



Dr. Rishikesh Shukla

UGC Dr. D.S. Kothari Post Doctoral Fellow
Department of Microbiology,
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PERSONAL DETAILS

Full Name Rishikesh Shukla
Fathers Name Pramod Kumar Shukla
Mothers Name Suman Lata Shukla
Date of Birth Jan 19th, 1983
Nationality Indian
Sex & Marital Status Male & married and having one girl child
Permanent Address Shukla Bhawan, Near Gopinath's Temple,
Dana Oli, Lashkar, Gwalior-474001, M.P.

RESEARCH INTERESTS

Microbiology, Protein
Biochemistry,
Immunology,
Carbohydrate chemistry,
Enzymology, Cell Biology
and Molecular Biology.

RESEARCH EXPERIENCE

May 2016 – Present: UGC Dr. D.S. Kothari Post-Doctoral Fellow in Central University of Haryana, Mahendergarh, Haryana.

(Project Title: Cost effective strategy for the production of Erythritol using crops byproducts.

July 2014 – May 2016: Research Associate in project entitled "Development of Seaweeds Biorefinery and Pilot Demonstration of Bioethanol Production".

July 2008- Jan 2014: PhD from Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati, Assam.

(Thesis title: Dextranase and dextran with anticancer properties from probiotic *Pediococcus pentosaceus* CRAG3 isolated from fermented cucumber)

Oct 2013- March 2014: SRF in project entitled "Molecular and functional characterization of dextran production in *Weissella* spp. - Superior dextran producers for cereal applications" funded jointly by the Academy of Finland and the Indian Department of Biotechnology (DBT).

ACADEMIC PERFORMANCES

Degree	Subject	Institution / Board	Year	Percent/ Grade
Ph. D.	Biotechnology	IIT Guwahati, Guwahati	2014	9.0 / 10.0
M. Sc.	Microbiology	Jiwaji University, Gwalior	2007	80%
B. Sc.	Microbiology	Jiwaji University, Gwalior	2005	67.5%

TECHNICAL PROFICIENCY AND RESEARCH SKILLS

Biochemical techniques: Gel-electrophoresis Native, (SDS-PAGE), Paper chromatography, Gel filtration, Ion exchange chromatography, TLC, FPLC, HPLC (Varian), NMR, FTIR, Spectrophotometric techniques: UV-Vis, Scanning Electron Microscopy (SEM), FE-SEM, Flow Cytometry, Confocal Laser Scanning Microscopy, Atomic Force Microscopy (AFM).

Molecular Biology techniques: Competent cell preparation, Transformation, Isolation & purification of plasmids, PCR amplification, cloning, etc.

Cell culture techniques: Seeding, trypsinisation, MTT assay etc.

Computational Skills: Microsoft application (Word, PowerPoint, Excel), Adobe Applications (Photoshop, Acrobat), Sigma plot, Graph pad, Math type, Chem draw.

ACADEMIC HONOURS

- Secured highest marks in my batch during M.Sc. with 80%.
- GATE-2007 qualified and got fellowship from Indian Institute of Technology, Guwahati, Assam.

PUBLICATIONS (PUBLISHED/ACCEPTED/IN PRESS)

1. **Rishikesh Shukla**, Manoj Kumar, Subhojit Chakraborty, Rishi Gupta, Savindra Kumar Dinabandhu Sahoo and Ramesh Chander Kuhad (2016) Process development for the production of bioethanol from waste algal biomass of *Gracilaria verrucosa*. *Bioresource Technology*. (Submitted).
2. Seema Patel, **Rishikesh Shukla** and Arun Goyal (2015) Probiotics in valorization of innate immunity across various animal models. *Journal of Functional Foods*, 14, 549-561 (IF: 3.574)
3. Arabinda Ghosh, Anil Kumar Verma, Jagan Mohan Rao T, **Rishikesh Shukla** and Arun Goyal (2015) Recovery and purification of oligosaccharides from coprameal by recombinant endo- β -mannanase and deciphering molecular mechanism involved and its role as potent therapeutic agent. *Molecular Biotechnology*, 57, 111-127. (IF: 1.876)
4. Jagan Mohan Rao T, Damini Kothari, **Rishikesh Shukla**, and Arun Goyal (2014) Structural and biocompatibility properties of dextran from *Weissella cibaria* JAG8 as food additive. *International Journal of Food Science and Nutrition*, 65, 686-691. (IF: 1.206)
5. **Rishikesh Shukla** and Arun Goyal (2014) Purified dextransucrase from *Pediococcus pentosaceus* CRAG3 as food additive. *Indian Journal of Experimental Biology*, 52, 1036-1044. (IF: 0.835)
6. **Rishikesh Shukla**, Ilia Iliev and Arun Goyal (2014) *Leuconostoc mesenteroides* NRRL B-1149 as probiotic and its dextran with anticancer properties. *Journal of Bioscience and Biotechnology* 3(1), 79-87.
7. **Rishikesh Shukla** and Arun Goyal (2014) Probiotic potential of *Pediococcus pentosaceus* CRAG3 a new isolate from fermented cucumber. *Probiotics and Antimicrobial Proteins*, 6, 11-21.
8. **Rishikesh Shukla** and Arun Goyal (2013) Novel dextran from *Pediococcus pentosaceus* CRAG3 isolated from fermented cucumber with anticancer properties. *International Journal of Biological Macromolecules*, 62, 352-357. (IF: 2.858)
9. **Rishikesh Shukla** and Arun Goyal (2013) Elucidation of structure and biocompatibility of levan from *Leuconostoc mesenteroides* NRRL B-1149 *Current Trends in Biotechnology and Pharmacy* 7 (2) 635-643.
10. Veselin Bivolarski, Tonka Vasileva, **Rishikesh Shukla**, Arun Goyal and Ilia Iliev (2012) Physiological studies of *Leuconostoc mesenteroides* strain NRRL B-1149 during cultivation on glucose and fructose media. *Journal of Bioscience and Biotechnology*, 1(3), 235-240.
11. **Rishikesh Shukla** and Arun Goyal (2012) Optimization and scale-up of fermentation of glucansucrase and branched glucan by *Pediococcus pentosaceus* CRAG3 using Taguchi methodology in bioreactor. *Journal of Bioscience and Biotechnology*, 1(1), 73-82.
12. Seema Patel, Damini Kothari, **Rishikesh Shukla**, Debasish Das and Arun Goyal (2011) Scale up of dextran production from a mutant of *Pediococcus pentosaceus* (SPAm) using optimized medium in a bioreactor. *Brazilian Archives of Biology and Technology*, 54(6), 1125-1133.
13. Mayur Agrawal, **Rishikesh Shukla** and Arun Goyal (2011) UV Mutagenesis of *Leuconostoc mesenteroides* NRRL B-640 for generation of a mutant (B-640M) with hyper-producing dextransucrase activity. *Current Trends in Biotechnology and Pharmacy*, 5 (4), 1445-1453
14. **Rishikesh Shukla**, Shraddha Shukla, Veselin Bivolarski, Ilia Iliev, Iskra Ivanova and Arun Goyal (2011) Production and structural characterization of insoluble dextran produced in the presence of maltose from *Leuconostoc mesenteroides* NRRL B-1149. *Food Technology and Biotechnology* 49(3), 291-296. (IF: 0.920)
15. **Rishikesh Shukla**, Ilia Iliev and Arun Goyal (2010) Purification and characterization of dextransucrase from *Leuconostoc mesenteroides* NRRL B-1149. *Biotechnology and Biotechnological Equipment* 24(SE); 576-580. (IF: 0.622)
16. Avishek Majumder, Anshuma Mangtani, Seema Patel, **Rishikesh Shukla** and Arun Goyal (2009) Gluco-oligosaccharides production from glucan of *Leuconostoc mesenteroides* NRRL B-742 by microwave assisted hydrolysis. *Current Trends in Biotechnology and Pharmacy* 3(4), 405-411.

PATENT

The mutant of *Leuconostoc mesenteroides* NRRL B-640 giving higher production of dextran. **Application no. 5/KOL/2010.**

AS A REVIEWER

A reviewer in **Annals of Microbiology**

CONFERENCE PAPERS

International

1. **Rishikesh Shukla** and Arun Goyal (2013) Purification and characterization of glucansucrase from *Pediococcus pentosaceus* CRAG3. International Conference on Advances in Biotechnology & Bioinformatics (ICABB-2013) & X Convention of The Biotech Research Society, India, November, 25-27 2013; Dr. D. Y. Patil Biotechnology & Bioinformatics Institute, Pune, Maharashtra, India.
2. Arabinda Ghosh, **Rishikesh Shukla** and Arun Goyal (2013) Production of manno-oligosaccharides from copra meal by recombinant endo β -mannanase: Their potential role as prebiotics and antitumorogenic agent. International Conference on Advances in Biotechnology & Bioinformatics (ICABB-2013) & X Convention of The Biotech Research Society, India, November, 25-27 2013; Dr. D. Y. Patil Biotechnology & Bioinformatics Institute, Pune, Maharashtra, India.
3. **Rishikesh Shukla**, Ilia Iliev, Iskra Ivanova and Arun Goyal (2012) *Leuconostoc mesenteroides* NRRL B-1149 as probiotic and its dextran displaying anti-cancer properties. 6th Annual Convention of Association of Biotechnology and Pharmacy (ABAP), ICEHT-2012, December 20-22, 2012; Sri Venkateswara University, Tirupati, A.P., India.
4. Veselin Bivolarski, Tonka Vasileva, **Rishikesh Shukla**, Arun Goyal and Ilia Iliev (2012) Physiological studies of strain *Leuconostoc mesenteroides* NRRL B-1149 during cultivation on glucose and fructose media. National Youth Conference, Oct 19-20, 2012; Plovdiv University Plovdiv, Bulgaria.
5. **Rishikesh Shukla** and Arun Goyal (2012) Purification and characterization of glucansucrase and glucan by *Pediococcus pentosaceus* CRAG3. 18th International Conference (Post ISCBC-2012), January 28-30, 2012; Institute of Advanced Study in Science and Technology (IASST), Guwahati, Assam, India.
6. **Rishikesh Shukla**, Rwivoo Baruah and Arun Goyal (2012) Molecular identification of a probiotic strain *Pediococcus pentosaceus* CRAG3 isolated from fermented cucumber. 18th International Conference (Post ISCBC-2012), January 28-30, 2012, Institute of Advanced Study in Science and Technology (IASST), Guwahati, Assam, India.
7. **Rishikesh Shukla** and Arun Goyal (2011) Probiotic potential and biochemical characterization of glucan producing *Lactobacillus plantarum* (RS3) isolated from fermented cucumber. 52nd Annual Conference of Association of Microbiologists of India (AMI), November 03-06, 2011; Panjab University, Chandigarh, India.
8. **Rishikesh Shukla**, Arun Dhillon, Ilia Iliev, Iskra Ivanova and Arun Goyal (2011) Purification and characterization of *Leuconostoc mesenteroides* NRRL B-1149 sucrose hydrolyzing enzymes and enzyme synthesized oligosaccharides. 52nd Annual Conference of Association of Microbiologists of India (AMI), November 03-06, 2011; Panjab University, Chandigarh, India.
9. **Rishikesh Shukla**, Rwivoo Baruah and Arun Goyal (2011) Production, purification and characterization of polysaccharides and oligosaccharides produced by hydrolysis from *Leuconostoc mesenteroides* NRRL B-1149; 26th Carbohydrate Conference; November 23-25, 2011; Indian Institute of Chemical Biology, Kolkata, West Bengal, India.
10. Tonka Vasileva, **Rishikesh Shukla**, Iskra Ivanova, Arun Goyal and Ilia Iliev (2011) Acceptor reactions of mannitol and lactitol with glucosyltransferases from *Leuconostoc mesenteroides* B-1149 and *Leuconostoc mesenteroides* URE 13. 9th Carbohydrate Bioengineering Meeting, May 15-18, 2011; Technical University of Lisbon, Lisbon.
11. **Rishikesh Shukla**, Seema Patel, Damini Kothari, Soumyadeep Chakraborty, Debasish Das and Arun Goyal; "Combined effects of pH and dissolved oxygen on dextran production from a mutant of soil isolate *Pediococcus pentosaceus*(SPAm)", "International Symposium on Recent Advances in Cross Disciplinary Microbiology: Avenues and Challenges & International Workshop on rRNA sequencing, phylogeny & Next Generation Genome Sequencing", 51st Annual Conference of Association of Microbiologists of India (AMI); December 14-17, 2010; Ranchi, Jharkhand, India.
12. **Rishikesh Shukla**, Seema Patel, Damini Kothari, Debasish Das and Arun Goyal; "Combined effects of freely available nitrogen substrates and carbon source on dextransucrase production from a mutant of soil isolate *Pediococcus pentosaceus* (SPAm)"; 7th BRSI Convention and International Conference on Genomic Sciences & Indo-Italian Workshop on Industrial and Pharmaceutical Biotechnology November 12-14, 2010; Madurai Kamraj University, Madurai, India.
13. **Rishikesh Shukla**, Shraddha Shukla, Ilia Iliev and Arun Goyal (2010) Production and structural characterization of insoluble glucan produced in the presence of maltose from *Leuconostoc mesenteroides* NRRL B-1149. 4th International Congress on Bioprocess in Food Industries, October 5-8, 2010; Curitiba, Brazil.
14. **Rishikesh Shukla**, Ilia Iliev and Arun Goyal (2010) Purification and characterization of dextransucrase from *Leuconostoc mesenteroides* NRRL B-1149. 2nd Balkan Conference on Biology, May 21-23, 2010; Plovdiv University, Plovdiv, Bulgaria.
15. **Rishikesh Shukla**, Mayur Agrawal and Arun Goyal; "Structural characterization of the two mutants B-640M1 and B-640M2 of *Leuconostoc mesenteroides* NRRL B-640 for the enhanced production of

dextran"; 6th BRSI Convention and International Conference on Emerging Trends in Biotechnology; December 4-6, 2009 BHU, Varanasi, India.

16. Seema Patel, **Rishikesh Shukla** and Arun Goyal; "Antibiotic sensitivity profiling and characterization of two new bacterial isolates of *Leuconostoc* sp"; North-East Conference of Medical Microbiologists; April 25-26, 2009, Down Town Hospital Complex, Guwahati, India.
17. Shadab Ahmed, **Rishikesh Shukla** and Arun Goyal; "Purification and biochemical characterization of bi-functional recombinant derivative (Lic26A-GH5) of cellulosomal cellulase from *Clostridium thermocellum*"; 5th BRSI Convention and International Conference on Bioprocesses in Food Industries; November 6-8, 2008; Osmania University, Hyderabad, India.

MEMBERSHIPS

1. Life member of **Indian Science Congress (ISC)** Membership no. **L19490**
2. Life member of **Biotech Research Society of India (BRSI)** Membership no. **LM-1305**
3. Life member of **Association of Microbiologists of India (AMI)** Membership no. **2396-2009**
4. Life member of **Society of Biological Chemists, India (SBCI)** Membership no. **2722**

REFERENCES

Prof. Arun Goyal (PhD Supervisor) Department of Biosciences and Bioengineering Indian Institute of Technology Guwahati Guwahati, Assam, India 781039 Email: arungoyl@iitg.ernet.in Phone: +91-361-2582208 Fax: +91-361-2582249	Dr. Debashish Das Associate Professor Department of Biosciences and Bioengineering Indian Institute of Technology Guwahati Guwahati, Assam, India 781039 Email: debasishd@iitg.ernet.in Phone: +91-361-2582221 Fax: +91-361-2582249	Prof. R. C. Kuhad (PI) Vice Chancellor Central University of Haryana Village-Jant Pali, Mahendergarh, Haryana-123029 Email: kuhad85@gmail.com Phone: +91- 1285-249333 Fax: +91 1285-249402
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SUMMARY OF PAST RESEARCH EXPERIENCE

PhD thesis work

Title: Dextranucrase and dextran with anticancer properties from probiotic *Pediococcus pentosaceus* CRAG3 isolated from fermented cucumber

A lactic acid bacterium was isolated from fermented cucumber based on its ability to produce higher glucansucrase and glucan. The isolate CRAG3 displaying maximum enzyme activity (2.7 U/ml) and maximum total carbohydrate content (4.5 mg/ml) was selected for identification and characterization. Morphological, biochemical and physiological studies of the isolate showed its relation with other *Pediococcus* sp. The isolate CRAG3 was identified as *Pediococcus pentosaceus* and assigned the Genbank accession number JX679020¹. The culture supernatant of *Pediococcus pentosaceus* CRAG3 showed maximum glucansucrase activity (4.35 U/ml) and total carbohydrate content (15.4 mg/ml) at optimum conditions of 25°C and 180 rpm. The crude glucansucrase (0.67 U/mg) after fractionation with PEG-1500 (10%, w/v) gave 20.0 U/mg specific activity which was further purified by gel-filtration using Sephacryl S-300HR column and gave the specific activity of 46.0 U/mg. The PEG-1500 purified enzyme gave two bands of 224 and 188 kDa band on denaturing SDS-PAGE. However, after PAS staining, a single band of 224 kDa was observed under non-denaturing condition served it as glucansucrase. The enzyme purified with gel filtration gave single band of 224 kDa confirming the presence of glucansucrase. The purified glucansucrase showed maximum activity (20.0 U/mg) in 5% sucrose in 20 mM sodium acetate buffer (pH 5.4) at 30°C. It showed K_m of 15.42 mM and V_{max} of 20.9 U/mg. The effects of various metal ions on glucansucrase activity showed that Co^{2+} (4 mM), Ca^{2+} (2 mM), Mg^{2+} (2 mM) and Zn^{2+} (1 mM) enhanced the activity by 56%, 44%, 14% and 12%, respectively. However, the glucansucrase activity was decreased by 89% and 90% in presence of Mn^{2+} (8 mM) and Ni^{2+} (8 mM) respectively. The glucansucrase was stable up to 40°C when incubated for 1 h. The storage stability of glucansucrase showed that it was most stable at -20°C with half-life of 307 days. It was found to be stable at pH range of 5.4-7.0 where the residual activity of glucansucrase at pH 5.4 was 82% after 3 h. Tween80 and glycerol enhanced the stability of glucansucrase at 30°C with half-life ($t_{1/2}$) of 85.5 h and 15.53 h, respectively as compared to control (6.9 h).

The glucan synthesised using purified glucansucrase displayed average molecular weight of 2.93×10^5 . The porous glucan consisted of only glucose monomers linked by 75% α -(1→6) linear chain and 25% α -(1→3) branching as reflected in FTIR and NMR spectra². *In vitro* cytotoxicity effect of *Pediococcus pentosaceus* CRAG3 glucan on HeLa and HT29 cell lines showed its anti-cancer activity against both cell lines. The effect of glucan on murine macrophage (J774A.1) lines showed increase in its growth by 20%². The optimisation of media components for maximum glucansucrase and glucan production using Taguchi methodology showed significant increase in both glucansucrase activity and glucan concentration in optimized medium (10 U/ml and 16 mg/ml, respectively) as compared to unoptimized medium (4.5 U/ml and 4.5 mg/ml, respectively)³. The maximum glucansucrase activity achieved in shake flask and bioreactor level was 10.1 U/ml 10.2 U/ml, respectively. The maximum glucan concentration at shake flask level was 16 mg/ml. The enhancement in glucan concentration (24 mg/ml) was observed at bioreactor level probably due to higher growth of cells.

The isolate was evaluated for probiotic properties by *in vitro* studies¹. It showed 33% hydrophobicity, 43% autoaggregation and 10% coaggregation with *E. coli* after 5 h which are related to cell adhesion properties and may also constitute an important host defence mechanism against infection. It displayed good adhesion property with mammalian cell lines (HeLa and J774A.1). The isolate resisted 100 μ g/ml lysozyme for 2 h with 75.0% survival, tolerance against simulated gastric juice (pH 3.0) with 58% survival and tolerance (73%, 63% and 50% survival) in presence of 0.3%, 0.5% and 1.0% (w/v) bile salts, respectively. It showed 1.04 U/ml bile salts hydrolase activity with yellow halo (6 mm) around colonies. It exhibited antibacterial activity against *E. coli*, *S. aureus* and *B. subtilis* which might be due to the production of lactic acid. *Pediococcus pentosaceus* CRAG3 displayed ability to utilize prebiotics such as inulin and raffinose with 37.5% and 6%, respectively relative cell growth.

1. Shukla and Goyal, *Probiotics and Antimicrobial Proteins*, 2013, DOI 10.1007/s12602-013-9149-8-2013; 2 Shukla and Goyal, *International Journal of Biological Macromolecules*, 2013, 62, 352-357; 3. Shukla and Goyal, *Journal of Bioscience and Biotechnology*, 2012, 1(1), 73-82.



Rishikesh Shukla