Structural characterization of xylanolytic enzymes and their application in the production of anti-cancer xylooligosaccharides

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Professor Arun Goyal

Submitted by

Kedar Sharma

Roll No: 146106031

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INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI

GUWAHATI – 781039, ASSAM, INDIA

SYNOPSIS

Introduction

The sustainable growth of any country mainly relies on three pillars, *i.e.*, development at the social, economic and environmental level. The socio-economic development of any country, whether it is developing or developed depends heavily upon the utilization of non-renewable resources or fossil resources (Linear fossil-based economy). These resources play a potential role in making life easy at various levels ranging from home appliances, in transportation, in various industrial processes for the fulfillment of our daily needs. Due to the limited availability with unsustainable use of these non-renewable resources, the interest in the steady shift from a linear fossil-based economy to circular bio-based economy or bioeconomy is increasing day by day. The conversion of renewable resources into value-added products such as food, animal feed, bio-based other value-added products and bioenergy production through a biotechnological intervention is termed as bioeconomy. Agricultural practices are the main component of bioeconomy. The need for food and energy supply increasing with the rise in population and it implies a tremendous pressure on agriculture farming and its further processing industries. The processing of these crops is producing about $1.3x10^{10}$ metric tons per annum of lignocellulosic waste worldwide and that can be used for the production of bio-based value-added products and bioenergy.

The organic material derived from plants or agricultural waste is termed as lignocellulosic biomass. Lignocellulosic biomass contains cellulose, lignin and hemicellulose as major components (De Wild, 2011). The major portion of plant polysaccharides is composed of cellulose, while the second most abundant plant polysaccharide is hemicelluloses. Hemicellulose constitutes 15-35% of total lignocellulosic biomass (Limayem & Ricke, 2012). Hemicelluloses are made up of monomeric sugars such as pentose (xylose, arabinose), hexoses (mannose and

glucose), acetylated sugars and uronic acids (Davison, 2013). The structure and composition of heteroxylan depend on the plant and its tissues and method used for the extraction (Petzold-Welcke et al., 2014). The backbone of heteroxylan is composed of β-(1→4)-linked xylose residues, which is often substituted with methylated α-D-glucopyranosyl uronic acid and α-L-arabinofuranosyl, ferulic acid, *p*-coumaric acid in the side chain (Ordaz-Ortiz and Saulnier, 2005). The complex xylan structure requires the synergistic action of an array of enzymes such as endo-β-xylanase, α-glucuronidase, β-xylosidase, α-arabinofuranosidase, *p*-coumaric acid esterase, acetyl xylan esterase and ferulic acid esterase for its complete hydrolysis (Sharma et al., 2018). Among all these enzymes, the endo-β-xylanase randomly (EC.3.2.1.8) hydrolyzes the xylan and produces xylooligosaccharides, which can be used as a food supplement for the improvement of probiotic bacterial growth (Goluguri et al., 2016). Currently, endo-1,4-β-xylanase are found in 16 GH families *viz.* 3, 5, 8, 9, 10, 11, 12, 16, 26, 30, 43, 44, 51, 62, 98 and 141 as single catalytic domain or as modular enzymes. β-xylosidase releases xylose residues from non-reducing ends of xylooligosaccharides. β-xylosidase are classified in family 1, 2, 3, 5, 10, 30, 39, 43, 51, 52, 54, 116 and 120 glycoside hydrolases. α-L-arabinofuranosidases catalyze the removal of α-L-arabinofuranosyl residue from the side chain of arabinoxylan. Currently, α-L-arabinofuranosidases are reported from several GH families *viz.* 2, 3, 5, 10, 43, 51, 54 and 62. However, a complete degradation requires synergistic action of acetyl esterase to remove acetyl substituents from β-1,4-linked D-xylose backbone of xylan (Wong and Saddler 1993; Coughlan and Hazlewood 1993).

Family 43 glycoside hydrolases

The family 43 glycoside hydrolase (GH43) contains mainly hemicellulolytic enzymes. As of today (September 2019), the family GH43 enzymes deposited in the CAZy database includes 19 archaeal origins, 13229 bacterial and 481 from eukaryotes, 5 from viruses and 28 are

unclassified. These family 43 glycoside hydrolases are further subclassified into 37 subfamilies (http://www.cazy.org/GH43.html). Till date, 13 arabinofuranosidase from various organisms reported GH43_16 subfamily had been biochemically characterized. Out of these, the crystal structure of only 1 modular arabinofuranosidase (*Bs*AXH-m2,3) from *Bacillus subtilis* containing GH43 domain as a catalytic module and family 6 carbohydrate-binding module (CBM6) as a non-catalytic module (PDB Id: 3C7E) have been reported and available in PDB (Vandermarliere *et al*., 2009).

Family 30 glycoside hydrolase

The family 30 Glycoside hydrolases (GH30) a variety of activities which include endo-β-1,4-xylanase (EC 3.2.1.8); β-xylosidase (EC 3.2.1.37) and glucuronoarabinoxylan endo-β-1,4-xylanase (EC 3.2.1.136). Till date (September 2019), family GH30 entries in CAZy database include 2376 bacterial and 105 protein entries from eukaryotes, while only 3 entries are as unclassified. These family 30 glycoside hydrolases are further subclassified into 9 subfamilies (http://www.cazy.org/GH30.html). Under GH30_8 subfamily 596 entries are listed and out of these the crystal structure of 6 proteins are already determined, but the role of linker peptide present within the protein is not determined so far.

1.3.3 Family 10 glycoside hydrolase

The family 10 glycoside hydrolase (GH10) mainly endo-1,4-β-xylanase (EC 3.2.1.8). The family GH10 contains a total of 3851 protein sequences in the CAZy database as of September 2019 (http://www.cazy.org/GH10.html). Out of 3851 sequences, 19 belong to archaea, 3015 belong to bacteria, 461 belong to eukaryote and 356 belongs to unclassified sequences. As of now (September 2019) 360 xylanases are characterized and out of 360 xylanases, only a few mesophilic bacterial xylanases belonging to family 10 glycoside hydrolase have been identified and

characterized [Fontes et al., 2000, Gallardo et al., 2010; Sharma et al., 2018). Out of these 360 characterized xylanases, the crystal structure of 45 xylanases is available in the PDB database. Majority of the crystal structures solved till date are of thermophilic xylanases, while the solution shape analysis of xylanases by Small Angle X-ray Scattering (SAXS) analysis is not reported till date.

These xylanolytic enzymes exist in nature either in a modular organization or as a single catalytic module. As, several questions remain unanswered about the conformational dynamics, molecular arrangement and protein stability of the modular and non-modular enzymes in solution. Therefore, the present study deals with the structural organization, dynamics and function characterization of modular and non-modular xylanolytic enzymes from *Clostridium thermocellum and Pseudopedobacter saltans.*

Present work

The present work entitled as "Structural characterization of xylanolytic enzymes and their application in the production of anti-cancer xylooligosaccharides" has been divided into 6 chapters.

Chapter 1 is a General Introduction, which mainly focuses on the brief review of literature dedicated to the bioeconomy, the lignocellulosic waste and the structural component of the plant cell wall with elaborated description of heteroxylan. This chapter describes different types of carbohydrate-active enzymes and their sequence-based classification. It includes a review of literature on families 43, 30 and 10 of glycoside hydrolases and their potential applications followed by significance of the work. The description of *Clostridium thermocellum* and *Pseudopedobacter saltans* has also been included in this chapter.

Chapter 2 describes the crystallization and the structure determination of α-L-arabinofuranosidase (EC 3.2.1.55) of family 43 glycoside hydrolases (*Ct*Abf43A) from *C. thermocellum.* The crystal structure of *CtAbf43A* comprises a five-bladed β-propeller fold typical of GH43 enzymes. *CtAbf43A* displays a highly compact architecture compatible with its high thermostability. The structural comparison of GH43_16 subfamily and GH43_29 subfamily enzymes reveals the presence of a highly conserved substrate-binding cleft, that reflects the topology of arabinoxylan. GH43_16 is thus unable to bind the arabinan backbone that presents a curved shape. This work presents novel information that contributes to understanding the molecular determinants of substrate binding specificity within one of the most biotechnological relevant CAZy families, GH43.

Chapter 3 mainly focuses on the analysis of conformational dynamics and modular arrangement of *Ct*Araf43 in solution. Enzymes participating in the hydrolysis of complex carbohydrates display a modular architecture. The significance of enzyme modularity in terms of flexibility and catalytic efficiency is not fully understood. α-L-arabinofuranosidase from *Clostridium thermocellum* (*Ct*Araf43) catalyzes the release of α(1→2)-, α(1→3)-, or α(1→5)-linked L-arabinose from arabinose decorated polysaccharides. In this study, the conformational dynamics and modular arrangement of *Ct*Araf43 in solution were described. The homology modeled structure of a family 43 modular α-L-arabinofuranosidase of *Clostridium thermocellum* revealed three distinct domains that are independently folded and achieve their stable conformation. The modeled structure of the full-length *Ct*Araf43 revealed that its N-terminal catalytic *Ct*Abf43A module displays a 5-fold β-propeller fold and the two CBMs display the typical jellyroll type β-sandwich folds. Ramachandran plot showed 98.5% residues in the favored region and only 1.5% residues in the disallowed region. Conformational dynamics analysis of

*Ct*Araf43 by MD simulation displayed its flexible nature and no change in secondary structure was observed. SAXS analysis of *Ct*Araf43 revealed its monodisperse nature at two concentrations, 1.2 mg/mL and 4.7 mg/mL. The SAXS analysis of *Ct*Araf43 at both concentrations suggested elongated structures with monomeric and dimeric conformations, respectively. Kratky plot revealed that *Ct*Araf43 retains a flexible state at both concentrations, which corroborated with MD simulation results. The pyDockSAXS displayed that the catalytic domain of two *Ct*Araf43 molecules interacted together and resulted in the dimerization of *Ct*Araf43. The *ab initio* derived molecular of *Ct*Araf43 showed the molecular envelope of Puppy shaped at 1.2 mg/mL and Genie lamp shape at 4.7 mg/mL, respectively, which superposed well with the MD simulated monomeric structure and dimeric structures of *Ct*Araf43 obtained from the pyDockSAXS analysis.

Chapter 4 describes the conformational flexibility, molecular organization and protein stability of *Ct*XynGH30 in solution. The secondary structure analysis of *Ct*XynGH30 by CD displayed the presence of 28.25% α helices and 40.5% β sheets. The melting analysis showed that the Ca^{2+} ions provide extra thermal stability to the *Ct*XynGH30 structure. The ITC and mass spectrometric analyses of individual modules, *Ct*Xyn30A and *Ct*CBM6, showed that even though there is no binding interaction between them, the two modules are required to be linked together for optimal function. The mixture of *Ct*Xyn30A and *Ct*CBM6 displayed lower xylanase activity as compared to the full-length *Ct*XynGH30 protein. The lower activity of *Ct*Xyn30A alone and the mixture (*Ct*Xyn30A and *Ct*CBM6) may be due to the absence of linker region, which may be functioning as a sliding hinge between the two modules, thereby facilitating the binding to the substrate and bringing about enhanced catalytic activity of the full-length enzyme. SAXS analysis of *Ct*XynGH30 revealed a boot-shaped, fully folded structure in solution. The SREFLEX refined model of *Ct*XynGH30 superposed well with the *ab initio* modeled structure. The optimal length

and composition of linker peptide is crucial for the catalytic activity. These results confirmed that the two domains are required to be attached via a linker peptide, but with enough flexibility to maintain a specific spatial organization to achieve optimal biological function.

Chapter 5 gave insights about the structural and functional characterization of endo β-1,4 xylanase (*Ps*GH10A) of a family 10, glycoside hydrolase from *Pseudopedobacter saltans*. 3D structure of *Ps*GH10A modeled by comparative modeling was compact and stable. The secondary structure by CD analysis of *Ps*GH10A displayed the presence of α-helices (31.75%), β-strands (20.00%) and random coil (48.25%). The *Ps*GH10A modeled structure showed $(\beta/\alpha)_8$ TIM barrel fold which is conserved in GH10 family. The docking studies revealed that the active site of *Ps*GH10A could accommodate linear xylooligosaccharides and arabinose substituted xylooligosaccharides. The residues, Glu153 and Glu263 are involved in catalysis. *Ps*GH10A melting analysis confirmed that the enzyme does not require any metal ion for its stability. SAXS analysis displayed the monomeric nature of *Ps*GH10A in solution form. Guinier analysis gave the radius of gyration between (Rg) 2.23±0.15 and 2.29±0.16 nm for all the protein concentrations. Kratky plot analysis of *Ps*GH10A displayed fully folded state in solution form. The *ab initio* derived DAM model of *Ps*GH10A superposed well with its comparative modeling based 3D-structure.

Chapter 6 describes the extraction of xylan, characterization and effect of xylooligosaccharide on colon cancer cells. Glucuronoxylan was isolated from neem sawdust. Carbohydrate composition and structural characterization analysis of extracted xylan displayed that the main chain is composed of xylose backbone, which is substituted with a 4-O-methyl glucuronic acid side chain. The hydrolysis of extracted xylan from neem sawdust by endo-β-1,4-xylanase (*Ps*GH10A) resulted in the release of xylooligosaccharides ranging from the degree of polymerization (DP) 2-7. *In vitro* cell cytotoxicity analysis of xylooligosaccharide on mouse fibroblast (L929) cells

displayed its biocompatible behavior. The HT-29 cells treated with xylooligosaccharides displayed the inhibition of cell growth. The mitochondrial membrane potential analysis of HT29 cells displayed reduction in the red/green fluorescence intensity confirming the disruption of mitochondrial membrane. FLICA, immunoblotting and nuclear fragmentation analysis revealed the caspase-3 mediated apoptotic killing mechanism of HT-29 cells. These results further contribute to the knowledge about xylooligosaccharides produced from extracted xylan gave better insights about their utilization in cancer therapy for the treatment of colon cancer.

List of publications

Published/accepted (*From Thesis*):

1. **Kedar Sharma**, Carlos M.G.A. Fontes, Shabir Najmudin and Arun Goyal **(2019).** Molecular organization and protein stability of the *Clostridium thermocellum* glucuronoxylan endo-β-1,4-xylanase of family 30 glycoside hydrolase in solution. *Journal of Structural Biology* 206**, 335-344.** (**JIF 3.75**)
2. **Kedar Sharma**, Inês Lobo Antunes, Vikky Rajulapati and Arun Goyal **(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 (JIF 4.78).**
3. Arun Goyal, Shadab Ahmed, **Kedar Sharma**, Vikas Gupta, Pedro Bule, Carlos M.G.A. Fontes and Shabir Najmudin **(2016).** Molecular determinants of substrate specificity revealed by the structure *Clostridium thermocellum* family 43_16 Arabinofuranosidase. ***Acta Crystallographica* D72, 1281-1289. (JIF 2.5)**

Submitted/to be submitted

4. **Kedar Sharma**, Carlos M.G.A. Fontes, Shabir Najmudin and Arun Goyal* **(2019)**. SAXS based structure, modelling and molecular dynamics analyses of family 43 glycoside hydrolase α-L-arabinofuranosidase (*Ct*Araf43) from *Clostridium thermocellum* **(Submitted)**
5. **Kedar Sharma**, Sudhir Morla, Kaustubh Chandrakant Khaire, Abhijeet Thakur, Vijay Suryakant Moholkar, Sachin Kumar and Arun Goyal **(2019)**. Extraction, characterization of xylan from neem sawdust and its application in xylanase mediated production of anticancer xylooligosaccharides. **(Submitted)**

Other Publications:

1. **Kedar Sharma**, Abhijeet Thakur, Rajeev Kumar and Arun Goyal (**2019**). Structure and biochemical characterization of glucose tolerant β-1,4 glucosidase (*Ht*Bgl) of family 1 glycoside hydrolase from *Hungateiclostridium thermocellum*. ***Carbohydrate Research*** **(Accepted) (JIF 1.87).**
2. **Kedar Sharma**, Vikky Rajulapati and Arun Goyal **(2019).** Green synthesis of arabinoxyloglucan coated antimicrobial copper nanoparticles eTCR (Trends in Carbohydrate Research), 11(1), 22-30 **(JIF 0.5)**
3. Karthika B[†]., **Kedar Sharma**[†] and Arun Goyal **(2019)** Structure and dynamics analysis of a new member heparinase II/III of family 12 polysaccharide lyase from *Pseudopedobacter saltans* by computational modelling and small angle x-ray scattering. *Journal of Biomolecular Structure and Dynamics* **Accepted** (**JIF 3.31)** [†]**Equal Contribution**
4. Priyanka Nath, Arun Dhillon, Krishan Kumar, **Kedar Sharma**, Sumitha Banu Jamaldheen, Vijay Suryakant Moholkar and Arun Goyal **(2019).** Development of bi-functional chimeric enzyme (CtGH1-L1-CtGH5-F194A) from endoglucanase (*Ct*GH5) mutant F194A and β-1,4-glucosidase (*Ct*GH1) from *Clostridium thermocellum* with enhanced activity and structural integrity. *Bioresource Technology*. **282, 494-501. (JIF 6.66)**
5. **Kedar Sharma**, Inês Lobo Antunes, Vikky Rajulapati and Arun Goyal **(2018)**. Molecular characterization of a first endo-acting β-1,4-xylanase of family 10 glycoside hydrolase (*Ps*GH10A) from *Pseudopedobacter saltans* comb. nov. *Process Biochemistry*. **70, 79-89. (JIF 2.88)**
6. **Kedar Sharma,** Arun Dhillon and Arun Goyal **(2018)**. Insight into Structure and reaction mechanism of β-mannanases. *Current Protein and Peptide Sciences* **19, 34-47. (JIF 1.88)**.
7. Arun Dhillon, **Kedar Sharma**, Vikky Rajulapati and Arun Goyal **(2018)**. The multi-ligand specific family 35 Carbohydrate-binding Module (CBM35) from *Clostridium thermocellum* targets rhamnogalacturonan I and also mediates binding through a second site. *Archives of Biochemistry and Biophysics*. **654, 194-208. (JIF 3.55)**
8. Sumitha Banu Jamaldheen, **Kedar Sharma**, Vijay S. Moholkar and Arun Goyal **(2018).** Comparative analysis of pretreatment methods on Sorghum (Sorghum durra) stalk agrowaste for holocellulose content. *Preparative Biochemistry and Biotechnology*. (In Press). **(JIF 1.11).**
9. Vikky Rajulapati, **Kedar Sharma**, Arun Dhillon and Arun Goyal **(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 (JIF 3.55)**.
10. Karthika B., **Kedar Sharma**, Aruna Rani, R. Vikky and Arun Goyal **(2018).** Deciphering the mode of action, structural and biochemical analysis of recombinant heparinase II/III (*Ps*PL12a) a new member of family 12 polysaccharide lyase from *Pseudopedobacter saltans*. *Annals of Microbiology*. **68(6), 409-418 (JIF 1.43).**
11. Aruna Rani, Arun Dhillon, **Kedar Sharma** and Arun Goyal **(2018)**. Insights into the structure and substrate binding analysis of chondroitin AC lyase (*Ps*PL8A) from *Pedobacter saltans*. *International Journal of Biological Macromolecules*. **109, 980-991 (JIF 4.78)**.

12. S.M. Khade, S.K. Srivastava, Krishan Kumar, **Kedar Sharma**, Arun Goyal and A.D. Tripathi **(2018).** Optimization of clinical uricase production by *Bacillus cereus* under submerged fermentation, its purification and structure characterization. *Process Biochemistry,* **75, 49-58**. **(JIF 2.88)**

13. Soumyadeep Chakraborty, **Kedar Sharma**, Joyeeta Mukherjee, Munishwar N. Gupta and Arun Goyal **(2015).** Structure, substrate binding analysis and stability studies of endo-pectate lyase (PL1B) of family 1 polysaccharide lyase from *Clostridium thermocellum*. ***Protein and Peptide Letters***. **22(6)**, **557-568. (JIF 1.1)**

14. Barnali Nath[†], **Kedar Sharma**[†], Komal Ahire, Arun Goyal and Sachin Kumar **(2019).** Structure analysis of the nucleoprotein of Newcastle disease virus: An insight towards it multimeric form. **(Submitted)** [†]**Equal Contribution**

15. Dishant Goyal, Krishan Kumar, **Kedar Sharma** and Arun Goyal **(2019)**. SAXS based structure, modeling and molecular dynamics analyses of a family 5 glycoside hydrolase first endo-mannanase (*Rf*GH5_7) from *Ruminococcus flavefaciens* FD-1 v3 **(Submitted)**

16. Priyanka Nath, **Kedar Sharma** and Arun Goyal **(2019)**. Combined SAXS and computational approaches for structure determination and binding characteristics of chimera (*Ct*GH-L1-*Ct*GH5-F194A) generated by assembling β-glucosidase (*Ct*GH1) and a mutant endoglucanase (*Ct*GH5-F194A) from *Clostridium thermocellum* **(Submitted)**

Book Chapters

1. Abhijeet Thakur, **Kedar Sharma**, Ruchi Mutreja, Vikky Rajulapati and Arun Goyal **(2019)** Thermostable enzymes from *Clostridium thermocellum*. Ed. Sonali Mohapatra, Microbial Fermentation and Enzyme Technology, CRC Press. (***submitted after revision***)
2. Abhijeet Thakur, **Kedar Sharma**, Kaustubh Khaire and Arun Goyal **(2018)** Enzymes: Key role in conversion of waste to biofuel. Ed Sonali Mohapatra, Microbial Fermentation and Enzyme Technology, CRC Press. (***submitted after revision***)
3. **Kedar Sharma**, Abhijeet Thakur and Arun Goyal **(2018)**, **Xylanases for food applications** "**Green Bio-Processes: Industrial Enzymes for Food Applications**" (Accepted).
4. Abhijeet Thakur, **Kedar Sharma** and Arun Goyal **(2018)**, ***α*-L-arabinofuranosidase: A potential enzyme for the food industry** "**Green Bio-Processes: Industrial Enzymes for Food Applications**" (***Accepted***).
5. Arun Dhillon, **Kedar Sharma**, Vikky Rajulapati and Arun Goyal **(2015)** Chapter 7, Proteolytic enzymes in "Current Developments in Biotechnology & Bioengineering", Volume 7: Production, Isolation and Purification of Industrial Products, Eds. Ashok Pandey, Sangeeta Negi, Poonam Nigam, Carlos Ricardo Soccol. http://dx.doi.org/10.1016/B978-0-444-63662-1.00007-5.

Awards

1. Recipient of Best Poster award in Research Conclave 2019 organized by Indian Institute of Technology Guwahati.
2. Recipient of IUCr Early Career Travel Support award to present a poster in AsCA'16 meeting at Hanoi, Vietnam.
3. Visiting Research Scholar in Department of Animal Production, Faculty of Veterinary Medicine, University of Lisbon, Portugal from May 2016-July 2016

List of conference/symposia attended

1. **Kedar Sharma**, Carlos M.G.A. Fontes, Shabir Najmudin and Arun Goyal (2019). Molecular organization and protein stability of the *Clostridium thermocellum* glucuronoxylan endo-β-1,4-xylanase of family 30 glycoside hydrolase in solution. Research Conclave 2019, *March 14-17, 2019*, **IIT Guwahati, India** (Best Poster Award)
2. **Kedar Sharma**, Abhijeet Thakur, Rajeev Kumar and Arun Goyal (2018). Structure and biochemical characterization of glucose tolerant β-1,4 glucosidase (HtBgl) of family 1 glycoside hydrolase from *Hungateiclostridium thermocellum*. Bioprocessing India 2018, Dec 16-18, 2018, IIT Delhi, India.
3. **Kedar Sharma** and Arun Goyal (2018) Structure characterization of endo-β-1,4 xylanase from *Pseudopedobacter saltans* by SAXS and Molecular Dynamics simulation. 59th Annual Conference of AMI, Dec 9-12, 2018, University of Hyderabad, India.
4. **Kedar Sharma**, Abhijeet Thakur, Kaustubh Khaire and Arun Goyal (2018) Molecular characterization of halo and organic solvent stable xylanase from *Pseudopedobacter saltans* and its application in xylooligosaccharides production from Kans grass biomass. International Conference on Biotechnological Research and Innovation for Sustainable Development, 15th BRSI convention. CSIR- Indian Institute of Chemical Technology (CSIR-IICT), Nov. 22-25, 2018, Hyderabad, India.
5. **Kedar Sharma** and Arun Goyal (2018) Green synthesis of copper nanoparticles using arabinoxyloglucan isolated from water hyacinth as stabilizing agent for antimicrobial applications. International Conference on Drug Discovery: Biotechnology and Pharma at Cross Roads, 15-17th Feb 2018 Thapar University, Patiala, INDIA
6. **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.
7. Arun Dhillon, **Kedar Sharma**, Vikky Rajulapati and Arun Goyal (2017) Rgl-CBM35 of family 35 Carbohydrate Binding Module (CBM) from *Clostridium thermocellum* represents first CBM targeting rhamnogalacturonan I and mediating binding by two sites. 23rd INPEC (International Network of Protein Engineering Centers) Meeting Protein Structure, function and Engineering, 9-11 Nov 2017, Bose Institute, Kolkata. (**Best Poster Award**)
8. **Kedar Sharma** and Arun Goyal (2017) Biochemical characterization and deciphering the mode of action of recombinant endo β-1,4 xylanase (*Ps*GH10) from *Pedobacter saltans* DSM12145. 14th BRSI Convention and International Conference (BRSI-2017), Oct 08-10, 2017, CSIR-NEERI, Nagpur.

9. **Kedar Sharma**, Shadab Ahmed, Carlos M.G.A. Fontes, Shabir Najmudin and Arun Goyal (2017) Low-resolution structure analysis of α-L-arabinofuranosidase (*Ct*GH43) by SAXS. 24th Congress & General Assembly of the International Union of Crystallography 2017 (IUCr 2017) August 21-28, Hyderabad, India.
10. Vikky Rajulapati, **Kedar Sharma**, Arun Dillon and Arun Goyal* (2017) Structural characterization of a recombinant pectin methylesterase (CtPME) of family 8 carbohydrate esterase (CE8) from *Clostridium thermocellum*. 45th National Seminar on Crystallography (NSC 45) July 9-12, 2017, IIT (BHU), Varanasi, India.
11. Sumitha Banu Jamaldheen, **Kedar Sharma**, Aruna Rani, Vijayanand S. Moholkar and Arun Goyal (2017) Evaluation of pretreatment methods and recombinant enzyme hydrolysis of sorghum stalk for bioethanol production. Bioenergy-Urja Utsav by Ministry of Petroleum and Natural Gas 2017, July 7-8, Pune, India.
12. Karthika B., Aruna Rani, **Kedar Sharma** and Arun Goyal (2017) Structural and biochemical characterization of recombinant Heparinase II/III of family 12 polysaccharide lyase (PL12) from *Pedobacter saltans*. 7th International Forum on Industrial Bioprocessing (IFIBiop 2017), May 21-24, Wuxi, China.
13. Shabir Najmudin, Shadab Ahmed, **Kedar Sharma**, Pedro Bule, Victor D. Alves, Carlos M.G.A. Fontes and Arun Goyal (2017) Molecular determinants of substrate specificity revealed by the structure of *Clostridium thermocellum* family 43_16 arabinofuranosidase. 6th National Meeting of Portuguese Synchrotron Radiation Users, May 19, 2017, National Laboratory of Energy and Geology, Alfragide, Portugal.
14. Aruna Rani, **Kedar Sharma** and Arun Goyal (2017) Insights into the structural characteristics of chondroitin AC lyase *Ps*PL8A from *Pedobacter saltans*. 12th Carbohydrate Bioengineering Meeting, April 23-26, 2017, Vienna, Austria.
15. **Kedar Sharma**, Anil Kumar Verma, Carlos M.G.A. Fontes, Shabir Najmudin and Arun Goyal (2017) Low-resolution structure of glucuronoxylan-xylanohydrolase (*Ct*XynGH30) of family 30 glycoside hydrolase from *Clostridium thermocellum* by SAXS. Annual Symposium of the Indian Biophysical Society, March 22-25, 2017, IISER Mohali, India.
16. **Kedar Sharma**, Anil Kumar Verma, Carlos M.G.A. Fontes, Shabir Najmudin and Arun Goyal (2016) Solution structure analysis of full length glucuronoxylan endo-β-1,4-xylanase from *Clostridium thermocellum* by Small Angle X-Ray Scattering. 14th International Conference of the Asian Crystallographic Association, 4-7 December 2016, Hanoi, Vietnam.
17. Arun Goyal, Shadab Ahmed, **Kedar Sharma**, Vikas Gupta, Pedro Bule, Victor D. Alves, Carlos M.G.A. Fontes and Shabir Najmudin (2016) Crystal structure and molecular determinants of substrate specificity of arabinofuranosidase from *Clostridium thermocellum*. 14th International Conference of the Asian Crystallographic Association, 4-7 December 2016, Hanoi, Vietnam.
18. **Kedar Sharma** and Arun Goyal (2016) Cloning, expression and characterization of a xylanase from family 10 Glycoside hydrolase (GH10) from *Pedobacter saltans* DSM12145. 57th International Annual Conference of the Association of Microbiologists of India (AMI-2016), Nov 24-27, 2016, Gauhati University and IASST, Guwahati, Assam India.
19. Karthika.B**, Kedar Sharma,** Aruna Rani and Arun Goyal (2016) Cloning, Expression, Purification and Biochemical Characterization of Heparinase II/III from *Pedobacter saltans* DSM12145. 57th International Annual Conference of the Association of Microbiologists of India (AMI-2016), Nov 24-27, 2016, Gauhati University and IASST, Guwahati, Assam India.

20. Inês Lobo Antunes, **Kedar Sharma**, Vikky Rajulapati and Arun Goyal (2016) Biochemical and structure characterization of xylanase of family 10 Glycoside Hydrolase (PsGH10B) from *Pedobacter saltans* DSM12145. CARBO-XXXI International Conference on "New Frontiers in Carbohydrate Chemistry & Biology", Nov 14-16, 2016, Department of chemistry, university of Delhi India.

21. **Kedar Sharma**, Bibari Boro and Arun Goyal (2015) *In silico* structure analysis of a family 12 polysaccharide lyase 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.

22. Arun Dhillon, **Kedar Sharma**, Vania O. Fernandes, Fernando M.V. Dias, José A.M. Prates, Luis M.A. Ferreira, Carlos M.G.A. Fontes, M.S.J. Centeno and Arun Goyal (2015) Biochemical characterization and deciphering the cleavage pattern of recombinant rhamnogalacturonan lyase (*Ct*RGL), a family 11 Polysaccharide Lyase (PL11) from *Clostridium thermocellum*. 56th International Annual Conference of Association of Microbiologists of India (AMI), December 7-10, 2015, Jawaher Lal Nehru University, New Delhi.

23. **Kedar Sharma** and Arun Goyal (2015) *In silico* structural characterization of a family 10 glycoside hydrolase 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.

24. Inês Lobo Antunes, Vikky Rajulapati, **Kedar Sharma** and 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.

25. **Kedar Sharma** and Arun Goyal (2014) *In silico* structure prediction of a family 10 glycoside hydrolase from *Pedobacter saltans* DSM12145. Indo-US Conference and Workshop on recent Advances in Structural Biology & Drug Discovery, October 9-11, 2014, Indian Institute of Technology Roorkee, Uttarakhand, India.

Workshops/courses attended

1. Participated in workshop on SAXS data analysis organized by "Anton Paar India Limited, on March 20-22, 2018.
2. Participated in workshop on Intellectual Property Rights and Patenting, organized by "Department of Biotechnology, Thapar Institute of Engineering and Technology Patiala and Punjab state council for Science and Technology (PSCST) Chandigarh, on February 17, 2018.
3. Participated in satellite meeting on "Phasing and Model Building" organized by IUCr Congress and Center for Chemical Biology, CSIR-Indian institute of Chemical Technology Hyderabad, on August 20-21, 2017.
4. Participated in GIAN Course "**Latest Methods in X-ray Crystallography: Lecture Series and Practical Course** organized by School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, INDIA under MHRD scheme on Global Initiative for Academic Network on **November 14th to 25th, 2016.**

5. Participated in "4th edition of **Computational Biotechnology at the Nanoscale: CCP4 Workshop 2016**" Jointly organized by CCP4, AIIMS, ICGEB, JNU, NII and UNESCO-Regional Centre for Biotechnology, at Regional Biotech Science Cluster NCR Biotech Science Cluster 3rd Milestone, Faridabad-Gurgaon Faridabad 121001 on 15/2/2016 - 20/2/2016.
6. Participated in "**Symposium cum Workshop on "Advances in Computational Biology and Computer Aided Drug Design**" organized by Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati on **June 24th to 26th, 2015.**