REYAZ AHMAD LONE

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Summary of Qualifications:

Ph. D. in Biotechnology **(2011- 2016)**

University : Periyar University, Salem Tamil Nadu
Guide : Dr. P. Indra Arulselvi, M.Sc., Ph. D.
Thesis title : Cloning and Expression of a Novel *cry2A* Gene from *Bacillus thuringiensis* SWK1 and its Insecticidal Activity

M. Sc. in Biotechnology **(2008 - 2010)**

University : Periyar University
Thesis title : Molecular Characterization of Psychrophilic, *Serratia marcescens*; Extraction of Protease, its Production and Industrial Application

Bachelor of Education **(2005 - 2007)**

College : Govt. College of Education, University of Kashmir, Srinagar.

Bachelor of Science **(2000 - 2003)**

College : Govt. Degree College Bemina, University of Kashmir, Srinagar.

Technical Skills Known:

- Handling whole genome sequences of bacterial cells
- Isolation techniques for nucleic acids, proteins from bacteria, plants and insects.
- Electrophoretic techniques (Agarose and Polyacrylamide gels).
- Cloning and transformation techniques such as plasmid DNA isolation, preparation of competent cells, transformation of bacteria and plants.
- Gene amplification by PCR technique.
- Master Mix preparation for PCR.
- Immunochemical techniques such as, immunodiffusion, ELISA.
- culture techniques such as anther culture, shoot tip culture, meristem tip culture
- Good knowledge on DNA and protein sequence databases and Bioinformatics tools (BLAST).
- Primer designing for PCR experiments.
- Antibody production
- Microbial staining procedures
- Isolation of microbes from the various kinds of samples such as soil, leaf, and spider webs etc.

- Maintenance of different kinds of lepidopteran insects (*H. armigera, S. litura, S. frugiperda*), dipteran insects (*B. cucurbitae, A. aegypti*, *C. quinquefasciatus A. stephensi*), nematode (*C. elegans*).

Research Directions:

1. Genetic Engineering
2. Microbial Biotechnology
3. Plant Tissue Culture and Transformation

Teaching Experiences in Department of Higher Education

Position	Employer	Courses Handled	From	To
Lecturer (contractual)	Govt. Degree College Handwara Kashmir, India	I. Plant Biotechnology II. Animal Biotechnology III. Genetic Engineering	April 2022	Dec 2023
Guest Faculty	University of Kashmir (North campus)	I. Introduction to Biotechnology (Multidisciplinary course)	March 2024	April 2024
Guest Faculty	Govt. Degree College Baramulla Kashmir, India	I. Recombinant DNA Technology II. Cell Signaling and Cancer III. Cell Biology, Microbiology and Immunology	March 2024	June 2024
Guest Faculty	University of Kashmir (North campus)	I. Introduction to Biotechnology (Multidisciplinary course) II. Introduction to Biochemistry (Multidisciplinary course)	Sep. 2024	Oct. 2024
Lecturer (contractual)	Govt. Degree College Baramulla (Autonomous) Kashmir, India	I. Biomolecules structure and Function II. Introduction to Biotechnology and Human Health (MD) III. Introduction to Biotechnology	Oct. 2024	Till date

Post Doctoral Research Experiences:

Designation : BIRAC Innovation Post Doctoral Fellow (**2019 - 2021**)

Project Title : Cloning and expression of a novel Bt protein isolated from an indigenous Bt isolate and validating its efficacy against lepidopteran and hemipteran insects.

Sponsor : Biotechnology Industry Research Assistant Council (BIRAC), Department of Biotechnology, India.

University : Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore, India.

Grant Amount : 23, 00000 INR

Project summary: Genomic DNA was extracted from indigenous *B. thuringiensis* strain T541 and the full length of the putative novel gene (*cry*-78 like) was amplified, purified and ligated with expression vector (pET28a) which was transferred into *E. coli* BL21 (DE3). The transformants were confirmed by colony PCR, restriction digestion and sequencing. Transformants were induced using IPTG and expression of novel protein was analyzed by SDS-PAGE. This protein was further purified using Amicon Centrifugal purification columns. Purified protein was used for bioassay purpose against lepidopteran insect pests: *Spodoptera frugiperda, Spodoptera litura, Helicoverpa armigera, Pectinophora gossypiella* and dipteran insect: *Bactrocera cucurbitae.* This protein was named as **Tpp80Ab1** by the **Bt nomenclature committee**. Further I raised antibodies against this protein and also synthesized codon optimized gene, Tpp80Ab1, which I cloned in Non-*Hind*III pCAMBIA2300 plasmid under the EnCaMV35S constitutive promoter. I mobilized it into the *Agrobacterium tumefaciens* LBA4404 from *E. coli* DH5α using helper strain *E. coli* pRK2013 via triparental mating and transformation of *Nicotiana tabacum* L *cv* Petit Havana. Putative transformed plants were confirmed using gene specific and *npt*II primers. I performed ELISA for the confirmed transgenic plants and the transgenic plants which have shown highest protein expression were chosen for the bioassay against whiteflies using clip-cage method.

Designation : National Post-Doctoral Fellow (NPDF) (**2016 – 2018**)

Project Title : Identification and characterization of *cry* gene pool from indigenous *Bacillus thuringiensis* strains against field evolved resistant population of pink bollworm (*Pectinophora gossypiella*) to *Bt* cotton (Bollgard-II) in India.

Sponsor : Science and Engineering Research Board (SERB), Department of Science and Technology (DST), India.

University : Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore, India.

Grant Amount : 19, 20000 INR

Project summary: In this project I collected pink bollworm (*Pectinophora gossypiella*) larvae from the Bollgard-II cotton fields of Tamil Nadu and Andhra Pradesh, India and maintained the populations on artificial diet in lab. I devised a new simple methodology to differentiate a field evolved resistant population (methodology published). I further characterized the

resistant populations of *P. gossypiella* by sequencing Cadherin genes using insect genomic DNA specific primers. I performed bioassay of indigenous Bt isolates (T4, T26, T32, T50, T78, T117, T400, T414, T444, T405, T541) along with a reference strain, Bt *subsp kurstaki* (HD1) against *P. gossypiella* Primary screening of insecticidal activity of 11 indigenous Bt strains with spore crystal–protein mixtures against the pink bollworm revealed toxicities in different ranges (10 to 100 percent mortality) on 14^{th} day of exposure on artificial diet. The isolates T32, T405 and T414, T541 showed 100% mortality on 10^{th} day like HD1. SDS-PAGE investigation of the spore–crystal mixture of 11 indigenous Bt strains demonstrated, these contained protein bands of various molecular weights ranging from 20kDa to 130kDa. The bioassay based selected isolates (T32, T405 and T414) were screened for *cry*1, *cry*2 and *cry*9 genes by PCR. All three isolates were found positive for *cry1* and *cry2* genes and negative for *cry*9 gene. Full length cloning of *cry2A* genes from the above three indigenous *Bt* strains (T32, T405, T414) was done. These were named as Cry2Aa19, Cry2Aa20 and Cry2Aa21 respectively by *Bacillus thuringiensis* delta-endotoxin nomenclature committee. Further, the whole genome sequencing of three Bt strains: T26 (50% mortality), T414 (100% mortality), T541 (100% mortality) was performed using MiSeq plateform. Whole Genome Shotgun sequences have been deposited at DDBJ/ENA/GenBank under the accessions RBKQ00000000, RBVK00000000, RAQV00000000 respectively. It was interesting to observe that even in whole genome sequencing, Bt strains such as T26 and T541 did not show any kind of known lepidotperan active *cry* genes (such as *cry1, cry2,* or *cry9*) but still had showed 50% and 100% mortality respectively against pink bollworm. This indicates the presence of novel insecticidal protein genes in these isolates.

Awards and Prizes:

First prize (Oral presentation): Department of Microbiology, Sree Amman Arts and Science College, Erode, India during a National Level Seminar on "Application of Current Techniques in Biological Science." **2014**

Journal Reviewer:

1. Scientific Reports
2. Microbial pathogenesis
3. Biocatalysis and Agricultural Biotechnology

Selected Publications:

1. **Reyaz A. L.,** Balakrishnan N. and Udayasuriyan V. Mining of a novel Tpp80 holotype using whole genome sequencing of a cotton pink bollworm (*Pectinophora gossypiella* Saunders) toxic novel *Bacillus thuringiensis* isolate T541. (Under preparation)
2. **Reyaz A. L.,** Balakrishnan N. and Udayasuriyan V. Expression studies of a novel holotype Tpp80 in model plant Tobacco nicotum. (Under preparation)
3. **Reyaz A. L.,** Balakrishnan N. and Udayasuriyan V. (2021). A novel *Bacillus thuringiensis* isolate toxic to cotton pink bollworm (*Pectinophora gossypiella* Saunders). Microbial Pathogenesis. IF: 3.84

4. **Reyaz A. L.,** Balakrishnan N. and Udayasuriyan V. (2019). Genome sequencing of *Bacillus thuringiensis* isolate T414 toxic to pink bollworm (*Pectinophora gossypiella* Saunders) and its insecticidal genes. Microbial Pathogenesis. *134*, p.103553. IF: 3.84
5. Kaviyapriya, M., **Lone, R.**, Balakrishnan, N., Rajesh, S. and Ramalakshmi, A., (2019). Cloning and characterization of insecticidal *cry*/*vip* genes from an indigenous *Bacillus thuringiensis* isolate T29 and evaluation of its toxicity to maize fall armyworm *Spodoptera frugiperda*. *J. Entomol. Zool. Stud*, *7*(3): 1314-1321.
6. **Reyaz A. L.,** Balakrishnan N., Udayasuriyan V. (2018). A new observation on feeding behaviour of pink bollworm and its application in screening Bt-resistant population. 3Biotech. 8:237 (DOI:10.1007/s13205-018-1262-7). IF: 2.8
7. Ganesh, K.N., **Reyaz, A.L.** and Balakrishnan, N., (2018). Molecular characterization of an indigenous lepidopteran toxic *Bacillus thuringiensis* strain T532. *Journal of Biological Control*, *32*(4), pp.246-251.
8. **Reyaz A.L.**, and P Indraarulselvi. (2017). Molecular Characterization and Evaluation of Two Potential Mosquitocidal Lysinibacillus Strains from Himalayan Valley Kashmir. Journal of Pure and Applied Microbiology 11 (4): 1811-1821
9. **Reyaz A.L.,** Gunapriya L., and Indra Arulselvi P. (2017). Molecular characterization of indigenous *Bacillus thuringiensis* strains isolated from Kashmir valley. 3Biotech. 7:143 (DOI: 10.1007/s13205-017-0756-Z). IF: 2.8
10. Tariq, A.L., Chandran, S.R., and **Reyaz A.L.** (2017). Molecular characterization and antifungal activity of extracellular chitinolytic enzyme producing *Paenibacillus elgii* TS33 isolated from shrimp shell waste. Int J Pharma Res Health Sci 5 (6): 2064-69
11. **Reyaz A.L.** and Indra Arulselvi P. (2016). Cloning characterization and expression of a novel haplotype cry2A type gene from *Bacillus thuringiensis* strain SWK1 native to Himalayan valley Kashmir. Journal of Invertebrate Pathology. 153: 1-6. IF: 2.84
12. Reyaz, A.L., Shahanaj, I., Gunapriya, L., Nancy, D., Karthik, C. and Indra Arulselvi P. (2013). Plasmid profiling of indigenous *Bacillus thuringiensis* isolated from Tamil Nadu and Kashmir. Journal of Pharmacy Research 6 (3): 325-330. IF: 2.1
13. Tariq A.L. and **Reyaz A.L.** (2012). Characterization of UTI associated protease from *Serratia marcescens* strain TW1 and its immunogenic properties. Int Res J Biotechnol 3: 88-95
14. Tariq, A.L., **Reyaz, A.L**. and John Prabakaran J. (2011). Purification and characterization of 56 KDa cold active Protease from *Serratia marcescens.* African Journal of Microbiology Research. 5(32): 5841-5847.
15. C Karthik, P Indra Arul Selvi, **AL Reyaz** (2011) Protein and Gene Profiling of Indigenous Bacillus thuringiensis Isolates. International Journal of Chemical and Pharmaceutical Science. 2 (2): 57-63

Presentations in seminars/conferences

1. **Reyaz A.L.,** I Shahanaj; characterization of crystal proteins of indigenous *Bacillus thuringiensis* isolates. 9th National Conference on Current Trends in Microbial

Technology held on 14th & 15th October, 2011. Department of Microbiology Periyar University Salem-11.

2. **Reyaz A.L.,** L. Gunapriya, K Sathiya, I Shahanaj & Indra Arulselvi. P *Bt* cotton in India from 2002 to 2012 a success story. A National Confernce on "New Horizons in Biotechnology and Bionanomedicines (NHBB-2012)'' on 24th October, 2012. Department of Biotechnology Periyar University Salem-11, India.
3. **Reyaz A.L.,** I Shahanaj and Indra Arulselvi P. Parasporin a new anticancer protein from *Bacillus thuringiensis*. A National seminar on Biotechnological Insights in Disease Management held on 8th & 9th Feb. 2012. Department of Biotechnology Periyar University Salem-11, India
4. **Reyaz A.L.** and Indra Arulselvi P. Detection of two *cry2A* genes in a *Bacillus thuringiensis* strain SWK1 Isolated from Kashmir. A National Level Seminar on "Application of Current Techniques in Biological Science" 30th January, 2014. Department of Microbiology, Sree Amman Arts and Science College, Erode, Tamil Nadu, India.
5. **Reyaz A. L.** and Indra Arulselvi P. Crystal protein profiling of indigenous *Bacillus thuringiensis* strains, isolated from Kashmir valley. A 2nd International seminar on Bioscience- "Elixir of Life" (BSEL-2016) on 5th February, 2016. Department of Microbiology and Biotechnology at Sri Ganesh College of Arts and Science, Salem, Tamil Nadu, India.

Workshop/ Training Attended:

1. **"Training on Next Generation Genomic Technologies (Next Generation Sequencing and Bioinformatics"** jointly organized by TransDisciplinary University (TDU) and Bengaluru Genomics Centre Pvt. Ltd. (BGC) from 25.06.2018 to 30.06.2018 (6 days).
2. **"Biodiversity, Biosystematics and Biocontrol"** 21 days (21.01.13 to 10.02.13) training programme held by National Bureau of Agriculturally Important Insects, ICAR, Bangalore, India.
3. **"Application of Molecular Techniques for Crop Improvement"** DBT sponsored 14 days workshop (07.03.12 to 22.03.12) held at Department of Biotechnology, Periyar University, Salem-11, India.
4. **"Biostatics using software packages"** a two days training program (30.09.14 to 01.10.14) organized by INAQ in association with Departments of Food Science and Nutrition and Computer Centre, Periyar University, Salem-11, India.
5. **"Interaction Programme on DST Schemes for Researchers"** Organized (12th June, 2015) by Centre for Nanoscience and Nanotechnology (CNSNT), Periyar University, Periyar Palkalai Nagar, Salem -11, India.
6. **"Scholarly Research Publications: Writing, Citation And Plagiarism (RPCP-16)"** an international workshop (01 February, 2016), organized by Department of Library and Information Science Periyar University Salem-11, India.

Books Published:

1. **Reyaz Ahmad Lone** & Indra Arulselvi Padikasan. *Bacillus thuringiensis* triumphant Cry1 Insecticidal Crystal Proteins. LAP Lambert Academic Publishing. 2015. (ISBN Number 978-3-659-74848-6).
2. Tariq Ahmad Lone & **Reyaz Ahmad Lone.** *Serratia marcescens* A prokaryote: Isolation and Identification. LAP Lambert Academic Publishing. 2015. (ISBN Number 978-3-659-75940-6).
3. Parveez Ahmad Para & **Reyaz Ahmad Lone.** Processed Meat Products-A Students Guide. LAP Lambert Academic Publishing.2017. (ISBN number: 978-3-330-35010-6).
4. Shabu Showket, Parveez Ahmad Para & **Reyaz Ahmad Lone.** Veterinary Antibiotic Residues in Food of Animal Origin. LAP Lambert Academic Publishing. 2017. (ISBN number: 978-3-659-52166-9).

Book Chapters Published:

1. Tariq Ahmad Lone & **Reyaz Ahmad Lone, "***Serratia marcescen,* a Handy Bacterium" *New Dimension in Microbiology* Ed. M.M. Abid Ali Khan, John K. Grandy, Egamberdieva Dilfuza, Murtaza Abid, Raaz K. Maheswari, T.S. Naqvi. Lenin media**.** 2015.
2. **Reyaz, A.L.,** Balakrishnan, N., Balasubramani, V. and Mohankumar, S. *'Bacillus thuringiensis* in Insect Pest Management' in Omkar (ed.) *Microbial Approaches for Insect Pest Management.* Springer Nature Singapore. 2021.

Extra Curricular Activities:

1. Participated in White water rafting course (26-06-05 to 02-07-05) conducted by the Directorate of Physical Education and Sports, University Of Kashmir at Sonmarg (River Sindh).
2. Participated in Adhoc Adventure course sponsored by the Nehru Yuva Kendra Sahgathan, Jammu and Kashmir from 27-11-06 to 01-12-2006.

Academic References:

Dr. Roohi Mushtaq, Ph.D. Professor and Head Department Biotechnology, Govt Degree College Baramulla, 193101 Jammu and Kashmir India Email: rohikhan400@gmail.com Mobile: +91 94439 71738	**Dr. Mohd Jamal Dar, Ph.D.** Principal Scientist Pharmacology Division CSIR – Indian Institute of Integrative Medicine,Canal Road, Jammu – 180001 Jammu and Kashmir India Email: jamal@iiim.res.in	**Dr. Ehab Salama** Lecturer, Faculty of Agriculture Saba Basha Alexandria University Alexandria – 21526 Egypt. E-mail: ehabsalama34@gmail.com Mobile: +91 9952369011

I, **Reyaz Ahmad Lone** hereby declare that the above mentioned particulars are true, complete and correct to the best of my knowledge and belief.

Place: Kashmir, India (REYAZ AHMAD LONE