JOSHI PRASHANT RATNAKAR

Flat No. 01,
Anuyog Apartment, Nandigram Colony, Garkheda,
Aurangabad 431009, Maharashtra, India
Email: joshpr183@gmail.com; Phone: +91- 9823167722
Gender: Male; Citizen of India
DOB: 26th September 1983

Career Summary / Research Expertise

Result-driven, detailed associate scientist, over 11 years of experience in the field of Enzymology, Molecular Biology, Genomics and drug discovery of novel anti-infective NCEs associated with research institute and pharmaceutical company in India. Research efforts led to the approvals of two US-IND targeting diverse target indications. A proven leader with strong project management and development abilities and a desire to achieve success and increase process productivity through process improvement.

EDUCATION

Doctorate in Philosophy (Perusing)

Under the supervision of Dr Smita Dharmadhikari, Government Institute of Sciences. Research titled as 'Spectrum gaps in recent Gram negative therapies: special emphasis on cephalosporin + beta lactamase inhibitor combinations'. Research aimed to decipher the dynamics of molecular resistance in *P. aeruginosa* for recent approved drugs such as ceftazidime + avibactam and ceftolozane + tazobactam. Specially assessing, how the enzymatic (variants in beta lactamases) and non-enzymatic (variable expression of efflux and outer membrane impermeability) resistance mechanisms contributing cephalosporin resistance in P. aeruginosa?

Post Graduate (M.Sc) in Biotechnology Score (%): 60/100
August 2005 - July 2007 Dr. Babasaheb Ambedkar Marathwada University, India

Post Graduate Diploma (B.Sc.) Applied Biotechnology Score (%): 67.5/100
August 2004 - June 2005 Pune University, Pune India

Under Graduate (B.Sc) in Microbiology, Chemistry and Botany Score (%): 64.5/100
July 2001 - June 2004 Swami Ramanand Teerth Marathwada University, India

OTHER TRAINING

Scientific training on Biochemical, Molecular and Proteiomic studies at Louis Stokes Veteran affairs Medical Center, Cleaveland, OH, USA 16th January - 08th February 2016

- This scientific training aims to unrevealing the mechanism of action of drug molecules, the studies involved enzyme kinetics, crystallography, proteomic analysis of interaction of drug candidate with bacterial enzymes. These studies were performed under the guidance of Dr. Robert Bonomo and Dr. Krisztina M. Papp-Wallace.

Hands on training on Genetic Analyzer 3130 (DNA sequencer), Applied Biosystems, New Delhi 2008

- Proficient in handling genetic analyzer for sequencing of DNA by Sanger method.

RESEARCH EXPERIENCE

Research Scientist June 2011– present
Drug Discovery Research, Wockhardt Research Centre, India

Strategic role in identifying molecular resistance mechanisms in pathogenic bacteria by employing genomics and proteomics tools. Additionally, establishing minimum inhibitory concentration (MIC) Determination, Antibiotic profiling of chemical agents against a variety of commonly prevalent organisms in the hospital and the community.
Mechanism of action of NCEs was determined by employing techniques such as post-antibiotic effect, pharmacokinetics, and pharmacodynamics studies.
Enzyme kinetics studies for enzymes, Inhibition, and determination of IC_{50}, Km, Vmax, Kcat.
More than nine years of research experience in gene identification, characterization of genes and proteins using a polymerase chain reaction, gene sequencing, and isoelectric focusing of the proteins/enzymes.
Preparing presentations for key opinion leaders for in-depth analysis of the projects and scientific scrutiny.
In-depth understanding of pharmacokinetic and toxicological behavior of each antibacterial drug class. Key member of team involved in structure activity relationship (SAR) with reference to *in vivo* efficacy and antibacterial activity.
Led to selection of pharmacokinetic optimized new chemical entities (NCEs) with less frequent dosing potential.
Screening optimization and lead refinement of Extended spectrum β-lactamases (ESBL) inhibitors and cephalosporins.
Development of microbiological assays to differentiate enzyme inhibitor molecules

- Assay established on cephalosporin based enzyme inhibition
- Assay based on diffusion potential of antibiotic
- Assay based on metabolic stability
- Differentials based on *in vivo* efficacy

Member of core team with responsibility of selecting novel antibiotic drug discovery project based on scientific, medical and commercial needs for Wockhardt' s future new drug discovery programs

- Evolving criteria for considering various cephalosporin classes
- In-depth evaluation of each criteria for ultimate selection

Senior Research Fellow March 2008– June 2011
Institute of Research, VP's School of Biotechnology, Baramati, Pune
Research project 1:
Morphometry and Phylogeography of Honeybees and Stingless bees in India

Key role in establishing the microsatellite marker identification and isolation, amplification of microsatellite region using simple sequence repeat (SSR) marker and fragment analysis using 3130 Genetic Analyzer. Phylogenetic analysis of four different species of honey bees collected from different geographical regions of India.
Principal investigator: Dr. Sushama Chaphalkar, Ph D, Director, VP's School of Biotechnology
Funding Agency: Department of Biotechnology, India.

Research project 2:

Construction of cDNA library from *Syzygium cumini* and *Aegle marmelos*

The project objective was to conserve imperative medicinal properties of medicinal plants through preserving vital genes which express protein of interest. Key role in this project was to isolate and purify RNA from medicinal plants by ascorbic acid method (published research article on optimization of RNA isolation method). Construction of first and second strand of cDNA from *Syzygium cumini* and *Aegle marmelos* using oligo(dT)/GSP and Clonetech kit. EST Sequencing of cloned cDNA in Applied Biosystems 3130 genetic analyzer. cDNA of total 230 genes were synthesized and preserve in repository for further use.

Principal investigator: Dr. Sushama Chaphalkar, Ph D, Director, VP's School of Biotechnology.
Funding Agency: Indian Council for Agriculture Research, New Delhi.

TECHNICAL EXPERTISE:

Enzymology: Nitrocefin-based enzyme inhibition assay, Determination of kinetics parameters such as enzyme efficiency, potency of enzyme inhibitors by calculating IC_{50}, Ki , enzyme turnover Kcat, binding affinity of a molecule by calculating association rate Kon and dissociation rates Koff.

Molecular biology:

- RNA, DNA and protein isolation from mammalian, plant and bacterial cells. RNA and DNA quantitation using nanodrop, Spectorophotometer. Protein quantitation Bradford and Lawry's method,
- Gene expression analysis by RT-PCR
- Iso electric focusing, Pulse field gel electrophoresis,
- Restriction fragment length polymorphism (RFLP)
- Simple sequence repeat (SSR) marker analysis,
- gene cloning and expression: Gene of interest was digested using BamHI and XbaI restriction endonucleases and cloned in to pBAD (promoter for E.coli arabinose operon) and pBin19 vectors for expression of gene in *E. coli* and in *Agrobacterium tuminfacian* bacterial cell respectively
- Sanger sequencing using genetic analyzer,
- Southern and northern blotting,
- cDNA library preparation from Animal, plant and bacterial RNA.

Mircobiology: Maintenance of various bacterial cultures (aerobic and anaerobic), minimum inhibitory concentration (MIC) determination, Well and disk diffusion assays, Time-kill studies, Biofilm eradication assay, Mutation Prevention Concentration and resistance development studies, *in vitro* and *in vivo* screening of New Chemical Entities (NCEs) to find out the antibiotic like properties in them.

Animal handling: The animal handling has done in routine experiments in *in vivo* laboratory, where oral infection , subcutaneous, intranasal and intraperitoneal infection of pathogenic strains in mice to test the efficacy of cephalosporin based drugs either alone or in combination with β-lactamase inhibitors.

Bioinformatics: Data retrieval from NCBI, Data mining, Databanks (GenBank, Swissprot, EMBL, etc.), Sequence Alignment (BLAST, FASTA, MSA), Phylogenetic Prediction using PHYLIP, mega3.1, Protein Classification & Structure viewing using Rasmol

RESEARCH ARTICLES PUBLISHED

- **In vitro activity of a Novel Benzoquinolizine Antibiotic, Levonadifloxacin (WCK 771) against Blood Stream Gram Positive Isolates from a Tertiary Care Hospital**. Dhruv Mamtora, Sanjith Saseedharan, Ritika Rampal, Prashant Joshi, Pallavi Bhalekar, Jaishid Ahdal, Rishi Jain. 2020. Journal of laboratory physician,

- **High prevalence of Escherichia coli clinical isolates in India harbouring four amino acid inserts in PBP3 adversely impacting activity of aztreonam/avibactam**. Periasamy H, **Joshi P**, Palwe S, Shrivastava R, Bhagwat S, Patel M. 2020. J Antimicrob Chemother 75:1650–1651.

- **In Vitro and In Vivo Activities of β-Lactams in Combination with the Novel β-Lactam Enhancers Zidebactam and WCK 5153 against Multidrug-Resistant Metallo- β -Lactamase-Producing Klebsiella pneumoniae.** Moya B, Barcelo IM, Cabot G, Torrens G, Palwe S, **Joshi P**, Umarkar K, Takalkar S, Periasamy H, Bhagwat S, Patel M, Bou G, Oliver A. 2019. Antimicrob Agents Chemotherapy 63:1–9.

- **WCK 5107 (Zidebactam, ZID): A pan Gram-negative β-lactam Enhancer Augmenting β-lactam Pharmacodynamics in Wild Type and Carbapenemase Producers (CP)**. SR Palwe, Prashant Joshi, HN Khande, SS Biniwale, SS Bhagwat, MV Patel. SUNDAY-438, ASM Microbe 2016, Boston, USA.

- WCK 5222 [Cefepime (FEP)-WCK 5107 (Zidebactam, ZID)]: ***In Vitro* and *In Vivo* Coverage of OXA-carbapenemases Expressing-Acinetobacter (OXA-AB)**. Prashant Joshi, HN Khande, SS Takalkar, AM Kulkarni, RP Chavan, VS Zope, SR Palwe, SS Biniwale, SS Bhagwat, MV Patel. SUNDAY-440, ASM Microbe 2016, Boston, USA.

- **Cefepime (FEP) and WCK 5107 (Zidebactam, ZID) Mediated Dual PBP Engagement at Sub-MIC Concentrations Drive Cidality Against Diverse β-lactamases Expressing Gram-negatives**. SR Palwe, SS Biniwale, HN Khande, Prashant Joshi, SS Bhagwat, MV Patel. SUNDAY-448, ASM Microbe 2016, Boston, USA.

- WCK 5999 (Carbapenem-WCK 4234): ***In Vitro* and *In Vivo* Activity of Novel Broader-spectrum β-lactam-β-lactamase Inhibitor (BL-BLI) Against Indian OXA Carbapenemase (OXA-CARB) Expressing Acinetobacter (AB)**. HN Khande, SR Palwe, SS Takalkar, KV Umarkar, JS Satav, Prashant Joshi, SS Biniwale, SS Bhagwat, MV Patel. MONDAY-424, ASM Microbe 2016, Boston, USA.

- WCK 5999 (Carbapenem-WCK 4234): ***In Vitro* and *In Vivo* Activity of Novel β-lactam-β-lactamase inhibitor (BL-BLI) Against OXA β-lactamase-producing Klebsiella (KP) and Pseudomonas (PA).** HN Khande, SR Palwe, SS Takalkar, KV Umarkar, JS Satav, RP Chavan, VS Zope, Prashant Joshi, SS Biniwale, SS Bhagwat, MV Patel. MONDAY-423, ASM Microbe 2016, Boston, USA..

- WCK 5999 (Carbapenem-WCK 4234): ***In vitro* Activity of Carbapenem in combination with WCK 4234 Against ESBLs, Class C, KPC-expressing Enterobacteriaceae (ENT) isolates.** Prashant Joshi, HN Khande, SR Palwe, SS Biniwale, SS Bhagwat, MV Patel. MONDAY-426, ASM Microbe 2016, Boston, USA.

- **WCK 4282 (High-dose Cefepime-Tazobactam): Complementary Features Drive Efficacy against KPC-producing Pathogens**. Hemant Khande, Prashant Joshi, Snehal Palwe, Kushal Umarkar, Swapna Takalkar, Sachin Bhagwat, Mahesh Patel, P1264, 26th ECCMID, Amsterdam.

- **WCK 4282 (High-Dose Cefepime-Tazobactam) – Assessment of Synergy Through Time-kill Curve and MIC Determination Against Psuedomonas aeruginosa Producing OXA and VEB β-lactamases.** Prashant Joshi, Hemant Khande, Sachin Bhagwat, Mahesh Patel, P1259, 26th ECCMID, Amsterdam

- ***In vitro* activity of WCK 771 – a new benzoquinolizine quinolone in development , against key bacterial groups from the USA and Europe**, M. Hackel, Sachin Bhagwat, Hemant Khande, Prashant Joshi, Mahesh Patel,Poster ICAAC 2015, F-1196

- **Biochemical Role of Ascorbic Acid During the Extraction of Nucleic Acids in Polyphenol Rich Medicinal Plant Tissues**, Tushar Borse, Prashant Joshi, Sushama Chaphalkar Journal of Plant Molecular Biology and Biotechnology, Vol 2, No 2 (2011).

- **Phytochemical and Molecular investigation of Nakshatra Tree viz. Aegle Marmelos and Syzygium cumini** Tushar Borse, **Prashant Joshi**, Sushama Chaphalkar, Priya Kakade Poster Presented at National Conference on biodiversity of Medicinal and Aromatic plants at Anand; Gujarat held at November 24-25 2010 and awarded as second prize for it.

- **cDNA library construction of Aegle Marmelos and Syzygium cumini,** Tushar Borse, Prashant Joshi, Sushma Chaphalkar, Poster Presented at INSA Platinum Jubilee International Symposium on Research in Molecular Medicine on Natural Resources and Traditional Knowledge held at National Chemical Laboratory, Pune during 21-23 November 2009.

COMPUTER/BIO-INFORMATIC SKILLS

MS Office, Endnote, Mendeley Reference Manager, GraphPad Prism

PROFESIONAL AND ENDUCATIONAL REFEREES

1. **Dr. Satish Bhavsar,**
   Associate Director, Drug-discovery Research
   Wockhardt Research Center, D4 chikalthana M.I.D.C., Aurangabad
   +91-240-6694212 (O)
   +91-9822022343 (M)
   Email: sbhavsar@wockhardt.com

2. **Dr. Sushma Chaphalkar,**
   Director, VP's School of Biotechnology,
   Vidya nagari, M.I.D.C., Baramati, Pune
   +91-2112-239385 (O)
   +91-9890337759 (M)
   Email: director.vsbt@gmail.com

3. **Dr. Robert Bonomo,**
   Professor of Medicine
   Case Western Reserve University School of Medicine,
   Veteran Affairs Medical Center,
   Cleveland, Ohio, USA
   +1 (216) 401 4672
   Robert.Bonomo@va.gov