

Gulshan Singh

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*Joining shortly (w.e.f. May, 2014) to Institute of Water & Waste Water Technology (IWWT), Durban University of Technology, Durban, South Africa

Academic Qualifications

- PhD in **Science**: Awarded from Academy of Scientific and Innovative Research(**AcSIR**)
 - ❖ **Title of PhD Dissertation**: Development of Molecular Techniques for the Rapid Culture-Free Detection of Bacteria in Environment
 - ❖ **CGPA (Course Grade Points)** : 8.00/12
 - ❖ **Course Credited**: Biostatistics, Computers & Bioinformatics, Basic Chemistry, Research Methodologies, Ethics and Safety, Biotechniques/Instrumentation, Molecular and Cellular Immunology, Stem cells, regeneration and aging, Gene-environment interactions, Nanobiology
- **M.Sc. (Biochemistry)**
Department of Biochemistry, University of Lucknow, UP, India **2005-2007**
First Division with 70% marks.
- **B.Sc. (Zoology, Botany, Chemistry)**
D.D.U Govt. P.G College, C.S.J.M.U Kanpur **2002-2005**
First Division with 73% marks.

Positions held

- **Research Associate** **From January 2014-Till date**
- **Senior Research Fellow** **From April 2011- December 2013**
Environmental & Nanomaterial Toxicology group,
CSIR-IITR, Lucknow
- **Project Assistant (Level-II)** **From March 2008- 2011**
Environmental & Nanomaterial Toxicology group
CSIR-IITR, Lucknow

Awards and Honours

- Invited for training at Institute of Photonic Technology, Germany (14 October to 25 November 2012) under EU New Indigo Project: AQUATEST
- Senior Research Fellowship, CSIR: April 2011- March 2014
- Best Oral/Poster/Paper Awards : 4

Research Interests

- Bacterial pathogenesis and host pathogen interactions.
- Emergence and dissemination of antibiotic resistance in the environment.
- Computational biology, micro-engineering.
- High throughput and nanotechnology based approaches for detection of pathogenic bacteria.
- Ecotoxicity: Interaction of bacteria and engineered nanomaterials.

Technical Skills

Molecular Biology Techniques

Isolation of DNA & RNA from cell suspensions, water and food matrices, quantitation of dsDNA, ssDNA and ssRNA using Spectrofluorometry, quantitative Real Time- PCR, Gene expression analysis based on Reverse Transcriptase PCR, Agarose gel Electrophoresis, Cloning and expression of gene, SDS-PAGE, Western Blot Analysis, Two-dimensional gel electrophoresis and Flow cytometry.

Microbiology Techniques

Isolation and characterization of bacteria from environment, handling and working with epifluorescence microscopy and UV-visible and fluorescence spectrometer/microplate reader, basic techniques like membrane filtration, concentration and reconcentration techniques for detection of microorganisms in low doses from viable but not culturable (VBNC) state.

Toxicity assessment

Characterization of Nanoparticles by Dynamic Light Scattering (DLS), Transmission electron Microscopy(TEM) and Scanning Electron Microscopy(SEM). Internalization of Nanomaterials in bacteria and Propidium iodide based viability assays by Flow cytometry. Live and Dead *BacLight* (Microscopic) assays, biochemical assays like measurement of Reactive Oxygen species (ROS) generation and expression of enzymes.

Computation skills

Retrieval of Nucleotide Sequence from NCBI Gen bank database, analysis of Nucleotide Sequence, Multiple Sequence alignments, computation of primers/probes with different softwares viz. Lasergene-DNASTAR, Primer 3, *Beacon Designer*. *In silico* simulations of

primers and qPCR probe chemistries such as Molecular Beacon, FRET, TaqMan, scorpion and SYBR Green chemistries (in *singleplex* and *multiplex* mode) for quantitation of pathogenic microorganism without enrichment from environmental samples.

Membership in Professional societies

Life member

Indian Nanoscience Society (INS), India

The Biotech Research Society of India (BRSI)

Student Member/Ordinary member

- Society of Free Radical and Research, India

Activities for personal growth to achieve Professional Excellence

Workshops Attended

- UGC NRCBS Winter School on “**Gene Cloning and Expression in Bacteria**” from September 16th – October 1st, **2010** at Madurai Kamraj University, Madurai.
- Workshop on **Mass and NMR techniques** from 28th -29th January- 2013, CSIR-SAIF Central Drug Research Institute, Lucknow.

Project Proposal Writing Skills

Wrote two project proposals and submitted to **AcSIR** (Academy of Council of Scientific and Industrial Research, India) for the partial fulfillment of doctoral research work:

- Real-Time PCR Array for Culture-Free Detection of Pathogenic Bacteria in Surface and Potable Water.
- Synergistic effect of metallic nanoparticles in Bacteria: Identification of differentially expressed novel proteins of *Escherichia coli*.

Research Publications

Total Publications (2008-2013) 12

- | | |
|--|---|
| A. Environmental Microbiology | 8 |
| B. Environmental and Nanomaterial Toxicology | 4 |

Total impact factor = 39.146 (Average impact factor = 3.26)

No. of Research article with impact ≥ 5 = 5

Total citations = 48

A: Environmental Microbiology

1. **Gulshan Singh**, Poornima Vajpayee, Saurabh Bhatti, Nirmala Ronnie, Nimish Shah , Peter McClure and Rishi Shanker. Determination of viable *Salmonellae* from potable and natural source water through PMA assisted qPCR. *Ecotoxicology & Environmental Safety*, 93(2013)121–127(Impact factor # 2.203).
2. Anurag Jyoti, **Gulshan Singh**, Poornima Vajpayee and Rishi Shanker. Toxicity and Safety assessment of bio-contaminants: Molecular Beacon based detection of *Salmonellae* in street food. *Journal of Translational Toxicology* (2014) [accepted in press].
3. **Gulshan Singh**, Poornima Vajpayee, Neetika Rani, Anurag Jyoti, Kailash C Gupta, and Rishi Shanker. Bio-capture of *S. Typhimurium* from surface water by aptamer for Culture-free quantification. (2012), *Ecotoxicology & Environmental Safety* 78.320–326. (Impact factor # 2.203).
4. *Anurag Jyoti, Poornima Vajpayee, **Gulshan Singh**, Chandra Bali Patel, Kailash Chand Gupta, and Rishi Shanker. Identification of Environmental Reservoirs of Nontyphoidal Salmonellosis: Aptamer-Assisted Bioconcentration and Subsequent Detection of *Salmonella Typhimurium* by Quantitative Polymerase Chain Reaction (2011) *Environmental Science & Technology*, 45.8996–9002 (Impact factor # 5.257).
5. ***Gulshan Singh**, Poornima Vajpayee, Siya Ram and Rishi Shanker (2010). Environmental Reservoirs for Enterotoxigenic *Escherichia coli* in South Asian Gangetic Riverine System (2010). *Environmental Science & Technology*, 44, 6475–6480(Impact factor # 5.257).
6. Anurag Jyoti, Siya Ram, Poornima Vajpayee, **Gulshan Singh**, Premendra D. Dwivedi, Swatantra K.Jain, Rishi Shanker. Contamination of surface and potable water in South Asia by *Salmonellae*: Culture-independent quantification with molecular beacon real-time PCR. (2010) *Science of Total Environment*. 408, 1256-63 (Impact factor # 3.25).
7. C B Patel, P Vajpayee, **G Singh**, A Jyoti, R S Upadhyay and R Shanker. Computation and in- Silico. Validation of a Real-Time PCR Array for Quantitative Detection of Fecal Coliforms in Surface and Potable Water. (2011) *International Journal of Biotechnology and Biosciences* 2,185-194.
8. Patel CB, Vajpayee P, **Singh G**, Upadhyay RS, Shanker R. Contamination of potable water by Enterotoxigenic *Escherichia coli*: qPCR based culture-free detection and quantification.(2011), *Ecotoxicology & Environmental Safety* 74,2292-8. (Impact factor # 2.203).

**Environmental Science Technology* (ES&T) ranked # 1 in Environmental Engineering and Environmental Sciences categories.

B: Environmental and Nanomaterial Toxicology

9. **Gulshan Singh**, Poornima Vajpayee, Imrana Khatoon, Anurag Jyoti, Alok Dhawan, K. C. Gupta, and Rishi Shanker. Chromium Oxide Nano-Particles Induce Stress in Bacteria: Probing Cell Viability **(2011)**, *Journal of Biomedical Nanotechnology* 7, 166-167(Impact factor # 5.256).
10. Poornima Vajpayee, Imrana Khatoon, Chandra Bali Patel, **Gulshan Singh**, Kailash Chand Gupta, and Rishi Shanker . Adverse Effects of Chromium Oxide Nano-Particles on Seed Germination and Growth in *Triticum aestivum* L. **(2011)** *Journal of Biomedical Nanotechnology*. 7, 205-206 (Impact factor # 5.256).
11. Imrana Khatoon, Poornima Vajpayee, **Gulshan Singh**, Alok K. Pandey, Alok Dhawan, K. C. Gupta, and Rishi Shanker. Determination of Internalization of Chromium Oxide Nano-Particles in *Escherichia coli* by Flow Cytometry. **(2011)***Journal of Biomedical Nanotechnology*. 7, 168-169 (Impact factor # 5.256).
12. Sandeep Kumar, Akanksha Sharma, Neelabh, **Gulshan Singh**, Alok K. Verma, Ruchi Roy, Anurag Tripathi, Mukul Das, Rishi Shanker, Premendra D. Dwivedi. Allergenic responses of green gram (*Vigna radiata* L. *Millsp*) proteins can be vitiated by induction of oral tolerance due to single acute dose in BALB/c mice. *Food Research International* 57 (2014) 130–141.(Impact factor # 3.005).

Research Papers under Communication

- **Gulshan Singh**, Poornima Vajpayee and Rishi Shanker.Molecular Signatures of antimicrobial resistance in *Escherichia coli* retrieved from surface water of sewage impacted rivers of Indian subcontinent.
- **Gulshan Singh**, Poornima Vajpayee, Neetika Rani, and Rishi Shanker . Prevalence of environmental reservoirs of β -lactamase resistance in Indo-Gangetic region.
- **Gulshan Singh**, Poornima Vajpayee and Rishi Shanker.Development of quantitative PCR Array for Simultaneous Detection of water and food borne Pathogens and applicability in Propidium monoazide based viable qPCR.

Book Chapter

- Rishi Shanker, *Gulshan Singh*, Anurag Jyoti, P.Dhar Dwivedi and Surinder Pal Singh. Nanotechnology and Detection of Microbial Pathogens. *Animal Biotechnology: Models in Discovery and Translation*, American Press,Edited by;Ashish S Verma and Anchal Singh. ISBN: 978-0-12-416002-6 pages 525-539, 2014.

Papers presented in National/International Conferences/Symposium

1. **Gulshan Singh**, Poornima Vajpayee, Neetika Rani, Nirmala Ronnie, Nimish Shah and Peter McClure and Rishi Shanker. Culture independent quantitative enumeration of selected water borne pathogens of importance in drinking water treatment presented in DST-New INDIGO Aquatest project meeting and International Workshop on Water Quality & Microbes: Detection to Remediation held at CSIR-Indian Institute of Toxicology research, India during 6- 7th November, 2013.
2. **Gulshan Singh**, Poornima Vajpayee, Imrana Khatoon, Neetika Rani, Saurabh Bhatti, Alok Pandey and Rishi Shanker. Chromium Oxide nano-particles induced oxidative stress in *Escherichia coli*, in the International conference on Advances in Free Radicals, Redox Signalling and Translational Antioxidant Research & XII Annual Meeting of the Society for free Radical Research-India (SFRR STAR 2013), held at CSIR-Indian Institute of Toxicology research, India during Jan 30- Feb 01, **2013**.
3. **#Gulshan Singh**, Poornima Vajpayee and Rishi Shanker. Prevalence of environmental reservoirs of β -lactamase resistance in Indo-Gangetic region. Presented in National conference on "The XXXII Annual Meeting of Society of Toxicology, India (STOX), Lucknow India and International Symposium on New Frontiers in Toxicology, Theme, New Paradigms in Toxicology (NPT-2012) held at CSIR-Indian Institute of Toxicology Research (CSIR-IITR) during 5-7 December, 2012. **#Awarded Best Poster Award**
4. **#Gulshan Singh**, Poornima Vajpayee, Chandra Bali Patel, Neetika Rani and Rishi Shanker. Predominance of genes responsible for resistance to multiple antimicrobials in *Escherichia coli* Presented in International conference on New Horizons in Biotechnology & 8th Annual Convention of The Biotech Research Society, India organized by NIIST, CSIR, Trivandrum during November 21-24, 2011. **#Awarded Best Poster Award**
5. **#Gulshan Singh**, Poornima Vajpayee, Imrana Khatoon, Anurag Jyoti, Alok Dhawan, K. C. Gupta, and Rishi Shanker. Chromium Oxide Nano-Particles Induce Stress in Bacteria: Probing Cell Viability. Presented in International Symposium on the Safe Use of Nanomaterials and Workshop on Nanomaterial Safety; Status, Procedure, Policy and Ethical Concerns at CSIR-Indian Institute of Toxicology Research, Lucknow during Feb 1-3, 2011. **#Awarded Best Oral Presentation Award**
6. Poornima Vajpayee, **Gulshan Singh**, Anurag Jyoti, Siya Ram and Rishi Shanker. Identification of Environmental reservoirs of global killer Enterotoxigenic *Escherichia coli* through fluorescent probe based real- time PCR. Presented in International Conference on "Biotechnology for Better Tomorrow" Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. (M.S.) India during Feb 6-9 **2012**.
7. **Gulshan Singh**, Poornima Vajpayee and Rishi Shanker, Culture- Free Molecular Beacon Based Quantitative Enumeration of Extended Spectrum β -Lactamases in Aquatic Environment. "Presented in International conference on Genomics Sciences" held at the Madurai Kamraj University, Madurai, Tamil Nadu during Nov. 12-14, 2010.

8. Neetika Rani, **Gulshan Singh**, Poornima Vajpayee, Rishi Shanker and K.C Gupta. Rapid and sensitive quantitative PCR for culture-independent detection of *Psuedomonas aeruginosa* in environmental samples. "Presented in International conference on Genomics Sciences" held at the Madurai Kamraj University, Madurai, Tamil Nadu during Nov. 12-14, 2010.
9. Chandra Bali Patel, Poornima Vajpayee, **Gulshan Singh** & Rishi Shanker. Environmental reservoirs of *Klebsiella pneumoniae*: culture independent detection and quantitative enumeration by q-PCR. "Presented in International conference on Genomics Sciences" held at the Madurai Kamraj University, Madurai, Tamil Nadu during Nov. 12-14, 2010.
10. Anurag Jyoti, Poornima Vajpayee, **Gulshan Singh**, Siya Ram, Premendra Dhar Dwivedi and Rishi Shanker. Detection of Nucleic Acid Signatures of Diarrheagenic *Escherichia coli*: Real-Time PCR Probes to Gold Nanoparticle Probes at International Conference on Nanomaterials and Nanotechnology, NAN0-2010 held at Rangasamy College of Technology, Tiruchengode, Tamil Nadu, India. December 13-16, 2010.
11. **Gulshan Singh**, Poornima Vajpayee, Siya Ram and Rishi Shanker. Quantitative Microbial Risk Assessment: Culture independent enumeration of Enterotoxigenic *Escherichia coli* from non-point sources Presented in International Symposium on Environmental Pollution, Ecology and Human Health" held at the Department of Zoology, Sri Venkateswara University, Tirupati during July, 25-27, 2009.
12. Anurag Jyoti, **Gulshan Singh**, Poornima Vajpayee and Rishi Shanker. Contamination of surface and potable water by *Salmonella* spp. Rapid detection and enumeration by Molecular Beacon Probes". Presented in International Symposium on Environmental Pollution, Ecology and Human Health" held at the Department of Zoology, Sri Venkateswara University, Tirupati during July, 25-27, 2009.
13. Anurag Jyoti, **Gulshan Singh**, Poornima Vajpayee and Rishi Shanker. Rapid Culture Independent Quantitative Enumeration of *Salmonella* in Water and Food by Molecular Beacon based Real-Time PCR presented in 77th Annual Meeting of the Society of Biological Chemists held at Indian Institute of Technology (Madras), Chennai (India) during 18th -20th Dec. 2008.

Research Experience

Inexorable urbanization and industrialization has led to the deterioration in the microbiological quality of water, adversely affecting human health. qPCR, an advance molecular tool, was used for quantification of virulent and multiple drug resistant microbes in the environment. In this context *Escherichia coli* isolates retrieved from surface water of river Ganga and Gomti were screened using PCR for the prevalence of fifteen antimicrobial resistant genes responsible for resistance to antimicrobial agents of five antimicrobial families (β -lactams, Aminoglycosides, Tetracycline, Phenicol, Sulfonamides). The potential environmental reservoirs of Enterotoxigenic *Escherichia coli* (ETEC) and TEM- β -lactam resistant bacteria in Indo-Gangetic region were explored targeting *LT1* and *bla*_{TEM} gene respectively using qPCR in molecular beacon (MB) format. Observations indicate that aquatic flora and cultivated leafy vegetables were environmental reservoirs for (ETEC) and aquatic sediments, water & street food items were contaminated by bacteria exhibiting *bla*_{TEM} gene. The sensitivity of qPCR declines several folds in presence of high nonspecific DNA background. A pre analytical step in the form of DNA aptamer to bio-capture *Salmonella enterica* serovar Typhimurium from surface water, prior to DNA extraction, enhances the sensitivity of MB based qPCR assay targeting *invA* gene. qPCR used for *Salmonella* detection could not discriminate between viable and nonviable cells. Propidium monoazide (PMA) that selectively penetrates membrane compromised cells was coupled with *ttr* gene specific qPCR for quantifying viable salmonellae in source/potable waters collected from a north Indian city. A SYBR Green based qPCR array comprising of six modules (Module 1: Enterotoxigenic *Escherichia coli*, Module 2: *Salmonella* spp., Module 3: *Vibrio cholerae*, Module 4: *Enterococcus* spp., Module 5: *Pseudomonas aeruginosa*, Module 6: *Staphylococcus aureus*) targeting eighteen molecular signatures has been developed exploiting available information on genomes of pathogenic bacteria for quick, reliable, inexpensive, flexible, multigene detection of water-borne bacterial pathogens for pathogen surveillance in water resources and developing pre-emptive monitoring plans for implementation of preventive measures to protect human health. The use of a small-volume, high-speed, and microchip device-integrated PCR developed at Institute of Photonic Technology (IPHT), Jena, Germany was explored, for fast, cost effective and automatic detection of ETEC. A societal project on "Conservation of the river Ganga: Microbiologically safe water in rural area was conducted. The project attempts to disseminate the observations 'on bench' to general public by building on the scientific data on microbial safety of the river Ganga, to ensure their active involvement in restoration of river Ganga.

References

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2. Professor Alok Dhawan

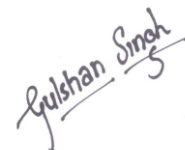
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3. Dr. P.D. Dwivedi

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Declaration

I here by declare that the above informations provided are true to the best of my knowledge.



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