloned, expressed and biochemically characterized. The modeled structure of *Ct*PME showed the right handed parallel β helices. Molecular Dynamic (MD) simulation confirmed the *Ct*PME stability. Small angle X-ray scattering analysis showed the globular shape of *Ct*PME similar to the modeled structure of *Ct*PME. *Ct*PME or *Ct*PL1B or mixture of both enzymes were explored for degumming of jute fiber and bioscouring of cotton fabric in textile industry. FESEM images revealed that the mixed enzymes treated jute fibers were having smooth surface against the rough surface of the control. Smooth surface of jute fibers facilitates their conversion into yarn. Enzyme treated cotton fabric showed enhanced water absorption capacity, which is highly desirable in textile industry. The enzymes treated jute fiber and cotton fabric gave higher Young's Modulus and ultimate tensile strength than the control. Enzymatic degumming and bioscouring are environment friendly alternatives to chemicals being utilized by textile industry in green process. Pectin was isolated from sweet orange peels by ultrasound-assisted method. Orange peels are waste and suitable source for extraction of pectin for its use in pharmaceutical industry. FESEM and AFM analysis of Extracted Orange Pectin (EOP) showed heterogeneous surface and wrinkled fiber net structure. The DLS analysis of EOP showed polydispersity. HPSEC analysis of EOP gave the molecular weights, 90 kDa and 1.3 kDa. DSC-TGA analyses of EOP showed thermal degradation at 225°C. XRD analysis showed semi-crystalline nature. FTIR and NMR analyses showed 68% esterification by galacturonic residues. EOP hydrolyzed by both pectinases (*Ct*PME or *Ct*PL1B) produced pectic oligosaccharides (POS) of mainly degree of polymerization (DP), DP2 and DP3. POS displayed anti-cancer activity.

Publications from thesis

1. **Rajulapati**, **V**., Sharma, K., Dhillon, A., & Goyal, A. (**2018**) SAXS and homology modelling based structure characterization of pectin methylesterase a family 8 carbohydrate esterase from *Clostridium thermocellum* ATCC 27405. **Archives of Biochemistry and Biophysics**, 641, 39-49.
2. **Rajulapati, V**., & Goyal, A. (**2017**) Molecular cloning, expression and characterization of pectin methylesterase (*Ct*PME) from *Clostridium thermocellum*. **Molecular Biotechnology**, 59(4-5), 128-140.
3. **Rajulapati**, V., Dhillon, A. & Goyal, A. Green process of degumming of jute fibers and bioscouring of cotton fabric by recombinant pectin methylesterase and pectate lyases from *Clostridium thermocellum.* **Manuscript under review**
4. **Rajulapati**, V., Dhillon, A. & Goyal, A. Extraction, characterization and anti-cancer activity of pectic oligosaccharides produced from agro-waste of Orange (*Citrus reticulate*). **Manuscript under preparation**

Other Publications

1. Kumar, A., Singh, S., **Rajulapati, V**., & Goyal, A. **(2020)**. Evaluation of pre-treatment methods for *Lantana camara* stem for enhanced enzymatic saccharification. **3 Biotech**, 10(2), 1-11. 112, 1104-1114.
2. Rani, A., **Rajulapati, V**., & Goyal, A. (**2019**). Antitumor effect of chondroitin AC lyase (PsPL8A) from *Pedobacter saltans* on melanoma and fibrosarcoma cell lines by *in vitro* analysis. **Pharmacological Reports**, 71(1), 167-174.
3. Dhillon, A., **Rajulapati, V**., & Goyal, A. (**2019**). Bio- scouring of cotton fabric and enzymatic degumming of jute fibres by a thermo- alkaline recombinant rhamnogalacturonan lyase, ctrglf from *Clostridium thermocellum*. **The Canadian Journal of Chemical Engineering**, 97(5), 1043-1047.
4. Sharma, K., Antunes, I. L., **Rajulapati, V**., & Goyal, A. (**2018**). Low-resolution SAXS and comparative modeling based structure analysis of endo-β-1, 4-xylanase a family 10-glycoside hydrolase from *Pseudopedobacter saltans* comb. nov. **International Journal of Biological Macromolecules**, 112, 1104-1114.
5. Sharma, K., Antunes, I. L., **Rajulapati, V.,** & Goyal, A. (**2018**). Molecular characterization of a first endo-acting β-1, 4-xylanase of family 10 glycoside hydrolase (PsGH10A) from *Pseudopedobacter saltans* comb. nov. **Process biochemistry**, 70, 79-89.
6. Dhillon, A., Sharma, K., **Rajulapati, V.** & Goyal, A. (**2018**). The multi-ligand binding first family 35 Carbohydrate Binding Module (CBM35) of Clostridium thermocellum targets rhamnogalacturonan I. **Archives of Biochemistry and Biophysics**, 654, 194-208.
7. Balasubramaniam, K., Sharma, K., Rani, A., **Rajulapati, V.,** & Goyal, A. (**2018**). Deciphering the mode of action, structural and biochemical analysis of heparinase II/III (*Ps*PL12a) a new member of family 12 polysaccharide lyase from *Pseudopedobacter saltans*. **Annals of Microbiology**, 68(6), 409-418.
8. Gupta, A., **Rajulapati**, V. Das, D. & Goyal, A. (2017). Comparative analysis of bioethanol production involving saccharification by mixed recombinant clostridial enzymes using sugarcane leaves and kans grass as sustainable feed stocks from north-east India. **Indian Journal of Biotechnology**, 16, 199-210.

Conferences/Symposia/Meetings

1. **Vikky Rajulapati** and Arun Goyal (**2018**) Extraction, characterization and anti-cancer activity of pectic oligosaccharides produced from agro-waste of Orange (*Citrus reticulate*). 29th International Carbohydrate Symposium - ICS 2018, July 13-19, 2018, Faculty of Sciences, University of Lisbon, Portugal.
2. Ajit Kumar, Shweta Singh, **Vikky Rajulapati**, Arun Goyal (**2018**) Optimization of pretreatment of *Lantana camara* stem as lignocellulosic biomass for bioethanol. Indo-Japan Bilateral Symposium on Future Perspective of Bioresource Utilization in North-Eastern Region, February 1- 4, 2018, IIT Guwahati.
3. **Vikky Rajulapati**, Arun Dillon and Arun Goyal (**2017**) Application of recombinant pectinolytic enzymes from *Clostridium thermocellum* in textile industry. Society of Biological Committee (SBC) conference organizing by JNU Delhi, November 21-23, 2017, New Delhi, India.
4. Kedar Sharma, **Vikky Rajulapati**, Inês Lobo Antunes and Arun Goyal (**2017**) SAXS analysis and structure modelling of endo β-1,4 xylanase (*Ps*GH10A) from *Pedobacter saltans*. 86th Annual Meeting of Society for Biological Chemists, India, Nov. 16-19, Jawaharlal Nehru University, New Delhi, India.
5. **Vikky Rajulapati** and Arun Goyal (**2017**) A new family member of Carbohydrate Esterase 8, pectin methyl esterase (*Ct*PME) from *Clostridium thermocellum* and its food applications. 12th Carbohydrate Bioengineering Meeting, April 23 - 26, 2017, Audi Max, Augasse, Vienna, Austria.

6. **Vikky Rajulapati** and Arun Goyal (**2016**) Biochemical characterisation of recombinant a pectin methyl esterase (*Ct*PME), a family 8 Carbohydrate Esterase (CE8) from *Clostridium thermocellum*. International Conference on Current Trends in Biotechnology (BRSI-ICCB), December 8 - 10, 2016, VIT University, Vellore, Tamil Nadu, India.
7. **Vikky Rajulapati**, Arun Dillon and Arun Goyal (**2016**) Ultrasound assisted extraction of pectin polysaccharide from the waste fruit peels of *Citrus preticulate*, Malus domestica and Ananas comosus. 57th International Annual Conference of Association of Microbiologists of India (AMI), November 24-26, 2016, Gauhati University and the Institute of Advanced Study in Science and Technology (IASST), Assam, India.
8. Inês Lobo Antunes, **Vikky Rajulapati**, Kedar Sharma, Arun Goyal **(2015)** Cloning, expression and characeterization of a xylanase from family 10 glycoside hydrolase (GH10) from *Pedobacter Saltans* DSM12145. 56th International Annual Conference of Association of Microbiologists of India (AMI), December 7-10, 2015, Jawaher Lal Nehru University, New Delhi.
9. **Vikky Rajulapati** and Arun Goyal **(2015)** Cloning, expression and purification of recombinant pectin methyl esterase (*Ct*PME) a family 8 Carbohydrate Esterase (CE8) from *Clostridium thermocellum*.14th FAOBMB Congress 84th Annual Meeting of SBC (I) on Current Excitements in Biochemistry & Molecular Biology for Agriculture and Medicine, 24 - 30 November 2015, Hyderabad, India.
10. **Vikky Rajulapati**, Vania Fernandes, Arabinda Ghosh, Carlos M.G.A. Fontes and Arun Goyal **(2014)** Cloning and expression of novel thermostable multi-substrate specific family 81 glycoside hydrolase (GH81) from *Clostridium thermocellum* ATCC 27405. 55th Annual International Conference of AMI and National Conference on Empowering Mankind with Microbial Technologies (AMI-EMMT-2014), November 12-14, 2014, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India.

Awards

1. Received the **International travel grant** by Department of Science and Technology **(SERB–DST)** to attend the **29th International Carbohydrate Symposium - ICS 2018,** July 13-19, 2018, Faculty of Sciences, University of Lisbon, Portugal.
2. Recipient of the partial STAF fund by Indian Institute of Technology Guwahati to attend the **29th International Carbohydrate Symposium - ICS 2018,** July 13-19, 2018, Faculty of Sciences, University of Lisbon, Portugal.
3. Received the **travel grant** by National Seminar Crystallography, to attend the **45th National Seminar Crystallography - NSC 2017,** July 9-12, 2017, School of material sciences and Technology, Indian Institute of Technology, Banaras Hindu University, Varanasi, India.

Workshops Attended

1. **Vikky Rajulapati** attended workshop on **Flow Cytometer and Real time PCR.** Jointly organized by BioRad Pvt Ltd, 30th October - 4th November 2017, at Indian Institute of Technology Guwahati, Assam.
2. **Vikky Rajulapati** attended workshop on **Advanced microscopy and Imaging Techniques.** Jointly organized by DSS Image tech Pvt Ltd and Olympus medical systems India Pvt Ltd, 18th - 20th April 2017, Indian Institute of Technology Guwahati, Assam.
3. **Vikky Rajulapati** attended Indo-Japan workshop on **Translational Agriculture- Avenues for International Cooperation.** Jointly organized by DBT India and Gifu University, Japan, 29th March 2017, Indian Institute of Technology Guwahati, Assam.