

Higher Education Commission Islamabad

Project Outcomes

1. Project/PI particulars

Title of Project: Role of Pest's Cellulases in Crop Damage
Name of PI: DR. AMTUL JAMIL SAMI
Project No: 568 **PI Email:** zamtuljamil@gmail.com
Duration: 36 Months **Total Cost:** 1624000
Start Date: 10-04-2006 **Completion Date:** 11/18/2020
University / DAI's: UNIVERSITY OF THE PUNJAB, LAHORE

2. Sector of Institution: Public

3. Is this project: Basic

4. Is output of the project commercialize able: No

5. Objectives of Project

Main objectives of the work were to screen the insect pests for cellulases and to look for the inhibitor molecules produced by the plants that could be used as bio-pesticides a number of insects were screened for the presence of animal cellulase activity in the whole body extract.

6. Summary of Project

The work reported here was carried out under a research project # 568 entitled "role of pest cellulases in crop damage", funded by the higher education commission Pakistan under national universities research program, at the institute of biochemistry and biotechnology, university of the Punjab, Lahore, under the supervision of dr. Amtul Jamil Sami (principal investigator) and Prof. Dr. A. R. Shakoori (co-principal investigator) during May 2006-April 2009. Main objectives of the work were to screen the insect pests for cellulases and to look for the inhibitor molecules produced by the plants that could be used as bio-pesticides a number of insects were screened for the presence of animal cellulase activity in the whole body extract. The cellulase activity was detected in insects. In *Oxya chinensis* and *Aulacophora* sp. The maximum cellulase activity was recorded in salivary glands and foregut. Multiple forms of endoglucanase activity were observed for both the insects. the endo-beta-1,4 glucanase of red pumpkin beetle, *Aulacophora foveicollis* (Lucas) was found to have optimum activity at pH 8.0, at 50 oc. the enzyme was inhibited by beta-mercaptoethanol, and its km value was 0.2g/l. *Oxya chinensis* is a serious paddy insect pest and responsible for major destruction of rice crop in the world. it has been reported that o. chinensis is completely dependent on the plant biomass for their nutritional requirement. Insect pest attacks all parts of the plants including root, stem, leaf and grain. a number of studies have been reported on the control of o. chinensis, but there is no report on the molecular basis of crop destruction by the pest. Here we report the presence and purification of an acidic and thermophilic endo-1, 4-beta -d-glucanase, identified in the crude insect extract. The enzyme was purified on sephadex g-200 column and cm-cellulose column after precipitation with acetone. The purified endoglucanase showed pH optima at 2.0 and temperature optima at 60 °c. The lower pH optima of the endo 1, 4-beta -d-glucanase could be related to the low digestibility and high ash content of the rice plant components. The production and characterization of endo-?-1, 4-glucanase from associated microbes was also studied. The microbial flora of beetles produced cellulase, xylanase enzyme activities and were able to produce polysaccharide, when grown in shake flask culture. Plant based inhibitors were identified from leaves of guava, mango, neem and grapes, which in their crude form were able to inhibit cellulase activity and also proved effective in insect repellent assay in our laboratory. The endoglucanase activity of *A. foveicollis* was completely inhibited by a flavonoid isolated from guava leaves. The compound was a polyhydric phenol with a molecular weight of 255 as determined by mass spectrometry. The km value of the inhibitor was 0.05µmol. the plants are known for production of certain metabolites for insect repellency. two glycosylated flavonoids - cyanidin-3-glycosides and quercetin-3-o-beta-d- glucosyl (1,2) beta glucoside were isolated from mango leaves, and used for studies on inhibition of cellulases of *Aulacophora foveicollis*. cyanidin-3- glucosides was able to completely inhibit the enzyme, while quercetin-3-o-beta-d- glucosyl (1,2) beta glucoside inhibited to a lesser extent. Rutin and c3g proved to be non-competitive inhibitors and had same inhibitor constant K_i 0.5µm, indicating the structural similarity at the binding site. Rutin and c3g were also able to retard the growth of two bacterial strains *pseudomonas* and *Staphylococcus*, isolated as associated microbes of *Aulacophora foveicollis*. It is concluded that the natural glycosylated flavonoids stand on good merit as potential insect cellulose hydrolyzing enzyme inhibitors. Neem was also found to be an excellent inhibitor for a number of cellulases from several insect pests. It stands good merit as soft insect repellent.

7. Out comes

i. No of Publications:

Research papers (National)		Research papers (International)		Total
In impact factor journals	In non-impact factor journals	In impact factor journals	In non-impact factor journals	
2	0	5	1	8

Please mention publication:

1. Sami AJ and Shakoori AR. (2006), Heterogeneity of cellulases among Insects pests cellulases Pakistan Journal of Zoology 38(4) 337-340 2. Sami A.J, and Haider M.K (2007), Identification of novel catalytic features of endo-?-1,4-glucanase produced by mulberry longicorn beetle *Apriona germari*, Journal of Zhejiang University Science B Vol 10-765-770 3. Sami AJ and Shakoori AR,(2007), Extracts of plant leaves have inhibitory effects on the cellulase activity of whole body extracts of insects - A possible recipe for bioinsecticides Proc. Pakistan Congr. Zool., Vol. 27 pages. 105-118 4. Sami,A.J., Yasmeen,N. and Shakoori,A.R. (2008), Cellulolytic activity of microbial flora of agricultural insects. Pakistan Journal of Zoology Vol 40. (1), 60-63. 5. Sami A.J., Awais M. and Shakoori A.R., (2008), Preliminary studies on the Production of endo-1, 4-?-D-glucanase activity produced by *Enterobacter cloacae*. African Journal of Biotechnology Vol 7 (9) 1318-1322. 6. Sami A.J. and Shakoori A.R. (2008), Biochemical characterization of endo-1, 4-?-D-glucanase activity of a green insect pest *Aulacophora foveicollis* (Lucas) Journal of Life Science Vol. 5. No. 2, 30-36 7. Fayyaz Ur Rehman, Mehwish Aslam, M. Ilyas Tariq, Ashraf Shaheen, Amtul Jamil Sami, Naima Huma Naveed and Aima Iram, Batool (2009). Isolation of cellulolytic activities from *Tribolium castaneum* (red flour beetle) African Journal of Biotechnology Vol. 8 (23), pp. 6710-6715. 8. Sami. A J Farhana Tabassum and A R Shakoori., (2010), Biodegradation of cellulase and xylane by a serious paddy pest by *Oxya chinensis* Annals of Biological sciences vol 21-12.

ii. Research Supervised (Number of students who have completed research degrees under said project)

Name of student	PhD/MS	Registration no	Thesis title	Year of degree notification	University	Department	Name of Supervisor
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1	Naseem BIBI	Ms	----	Molecular Basis of Cellulose Hydrolysis by Beetles and associated Bacteria	2007	University of the Punjab	Institute of Biochemistry and Biotechnology	Prof. Dr. Amtul Jamil Sami
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iii. **Patents (Numbers)**

Local			Foreign			Total
Submitted	Granted	Income/royalties	Submitted	Granted	Income/royalties	
0	0	0	0	0	0	0

iv. **Major Equipment** (Please give detail of equipment purchased under said project along with verified copy of entry page of university stock register)

S. NO	Name of equipment	Cost of equipment	Entry Page in University Stock Register
1	non	0	----

v. **Linkages with R&D organizations, universities and industries**

S. NO	Counterpart organization	Type of linkage
1	nil	nil
2	non	non

8. **Other out puts**

No of products		No of process/methods		No of Crop varieties		Any other out put please specify	
Developed	Marketed	Developed	Marketed	Developed	Marketed	Developed	Marketed
0	0	1	1	0	0	0	0

9. **Have you considered communicating any interest in transferring research outcomes/products to (Please fill at least one):**

- Private Companies: *
Nil
- Government organization: *
Nil
- None-government organization: *
Nil
- ORICs organization:
Nil
- others (please specify):
- None of the above:

10. **Paper presented under the said NRPJ project:**

S.NO	Title of conference	Level	
		International	National
1	18th International FAOBMB Conference, Pakistan, 2006.	International	-
2	, Mechanism and Bioprocesses-A Biochemical Society Meeting, Manchester, UK, 2006.	International	-
3	, Biochemical Society meeting, july 23-27. 2006, Glasgow,UK	International	-
4	Abstract no. 558, Bioscience, Biochemical Society meeting, july 23-27 2006, Glasgow,UK	International	-
5	international symposium on nanochemistry: chemistry, biochemistry, molecular biology and bioinformatics of enzymes . Sept 20-21. 2006., School of Biological Sciences University of the Punjab Lahore Pakistan.	International	-
6	Cellulose hydrolyzing activity of blue beetle Aulacophora atritennis Royal Society of Entomology Manchester meeting July 2007 Published online.	International	-

11. **Benefit of the project to the Community (Please mention the target group of the community, if any):**

Community is aware of the characteristics of the pest digestive enzymes and their inhibition by plant derived molecules.

12. **Major problems hindering in the execution of the project, if any:**

Nil