CURRICULUM VITAE

Name	:	Anil K. Tyagi
Designation	:	**Vice Chancellor**
Institution and Address	:	Guru Gobind Singh Indraprastha University Sector 16-C, Dwarka New Delhi 110078
Phone & FAX	:	+91-11-25302104, 25302105 Fax: +91-11-28035243
Electronic Mail Address		aniltyagi@ipu.ac.in; aniltyagi@south.du.ac.in; akt1003@rediffmail.com
Website	:	http://ipu.ac.in, http://www.aniltyagi.org

Honours/ Awards

- Shanti Swarup Bhatnagar Prize by CSIR (1995)
- J.C. Bose National Fellow, Department of Science and Technology, GOI (2010)
- Vigyan Gaurav Samman Award by UP Government. (2010)
- Vice President, Society of Biological Chemists (India) from 2004-2006
- Ranbaxy Research Award by Ranbaxy Science Foundation (1999)
- P.S. Sarma memorial award by the Society of Biological Chemists (India) (1993)
- Dr. Nitya Anand Endowment Lecture Award by INSA (1999)
- C.R. Krishnamurthy Memorial Oration Award by CDRI, Lucknow (2007)
- Prof. S.H. Zaidi Oration Award by ITRC, Lucknow (2005)
- Dr. Kona Sampath Kumar prize by the University of Delhi (1983)
- Fellow of the National Academy of Sciences, India
- Fellow of the Indian Academy of Sciences, India
- Fellow of the Indian National Science Academy, India
- Fellow of the Society for Immunology and Immunopathology, India

Membership to professional associations/societies

- Member of Guha Research Conference
- Life Member of the Society of Biological Chemists (India)
- Life Member of Indian Society of Cell Biology
- Life Member of Association of Microbiologists of India

Education

Degree	University	Subject	Division	Year
Ph.D.	University of Delhi	Medical Biochemistry	-	1977
M.Sc.	University of Allahabad	Biochemistry	First	1972
B.Sc.	University of Meerut	Zoology, Botany, Chemistry	First	1970

Positions

Duration	Designation	Institution
August 2011 onwards	**Professor**	Department of Biochemistry, University of Delhi, South Campus, New Delhi-110021
August 2008 - August 2011	**Professor & Head**	Department of Biochemistry, University of Delhi, South Campus, New Delhi-110021
August 1999 - August 2008	**Professor**	Department of Biochemistry, University of Delhi, South Campus, New Delhi-110021
August 1996 - August 1999	**Professor & Head**	Department of Biochemistry, University of Delhi, South Campus, New Delhi-110021
May 1993 - August 1996	**Professor of Biochemistry**	Department of Biochemistry, University of Delhi, South Campus, New Delhi-110021
August 1990 - May 1993	**Head of the Department**	Department of Biochemistry, University of Delhi, South Campus, New Delhi-110021
June 1986 - August 1990	**Reader**	Department of Biochemistry, University of Delhi, South Campus, New Delhi-110021
June 1983 - June 1986	**Lecturer**	Department of Biochemistry, V.P. Chest Institute, Delhi-110007
May 1980 - June 1983	**International Visiting Associate**	Laboratory of Biochemical Pharmacology, NIADDK, NIH, Bethesda, MD USA
May 1978 - April 1980	**International Visiting Fellow**	National Cancer Institute, NIH, Bethesda, MD USA
January 1973 - April 1978	**CSIR – JRF SRF, PDF**	Department of Biochemistry, V.P. Chest Institute, Delhi-110007

Public Service / University Service / Administrative Experience / Consulting Activity

Member Scientific Advisory Committees of National Institutions

1. Member, Scientific Advisory Group, Translational Health Science and Technology Institute (THSTI), Udyog Vihar, Gurgaon from 2010 onwards.
2. Member Expert, Research Council of Institute of Genomics and Integrative Biology, Delhi, 1st January 2004-2007.
3. Member, Research Advisory Committee, Central Institute of Fisheries Technology (CIFT), Cochin, 2004-2007.
4. Member of Scientific Advisory Committee, National Centre for Cell Sciences (NCCS), Pune, 2003 -2010.
5. Member, Apex Committee of the Department of Biotechnology, Government of India on "New Programme Support in High Priority Area of Biology 2002-2007" at Indian Institute of Science, Bangalore.
6. Member of Scientific Advisory Committee, National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, 2001-2004.
7. Member of the Research Area Panels and Scientific Advisory Committee, Centre for DNA Finger Printing and Diagnosis (CDFD), Hyderabad, 1999-2011.
8. Member of Scientific Advisory Committee, Institute of Pathology, Indian Council of Medical Research, Safdarjung Hospital, New Delhi, 1998-2003.
9. Member of Scientific Advisory Committee, Tuberculosis Research Centre, Indian Council of Medical Research, Chennai, 1998-2004.
10. Member of the Project Advisory Committee on "Biochemistry, Biophysics and Molecular Biology", Department of Science and Technology, Government of India, 1998-2001.
11. Member of the Research Committee on "Animal Science and Biotechnology" Council of Scientific and Industrial Research, New Delhi, 1998-2001.
12. Member of the Research Council of Centre for Biochemical Technology, New Delhi, 1998-2001.
13. Member, Research Area Panels and Scientific Advisory Committee, National Institute of Immunology, New Delhi, 1996-2008.
14. External expert on the Board of Studies for Biotechnology, Banaras Hindu University, Varanasi, 1995-1996.
15. Expert Consultant to the Tuberculosis Research Programme (TBRU) of the National Institutes of Health, USA, 1993-1999.
16. Member Board of Studies for Biochemistry, Aligarh Muslim University, Aligarh, 1993-1995.
17. External expert on the Board of Research Studies in Science, The University of Kashmir, Srinagar, 1992-1995.
18. Member, Board of Research Studies, Faculty of Inter Disciplinary and Applied Sciences, University of Delhi, 1986-2006 and then 2008-2012.

Member of National / International Committees for evaluation / funding / review of scientific research

19. Member, APEX Committee, Vaccine Grant Challenge Programme, Department of Biotechnology, Government of India, New Delhi from 2011 onwards.
20. Member of Expert Committee for North Eastern Region Biotechnology Programmes, Department of Biotechnology, Government of India, 2009 onwards.
21. Member, Technical Advisory Committee (TAC) for advising, evaluating, reviewing and monitoring activities of National Research Development Corporation (NRDC), New Delhi for activities funded by DSIR, 2007-09.
22. Member, Task Force for Vaccines and Diagnostics in the areas of health care, Department of Biotechnology, Government of India, New Delhi, 2005-08.
23. Member, Task Force for Infectious Disease Biology, Department of Biotechnology, Government of India, New Delhi, 2005-08.
24. Member, Expert Committee, University Grants Commission (UGC), New Delhi for evaluation of major research projects, 2003-09.
25. Member, Task Force on International Collaborations, Department of Science and Technology, Government of India, 2001-05.
26. Member of the Task Force on Basic Research in Modern Biology, Department of Biotechnology, Government of India, 2000-2004.
27. Member of the International Programme Approval Committee (IPAC), Department of Biotechnology, Ministry of Science and Technology, New Delhi, 1998-2008.
28. Member of Research Council of Human Research Development Group, Council of Scientific and Industrial Research, New Delhi, 1998-2000.
29. Member, Project Review Committee on “Leprosy and Tuberculosis and Other Chest Diseases”, Indian Council of Medical Research, 2001-07.
30. Member of the Project Advisory Committee on "Biochemistry, Biophysics and Molecular Biology”, Department of Science and Technology, Government of India, 1998-2001.

Member Governing Bodies of Institutions

31. Chairman, Governing Body, Miranda House, University of Delhi from 2014 onwards.
32. Member, Governing Body, Moti Lal Nehru College, University of Delhi from 2014 onwards.
33. Member, Governing Body, Shivaji College, University of Delhi from 2011-2013.
34. Member, Governing Body, Ram Lal Anand College, University of Delhi from 2011-2013.
35. Member, Governing Body, University College of Medical Sciences (UCMS), University of Delhi from 2010-2012.
36. Member of Academic Council of University of Delhi, 1990-1993; 1996-1999; 2009-12.
37. Member, Governing Body, Acharya Narendra Dev College, New Delhi, 2008-2011.
38. Member, Governing Body, V.P. Chest Institute, University of Delhi, Delhi, 2008 onwards.
39. Member, Governing Body, ARSD College, University of Delhi, Dhaula Kuan, New Delhi, 2008-2010.

40. Member, Governing Body, Dayal Singh College, New Delhi, 2005-2008.
41. Member, Governing Body, Maulana Azad Medical College, New Delhi, 2005-2006.
42. Member, Governing Body, Sri Venkateswara College, New Delhi, 2003-2005.
43. Member, Governing Body, Rajkumari Amrit Kaur College of Nursing, New Delhi, 2001-2003.
44. Member, Governing Body, Lady Harding Medical College, New Delhi, 2000-2002.
45. Member, Governing Body, Acharya Narendra Dev College, New Delhi from 2000-2002.
46. Member, Governing Body of Sri Venkateswara College, University of Delhi, New Delhi, 1998-2000.
47. Member, Governing Body of Moti Lal Nehru College, University of Delhi, 1995-1997.
48. Member, Governing Body of Maitreyi College, University of Delhi, New Delhi, 1993-1995.

Member of Academic Committees of Scientific Institutions

49. Member, Academic Committee, Translational Health Science and Technology Institute, Gurgaon from August 2013 onwards.
50. Member, Academic Committee, National Institute of Immunology, New Delhi from 2013 onwards.
51. Member, Academic Committee, International Centre for Genetic Engineering and Biotechnology, New Delhi, January 2008-10.
52. Member, Advisory Committee of DRS Programme, Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, May 2007 to March 2012.
53. Member of Special Committee of the Special Centre of Molecular Medicine, Jawahar Lal Nehru University, New Delhi, 2004-2007.
54. Member of Special Committee, School of Life Sciences, Jawaharlal Nehru University, New Delhi, 2002-2005.
55. Member of the Academic Committee, Central Drug Research Institute, Lucknow, 2002-2005.
56. Member of Academic Committee, Centre for Biotechnology, Banaras Hindu University, Varanasi, 2001-2003.
57. Member of the Academic Committee of the International Centre for Genetic Engineering and Biotechnology, New Delhi, 1997-2001.
58. Member of the Academic Committee, Institute of Microbial Technology, Chandigarh, 1996-2004.
59. Member of the Academic Committee, National Institute of Immunology, New Delhi, October 1994-2009.
60. Member of Special committee for Centre of Biotechnology, Jawaharlal Nehru University, New Delhi, 1993-1996.
61. Member of academic committee for Biochemistry - Kurukshetra University, 1991-1994.

Other services

62. Member Committee of Courses for M.Phil. Biotechnology for designing, reviewing and running of various courses concerning M.Phil Biotechnology at University of Delhi, 1987 onwards.
63. Member, Institutional Biosafety Committee, National Institute of Immunology, New Delhi, 1999 onwards.
64. Member, Management Committee of Bakson Homoeopathic Medical College, Greater NOIDA, Gautam Budh Nagar, U.P., 2008-2011.
65. Member Committee of Courses for Biochemistry for designing, reviewing and modification of various curriculum of the University of Delhi pertaining to Biochemistry, 1983-2011.
66. Member, Sectional Committee IX (General Biology), Indian National Science Academy, New Delhi, 2004-2006.
67. Member, Sectional Committee X (General Biology), Indian National Science Academy, New Delhi, 2012-13
68. Member, Sectional Committee M-2 (Multidisciplinary Committee for Engineering and Applied Sciences), Indian National Science Academy, New Delhi, 2005-2007.
69. Member of the Biosafety Committee for the Ranbaxy Laboratories, Gurgaon, India, 2000-2002.
70. Member of the Biosafety Committee for the Jawahar Lal Nehru University, New Delhi, 1994-1997.
71. Member of the Biosafety Committee for the Centre for Biochemical Technology, Delhi, 1994-1997.
72. Member of the University - Industry interaction Cell, University of Delhi, 1991-1994.
73. Chairman, Institutional Animal Ethics Committee, University of Delhi South Campus, 2008-13.

Delivered invited lectures at:

1. International Conference on Plant Biotechnology, Molecular Medicine and Human Health, Department of Genetics, UDSC, New Delhi, Chaired a session and delivered a talk, 18th to 20th October 2013.
2. Zoonotic Mycobacterial Infections and their Impact on Public Health, AIIMS, New Delhi, 25th-27th February 2013.
3. Refresher Course in Life Science, UDSC, New Delhi, 15th March 2013
4. Science, Technology and Innovation (STI) Policy – a Brainstorming conference on implementation aspects, National Institute of Plant Genome Resarch, New Delhi, 2nd March, 2013
5. Symposium on “Vaccines for India: Innovations and Roadmap”, St. Johns Research Institute, Bangalore, 5th February 2013.
6. National Symposium on Microbes in Health and Agriculture, JNU, New Delhi, 12th and 13th March 2012.

7. Indo-Swedish Conference on "Post Genomic Opportunities in Tuberculosis and Other Mycobacteria Diseases, Unchagaon Fort, Bulandshahr, 29th – 31st January 2012.

8. International Symposium on "Vaccine to Translation", Suraj Kund, Faridabad, 14th – 17th November 2011.

9. "Celebration of 100 years of Chemistry", special lecture on "Development of TB Vaccines", Hans Raj College, University of Delhi, 26th March 2011.

10. UGC-SAP workshop on "Advances in Molecular Biologyand Biotechnology", Department of Plant Molecular Biology, UDSC, New Delhi, 25th March 2011.

11. Key note Lecture delivered in the Indo-Canada symposium on "Redox Status and Control in TB: From Basic Research to Drug Development", January 30th to February 1st, 2011, Hyderabad.

12. Rama-Robbins Lecture delivered during the annual meeting of the Indo-US Vaccine Action Programme, New Delhi 17th November 2010.

13. National Symposium on "Emerging Trends in Biotechnology", Indian Institute of Advanced Research, Gandhinagar, Ahmedabad, Gurjrat, 27th-28th April 2010.

14. International symposium on "Understanding and Managing the Pathogenic Microorganisms", Institute of Microbial Technology, Chandigarh, 22-24 January 2010.

15. International symposium on Trends in Drug Discovery and Development, Department of Chemistry, University of Delhi, 5th – 8th January 2010.

16. Inaugural Lecture for the Annual Function of Biochemistry Society, Institute of Home Economics, Hauz Khas, New Delhi, 15th December 2009.

17. International symposium on Emerging Trends in Biotechnology, Banaras Hindu University, Varnasi, 4th – 6th December 2009.

18. Indo-US Tuberculosis Consultation Meeing, National Institute of Immunology, New Delhi, July 2009.

19. 77th Annual Meeting of the Society of Biological Chemists (India), IIT Madras, Chennai, 18th – 20th December 2008.

20. Ranbaxy Science Foundation's 22nd Round Table Conference on "Challenges of MDR/XDR Tuberculosis in India", New Delhi, 13th December 2008.

21. International Symposium on Emerging Trends in Tuberculosis Research: Biomarkers, Drugs and Vaccines, ICGEB, New Delhi, 1st-3rd December 2008.

22. 49th Annual Conference of Association of Microbiologists of India – International Symposium on Microbial Biotechnology: Diversity, Genomics and Metagenomics, Delhi, 18th – 20th November 2008.

23. 22nd Meeting of the Joint Working Group of INDO-US Vaccine Action Programme, New Delhi, 23rd – 24th October 2008.

24. 32nd Annual Conference of Indian Association of Medical Microbiologists (IAMM), A CME on "Vaccinology - an update", AFMC, Pune, 22nd October 2008.

25. Symposium on Industrial application of microbial proteomics, Indian Institute of Advanced Research, Gandhi Nagar, Gujarat, 2nd-4th June 2008.
26. Symposium on Recent Trends in Biotechnology, Aligarh Muslim University, Aligarh, 16th January, 2008.
27. Indo-German Workshop on infectious diseases at INSA, New Delhi, 24th November 2007.
28. B.R. Ambedkar Centre, University of Delhi, Delhi, 10th July 2007.
29. Dr. C.R. Krishnamurthy Memorial Oration, ITRC, Lucknow, 5th June 2007.
30. Foundation Day Lecture at JALMA National Institute of Leprosy and Other Mycobacterial Diseases, Agra, 17th April 2007
31. Department of Genetics, University of Delhi South Campus, New Delhi-110021, 4th April 2007
32. Department of Biochemistry, Faculty of Science, MS University, Baroda, 7th March 2007.
33. International symposium on New Frontiers in Tuberculosis Research, ICGEB, New Delhi, 4th –6th December 2006.
34. Indo-UK Meeting organized by Royal Society, London, UK, 12th –14th September 2006.
35. Indo-Europe Meeting on Infectious Diseases, Bangalore, 5th –6th June 2006
36. International Conference on Opportunistic Pathogens in AIDS, New Delhi, 27th –29th March 2006.
37. Third Indo-Australian Conference on "Vaccines for Cancer, Infectious Diseases, Lifestyle and Degenerative Diseases" Hyderabad, 6th –8th March 2006.
38. 24th Biennial Conference of the Indian Association of Leprologists, JALMA, Agra, 12th - 14th November 2005.
39. Annual Meeting of the Society of Biological Chemist(s) and Molecular Biologists, India, Lucknow, 7th –10th November 2005.
40. Brainstorming workshop on Tuberculosis, ICGEB, New Delhi, 19th – 21st May 2005.
41. Prof. S.H. Zaidi Oration at Industrial Toxicology Research Centre, Lucknow 3rd November 2005.
42. Symposium on Tuberculosis Research – An Indian Perspective (TRIP), AstraZeneca Bangalore, India, 20th October 2005.
43. INDO-Australian Symposium, "Modern Biological Approaches for the Diseases caused by Mycobacteria and Helicobacter" CDFD, Hyderabad, 5th March 2005.
44. 59th National Conference on Tuberculosis and Chest Diseases, New Delhi, 3rd-6th February 2005.
45. Asian Regional Workshop on International Training and Research in Emerging Infectious Diseases, JNU, New Delhi, 8th –11th March 2005.

46. Ranbaxy Science Foundation's 15th Round Table Conference on "HIV and Tuberculosis: Co-Infections", New Delhi, 8th January 2005.

47. International symposium on "Emerging Trends in Tuberculosis Research", 15th –17th November 2004, New Delhi, India

48. INDO-US Workshop on "AIDS in India: A workshop-symposium on Research, Trials and Treatment", 2nd – 4th August 2004, Bangalore, India.

49. INDO-UK Tuberculosis Meeting organized by the Royal Society London and DST, India, Hyderabad, 12th –13th January 2004.

50. ICMR-INSERM Workshop on Tuberculosis, Agra, India, 12th – 14th December 2003.

51. 10th Congress of Federation of Asian and Oceanian Biochemists and Molecular Biologists, Bangalore, India, 7th –11th December 2003.

52. Global challenges in TB: An update. V.P. Chest Institute, Delhi, 6th April 2003.

53. Tuberculosis Discussion Meeting organized by Royal Society, London, UK, 9th -10th December 2002.

54. INDO-German Workshop on Infectious Diseases, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, 11th -13th December 2002.

55. BCG Group Meeting for the development of a vaccine against AIDS, International AIDS Vaccine Initiative, New York, 19th June 2002.

56. Symposium on "The Frontiers of Molecular Medicine", Special Centre for Molecular Medicine, Jawaharlal Nehru University, New Delhi, 2nd February 2002.

57. Refresher Course for teachers in Biochemistry, B.R. Ambedkar Centre, University of Delhi, Delhi, 6th October 2001

58. 1st Conference of Biotechnology Society of India, "Biotecon-2001", New Delhi, 4th – 6th October 2001.

59. International symposium on "Mycobacterial Diseases: Pathogenesis, Protection and Control", Calcutta, January 2001.

60. Annual meeting of the Association of Microbiologists of India (AMI), Jaipur, November 2000.

61. ATA-Apollo Millennium Medical Conference, Hyderabad, December 2000.

62. INDO-GERMAN Workshop on Tuberculosis Braunschweig, Germany, 18th –20th September 2000

63. ILTP Workshop – INDO-RUSSIAN Collaboration in Biotechnology, Moscow, Russia, 24th – 30th June 2000.

64. The first Sir Dorabji Tata Symposium – Status of tuberculosis in India, March 11-12, 2000.

65. 5th International Conference on Emerging Infectious Diseases in the Pacific Rim, Chennai, 7th – 9th January 2000.

66. Dr. Nitya Anand Endowment Lecture 1999 (awarded by INSA), Tata Institute of Fundamental Research, Bombay, 27th December 1999.

67. International training and research in emerging infectious diseases - Asian Regional Workshop on Intracellular Pathogens, New Delhi, 6th – 10th December 1999.

68. WHO/IUIS Refresher Course on immunology, vaccinology and biotechnology applied to infectious diseases, Pune, 24th November – 10th December1999.

69. Indo-US Vaccine Action Programme, Joint workshop on Novel Vaccine Technologies, 26th – 27th October 1999.

70. Indo-French Symposium on Multiple Drug Resistance and Emerging Diseases, New Delhi, March 1999.

71. Annual Meeting of the Society of Biological Chemists, India, New Delhi, December 1998.

72. 12th International Congress of Immunology, New Delhi, November 1998.

73. HIV Vaccine Development Initiative by India - Seminar arranged by NACO and Ministry of Health, New Delhi, November 1998.

74. Department of Biological Sciences, Institute of Bacteriophages, University of Pittsburgh, Pittsburgh, USA, October 1998.

75. “Reemerging Infectious Diseases” - symposium held during the meeting of Indo-US Vaccine Action Programme, Washington, DC, USA, October 1998.

76. "Mycobacterial Genome" August - symposium arranged by : Bioinformatics Centre, JNU, August 1998.

77. Host Pathogen defences in Mycobacterium tuberculosis and HIV Infections: Emerging scenario, National Institute of Immunology, New Delhi, 1998.

78. Brain Storming Session on "Development and deployment of target molecules from New Bioactive Substances" held at CCMB, Hyderabad, 1st – 2nd August 1998.

79. Indo-European Commission Symposium on Tuberculosis Research: Into the 21st Century, Chennai, 3rd – 5th February 1998.

80. ASTRA-CCMB Symposium on Molecular Aspects of Microbial Pathogenesis, Hyderabad, 11th – 13th January 1998.

81. 38th Annual Meeting of the Indian Science Congress, Hyderabad, 3rd – 6th January 1998.

82. Centre for Genetic Engineering, MK University, Madurai, March 1997.

83. Department of Biochemistry, M.S. University, Baroda, February 1997.

84. 37th Annual Meeting of the Association of Microbiologists of India, Chennai, 4th – 6th December 1996.

85. Department of Biochemistry, North-Eastern Hill University, Shillong, September 1996.

86. International conference on Eukaryotic Expression Vector Systems: Biology and Applications, National Institute of Immunology, New Delhi, February 1996.

87. Institute of Nuclear Medicine and Allied Sciences, New Delhi, January 1996.

88. Workshop on Infectious diseases: diagnostics, prophylactics, and therapeutics, National Institute of Immunology, December 1995.

89. International Symposium on Trends in Microbiology, Bose Institute, Calcutta, December 1995.

90. Annual meeting of the Society of Biological Chemists, India, Lucknow, October 1995.

91. Symposium on Pasteur's Heritage: from Molecular asymmetry/Industrial fermentation to causality and cure of infectious diseases, Institute of Microbial Technology, Chandigarh, September 1995.

92. Albert Einstein Medical College, New York, USA, April 1995.

93. Institute of Public Health Services, New York, USA, April 1995.

94. John L. McClellan Memorial Veteran's Hospital, Little Rock, USA, April 1995.

95. XI National Symposium on Developmental Biology, Maharshi Dayanand University, Rohtak, March 1995.

96. First Congress of Federation of Indian Physiological Societies, New Delhi, March 1995

97. XVIII All India Cell Biology Conference and Symposia, National Botanical Research Institute, Lucknow, February 1995.

98. Third Asian Conference on Transcription, Bangalore, September 1994.

99. Institute of Microbial Technology, Chandigarh, August 1994.

100. Department of Biochemistry, Banaras Hindu University, Varanasi, July 1994.

101. UGC sponsored Refresher course in Biochemistry at Sri Venkateswara College, University of Delhi, April 1994.

102. Annual Meeting of the Society of Biological Chemists, India, Madurai, December 1993.

103. Department of Biochemistry, North Eastern Hill University, Shillong, December 1993.

104. UGC sponsored Refresher course in Biochemistry at Daulat Ram College, University of Delhi, July 1993.

105. Annual meeting of the Society of Biological Chemists, India, Hyderabad, December 1992.

106. National Chemical Laboratory, Pune, May 1992.

107. National Institute of Immunology, New Delhi, April 1992.

108. Department of Biochemistry, University of Allahabad - March 1992

109. Brain Storming session on Molecular Biology sponsored by TAB - CSIR Centre for Biochemicals, Delhi, March 1992.

110. Annual meeting of the Tuberculosis Association of India, New Delhi, January 1992.

111. International symposium on gene expression at Indian Institute of Science, Bangalore, December 1991.

112. Department of Plant Molecular Biology, University of Delhi, March 1991.

113. Symposium on Molecular Genetics, at the annual meeting of the Indian Science Congress, Indore - January 1991.

114. Laboratory of Biochemical Pharmacology, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, USA, May 1990.

115. The annual meeting of the Society of Biological Chemists India, New Delhi, October 1984.

Scientific meetings/Conferences attended/work presented

- International Conference on Plant Biotechnology, Molecular Medicine and Human Health, Department of Genetics, UDSC, New Delhi, Chaired a session and delivered a talk, 18th to 20th October 2013.
- Biotechnology Industry Research Assistance Council (BIRAC) Foundation Day and BIRAC Grand Challenge Meet, Indian Habitat Centre, New Delhi, 20th – 22nd March 2013.
- International Symposium on "Rotavirus Vaccines for India – The Evidence and the Promise" New Delhi, 14th & 15th May 2013.
- Zoonotic Mycobacterial Infections and their Impact on Public Health, AIIMS, New Delhi, 25th-27th February 2013.
- Refresher Course in Life Science, UDSC, New Delhi, 15th March 2013
- National Symposium on "Ramachandran Manifestation: Peptide to Proteome", UDSC, New Delhi, 14th-15th March 2013.
- Science, Technology and Innovation (STI) Policy – a Brainstorming conference on implementation aspects, National Insitute of Plant Genome Resarch, New Delhi, 2nd March, 2013
- Symposium on "Vaccines for India: Innovations and Roadmap", St. Johns Research Institute, Bangalore, 5th February 2013.
- National Symposium on Microbes in Health and Agriculture, JNU, New Delhi, 12th and 13th March 2012.
- Indo-Swedish Conference on "Post Genomic Opportunities in Tuberculosis and Other Mycobacteria Diseases, Unchagaon Fort, Bulandshahr, 29th – 31st January 2012.
- International Symposium on "Vaccine to Translation", Suraj Kund, Faridabad, 14th – 17th November 2011.

- "Celebration of 100 years of Chemistry", special lecture on "Development of TB Vaccines", Hans Raj College, University of Delhi, 26th March 2011.
- UGC-SAP workshop on "Advances in Molecular Biologyand Biotechnology", Department of Plant Molecular Biology, UDSC, New Delhi, 25th March 2011.
- Key note Lecture delivered in the Indo-Canada symposium on "Redox Status and Control in TB: From Basic Research to Drug Development", January 30th to February 1st, 2011, Hyderabad.
- Rama-Robbins Lecture delivered during the annual meeting of the Indo-US Vaccine Action Programme, New Delhi 17th November 2010.
- National Symposium on "Emerging Trends in Biotechnology", Indian Institute of Advanced Research, Gandhinagar, Ahmedabad, Gujrat, 27th-28th April 2010.
- International symposium on "Understanding and Managing the Pathogenic Microorganisms", Institute of Microbial Technology, Chandigarh, 22-24 January 2010.
- International symposium on Trends in Drug Discovery and Development, Department of Chemistry, University of Delhi, 5th – 8th January 2010.
- Inaugural Lecture for the Annual Function of Biochemistry Society, Institute of Home Economics, Hauz Khas, New Delhi, 15th December 2009.
- International symposium on Emerging Trends in Biotechnology, Banaras Hindu University, Varnasi, 4th – 6th December 2009.
- Indo-US Tuberculosis Consultation Meeing, National Institute of Immunology, New Delhi, July 2009.
- 77th Annual Meeting of the Society of Biological Chemists (India), IIT Madras, Chennai, 18th – 20th December 2008.
- Ranbaxy Science Foundation's 22nd Round Table Conference on "Challenges of MDR/XDR Tuberculosis in India", New Delhi, 13th December 2008.
- International Symposium on Emerging Trends in Tuberculosis Research: Biomarkers, Drugs and Vaccines, ICGEB, New Delhi, 1st-3rd December 2008.
- 49th Annual Conference of Association of Microbiologists of India – International Symposium on Microbial Biotechnology: Diversity, Genomics and Metagenomics, Delhi, 18th – 20th November 2008.
- 22nd Meeting of the Joint Working Group of INDO-US Vaccine Action Programme, New Delhi, 23rd – 24th October 2008.
- 32nd Annual Conference of Indian Association of Medical Microbiologists (IAMM), A CME on "Vaccinology - an update", AFMC Pune, 22nd October 2008.
- Symposium on Industrial application of microbial proteomics, Indian Institute of Advanced Research, Gandhi Nagar, Gujarat, 2nd- 4th June 2008.
- Symposium on Recent Trends in Biotechnology, Aligarh Muslim University, Aligarh, 16th January 2008.

- Indo-German Workshop on infectious diseases at INSA, New Delhi, 24th November 2007.
- International symposium on New Frontiers in Tuberculosis Research, ICGEB, New Delhi, 4th – 6th December 2006.
- Indo-UK Meeting organized by Royal Society, London, UK, 12th–14th September 2006.
- International Conference on Opportunistic Pathogens in AIDS, New Delhi, 27th – 29th March 2006.
- Indo-Europe Meeting on Infectious Diseases, Bangalore, 5th – 6th June 2006.
- Third Indo-Australian Conference on "Vaccines for Cancer, Infectious Diseases, Lifestyle and Degenerative Diseases" Hyderabad, 6th – 8th March 2006.
- 24th Biennial Conference of the Indian Association of Leprologists, JALMA, Agra, 12th - 14th November 2005.
- Annual Meeting of the Society of Biological Chemist(s) and Molecular Biologists, India, Lucknow, 7th – 10th November 2005.
- Symposium on Tuberculosis Research – An Indian Perspective (TRIP), AstraZeneca Bangalore, India, 20th October 2005.
- Brainstorming workshop on Tuberculosis, ICGEB, New Delhi, 19th – 21st May 2005.
- INDO-Australian Symposium, "Modern Biological Approaches for the Diseases caused by Mycobacteria and Helicobacter", CDFD, Hyderabad, 5th March 2005.
- 59th National Conference on Tuberculosis and Chest Diseases, New Delhi, 3rd - 6th February 2005.
- Asian Regional Workshop on International Training and Research in Emerging Infectious Diseases, JNU, New Delhi, 8th – 11th March 2005.
- Ranbaxy Science Foundation's 15th Round Table Conference on "HIV and Tuberculosis: Co-Infections", New Delhi, 8th January 2005.
- International symposium on "Emerging Trends in Tuberculosis Research", New Delhi, India 15th –17th November 2004
- Genetics – The Expanding Horizon, Department of Genetics, University of Delhi South Campus, New Delhi, 13th – 14th October 2004.
- INDO-US Workshop on "AIDS in India: A workshop-symposium on Research, Trials and Treatment", 2-4 August 2004, Bangalore, India.
- INDO-UK Tuberculosis Meeting organized by the Royal Society London and DST, India, Hyderabad, 12th –13th January 2004.
- ICMR-INSERM Workshop on Tuberculosis, Agra, India, 12th – 14th December 2003.
- 10th Congress of Federation of Asian and Oceanian Biochemists and Molecular Biologists, Bangalore, India, 7th – 11th December 2003.
- Global challenges in TB: An update. V.P. Chest Institute, Delhi, 6th April 2003.

- Tuberculosis Discussion Meeting organized by Royal Society, London, UK, 9th -10th December 2002.
- INDO-German Workshop on Infectious Diseases, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, 11th - 13th December 2002.
- BCG Group Meeting for the development of a vaccine against AIDS, International AIDS Vaccine Initiative, New York, 19th June 2002.
- Symposium on "The Frontiers of Molecular Medicine", Special Centre for Molecular Medicine, Jawaharlal Nehru University, New Delhi, 2nd February 2002.
- Expert Advisory Group Committee Meeting under INDO-US VAP Programme, Paris, 3rd November 2001.
- 1st Conference of Biotechnology Society of India, "Biotecon-2001", New Delhi, 4th – 6th October 2001.
- International symposium on "Mycobacterial Diseases: Pathogenesis, Protection and Control", Calcutta, January 2001.
- Annual meeting of the Association of Microbiologists of India (AMI), Jaipur, (Delivered a lecture and chaired a session), November 2000.
- ATA-Apollo Millennium Medical Conference, Hyderabad, December 2000.
- INDO-GERMAN Workshop on Tuberculosis Braunschweig, Germany, 18th-20th September 2000
- ILTP Workshop – INDO-RUSSIAN Collaboration in Biotechnology, Moscow, Russia, 24th – 30th June 2000.
- The First Sir Dorabji Tata Symposium – Status of tuberculosis in India, March 11th – 12th, 2000.
- 5th International Conference on Emerging Infectious Diseases in the Pacific Rim, Chennai, 7th – 9th January 2000.
- International training and research in emerging infectious diseases - Asian Regional Workshop on Intracellular Pathogens, New Delhi, 6th–10th December, 1999.
- WHO/IUIS Refresher Course on immunology, vaccinology and biotechnology applied to infectious diseases, Pune, 24th November – 10th December 1999.
- Indo-US Vaccine Action Programme on Novel Vaccine Technologies, October 1999.
- Indo-French Symposium on Multiple Drug Resistance and Emerging Diseases, New Delhi, March 1999.
- Fourth International Meeting on the Pathogenesis of Mycobacterial Infections, Stockholm, Sweden, July 1999.
- Annual Meeting of the Society of Biological Chemists, India, New Delhi, December 1998.
- 12th International Congress of Immunology, New Delhi, (Delivered seminar and chaired a session), November 1998.

- HIV Vaccine Development Initiative by India - Seminar arranged by NACO and Ministry of Health, New Delhi, November 1998.
- "Reemerging Infectious Diseases" - symposium held during the meeting of Indo-US Vaccine Action Programme, Washington D.C., USA, October 1998.
- Mycobacterial Genome: Bioinformatics Centre, JNU, 25th August 1998.
- Host Pathogen defences in Mycobacterium tuberculosis and HIV Infections: Emerging scenario, National Institute of Immunology, New Delhi-110067, 10th – 11th August 1998.
- Brain Storming Session on "Development and deployment of target molecules from New Bioactive Substances" held at CCMB, Hyderabad, 1st – 2nd August 1998.
- Annual Meeting of the Tuberculosis Research Unit of NIH, Cleveland, USA, 14th – 15th June 1998.
- Indo-Eurpean Commission Symposium on Tuberculosis Research: Into the 21st Century, Chennai, 3rd – 5th February 1998. (*Delivered a seminar and chaired a session*).
- ASTRA-CCMB Symposium on Molecular Aspects of Microbial Pathogenesis, Hyderabad, 11th – 13th January 1998.
- Annual Meeting of the Indian Science Congress, Hyderabad, 3rd – 6th January 1998.
- 38th Annual Meeting of the Association of Microbiologists of India, New Delhi, 12th – 14th December 1997.
- IBY2K (Indian Biology beyond the year 2000) Symposium at CCMB, Hyderabad, 24th – 27th November 1997 (*Chaired a session*).
- Diversity in Modern Biology - An Interdisciplinary Symposium held at New Delhi, 21st – 22nd September 1997.
- WHO Meeting on the Diagnosis of Tuberculosis, Cleveland, USA 26th June 1997.
- Annual Meeting of the Tuberculosis Research Unit of NIH, Cleveland, USA, 24th – 25th June 1997.
- 32nd US-Japan Co-operative Medical Science Programme Tuberculosis-Leprosy Research Conference held at Cleveland, USA, 21st – 23rd June 1997.
- Bimal K. Bachhawat Symposium on Genomic Research Emerging Ethical, Legal, Social and Economic issues Sarovar Park Plaza Resort, Goa, 22nd – 25th May 1997.
- 37th Annual Meeting of the Association of Microbiologists of India, Chennai, 4th – 6th December 1996.
- International conference on Eukaryotic Expression Vector Systems: Biology and Applications, National Institute of Immunology, New Delhi, 4th - 8th February 1996.

- Workshop on Infectious diseases: diagnostics, prophylactics, and therapeutics, National Institute of Immunology, 21st - 22nd December 1995.
- International Symposium on Trends in Microbiology, Bose Institute, Calcutta, 4th - 8th December 1995.
- Symposium on Pasteur's Heritage: from Molecular asymmetry/Industrial fermentation to causality and cure of infectious diseases, Institute of Microbial Technology, Chandigarh, 27th - 29th September 1995.
- XI National Symposium on Developmental Biology, Maharshi Dayanand University, Rohtak, 25th - 27th March 1995.
- First Congress of Federation of Indian Physiological Societies, New Delhi, 1st - 3rd March 1995**.**
- XVIII All India Cell Biology Conference and Symposia, National Botanical Research Institute, Lucknow, 13th - 15th February 1995.
- Third Asian Conference on transcription, Indian Institute of Science, Bangalore, 25th - 27th September 1994.
- 16th International Congress of Biochemistry and Molecular Biology, New Delhi, India, 19th - 22nd September, 1994.
- 2nd International Conference on the pathogenesis of mycobacterial infections, Stockholm, Sweden, 2nd - 4th July, 1993**.**
- World Congress on tuberculosis, Bethesda, Maryland, USA**.** 16th-19th Nov. 1992**.**
- The annual meeting of the Tuberculosis association of India, New Delhi - 1992.
- The National Symposium on Liposome Research, University of Delhi South Campus, New Delhi, 1988, 1989, 1991, 1992**.**
- Brain Storming session on Molecular Biology, sponsored by TAB, held at the CSIR Centre for Biochemicals, New Delhi, March 1992**.**
- The annual meeting of the Clinical Biochemists of India, New Delhi, February 1992.
- The XV All India Cell Biology Conference and Symposia held at the University of Delhi South Campus, New Delhi, February 1992.
- Symposium on molecular genetics at the Annual meeting of the Indian Science Congress, Indore, India, January 1991.
- International Symposium on gene expression, Indian Institute of Science, Bangalore, December 1991
- Guha Research Conference, India, 1989, 1991, 1992, 1993, 1996, 1998, 2000, 2002, 2003. 2004, 2006, 2009, 2011.
- The International Symposium on eukaryotic cell surface macromolecules, University of Delhi South Campus, New Delhi, 1987.

- The Annual meeting of the American Society of Biochemists and Molecular Biologists, USA - 1980, 1981, 1982, 1990.
- Annual Meeting of the Society of Biological Chemists (India) - 1974, 1975, 1976, 1977, 1983, 1984, 1988, 1990, 1992, 1993, 1995, 1998, 2003, 2005, 2008.
- Gorden Research Conference on Polyamines - New Hampshire USA, 1981.
- The annual meeting of the American Association of Cancer Research, New Orleans, USA, 1979.
- International symposium on Biomembranes - Madurai Kamraj University, Madurai, December 1973.

Editorial Work

Academic Editor, PLoS ONE from 2009 onwards, published by Public Library of Science.

Member of Editorial Advisory Board for the journal Tuberculosis from 2012 onwards.

Member of the Editorial Board for the Journal "Indian Journal of Medical Research" published by ICMR, New Delhi, 2003 onwards.

Member of Editorial Board for the journal "Tuberculosis" published by Elsevier Press, 2003-2007.

TEACHING EXPERIENCE

M.Sc., BIOCHEMISTRY	:	Molecular biology, Molecular genetics, Recombinant DNA technology, enzymes, carbohydrate metabolism
M.Sc., GENETICS	:	Molecular biology
M.Sc., MICROBIOLOGY	:	Molecular biology
M.Phil., BIOTECHNOLOGY	:	Molecular genetics and Molecular biology

DETAILS OF TEACHING EXPERIENCE
Total teaching experience = 35 years

M.Phil. Biotechnology	1988-2013	Molecular Biology
M.Sc. Microbiology	1994-2009	Molecular Biology
M.Sc. Genetics	1986-1989	Recombinant DNA Technology
M.Sc. Genetics	1986-2009	Molecular Biology
M.Sc. Biochemistry	1985-1989	Recombinant DNA Technology
M.Sc. Biochemistry	1985-2013	Molecular Biology
M.Sc. Biochemistry	1985-1987	Molecular genetics
M.Sc. Biochemistry	1983-1987	Enzymes, Carbohydrate metabolism

*M.D. Medical Biochemistry	1974-1978	Enzymes
*M.Sc. Medical Biochemistry	1974-1978	Enzymes, metabolism

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These classes were taught while working as JRF/SRF during Ph.D. and during the post-doctoral period.

Development of curriculum for various courses

Major contribution in developing the curriculum for the following courses

- Development of new revised syllabus for B.Sc. (Hons) Biochemistry, University of Delhi, 2010.
- Development of new/revised curriculum for M.Sc. Biochemistry, University of Delhi, 2009.
- Development of revised curriculum for B.Sc. (Hons) Biochemistry for Delhi University, 1998.
- Development of revised curriculum for post-graduate diploma in Molecular and Biochemical Technology, University of Delhi, 1998.
- Development of Curriculum for M.Sc. Biochemistry, Kurukshetra University, 1991.
- Development of curriculum for postgraduate diploma course in Biochemical Technology, University of Delhi, 1990.
- Development of revised/advanced curriculum for M.Sc. Biochemistry, University of Delhi, 1989.
- Development of Curriculum for M.Phil Biotechnology, University of Delhi, 1988.
- Development of curriculum for B.Sc.(Hons) Biochemistry Course for Delhi University, 1987.
- Development of new/revised curriculum for M.Sc. Biochemistry, University of Delhi, 1985.

Meetings / Symposia / Refresher courses organized

- Co-Convenor of the National Symposium on "Ramachandran Manifestation: Peptide to Proteome", UDSC, New Delhi, 14^{th}-15^{th} March 2013.
- Co-Convenor of the symposium on "Systems Biology" held at the Department of Biochemistry, University of Delhi South Campus, New Delhi, 26^{th} March 2012.
- Co-Convenor of the symposium-cum-workshop on "Next Generation Sequencing Data Analysis" held at the Department of Biochemistry, University of Delhi South Campus and JNU, New Delhi, 28^{th} – 29^{th} January 2011.
- Co-Convenor of the national conference on "Drug Discovery and Development" held at the University of Delhi South Campus, New Delhi, organized by Bioinformatics Centre, Sri Venkateswara College in association with Bioinformatics Centre, DISC, University of Delhi South Campus, 21^{st} – 23^{rd} January 2009.

- Co-Convenor of the symposium-cum-workshop on "Computational Biology – Construction of databases" held at the Department of Biochemistry, University of Delhi South Campus and JNU, New Delhi, 14th - 15th March 2008.
- Co-Convenor of the symposium on "Systems Biology" held at the Department of Biochemistry, University of Delhi South Campus and JNU, New Delhi, 12th - 13th March 2006.
- Chairman, Organizing Committee for Brain Storming Session on Tuberculosis held at ICGEB, New Delhi, 19th - 21st May 2005.
- Co-Convenor of the workshop entitled, "Machine Learning Techniques in Bioinformatics" held at the Department of Biochemistry, University of Delhi South Campus and JNU, New Delhi, 28th - 29th March 2005.
- Co-Convenor of the Workshop entitled, "Biological databases – Mining of Information" held at the Department of Biochemistry, University of Delhi South Campus and JNU, New Delhi, 28th - 29th March 2003.
- Co-Convenor of the Workshop entitled, "Applications of Genomics and Proteomics" held at the Department of Biochemistry, University of Delhi South Campus and JNU, New Delhi, 1st - 3rd February 2002.
- Convenor of the Workshop entitled, "Bioinformatics and its Application to Biology" held at the Department of Biochemistry, University of Delhi South Campus, New Delhi, 22nd - 23rd March 2000.
- Joint-convenor of the meeting - TRendys in Biochemistry, held at the University of Delhi South Campus, New Delhi, 23rd – 24th November 1999.
- Convener of the symposium on "Microbial Infections: Diagnostics, Prevention and Cure" during the 38th Annual Meeting of the Association of Microbiologists of India held at New Delhi, 12th – 14th December 1997.
- Joint-Convener of "Diversity in Modern Biology - an Interdisciplinary Symposium" held at University of Delhi South Campus, 21st – 22nd September 1997
- Course in charge for the refresher course in biochemistry sponsored by the University Grants Commission, 28th June – 17th July 1993.
- Co-convener of the Guha Research Conference held at Dalhousie, 17th – 20th May 1993.
- Course Incharge for the refresher course in Immunology sponsored by the University Grants Commission, 28th September - 17th October 1992.
- Course in charge for the refresher course in Biochemistry sponsored by the University Grants Commission, 31st March – 19th April 1991.
- Course-Incharge for the workshop on Nucleic Acid Probes held on the auspices of annual meeting of the Clinical Biochemists of India, at G.T.B. Medical College, New Delhi, February 1991.
- Convener of the Annual meeting of the Society of Biological Chemists (India), New Delhi, 1984.

DETAILS OF RESEARCH EXPERIENCE

Current Research Activities

The current research activities are focused on understanding the molecular biology of mycobacteria and developing strategies for prevention and control of tuberculosis. Techniques of molecular biology, structural biology, immunology, purification and characterization of proteins, DNA protein interactions, gene knock-outs, vaccine development strategies and animal experiments are the main tools employed. Various aspects of current research activities are:

- Vaccine development programme - Development of new vaccines against tuberculosis and evaluation of their efficacy in animal models.
- Drug discovery programme - Characterization and validation of potential drug targets of *Mycobacterium tuberculosis* and identification of new inhibitors for treatment of tuberculosis.
- Study of genes involved in the pathogenesis of *Mycobacterium tuberculosis*

Supervision of Research Work

Ph.D. awarded	:	23
Ph.D. thesis submitted	:	2
Ph.D. students currently working	:	7
M.Phil. (Biotechnology) awarded	:	2
M.D. (Medical Biochemistry) awarded	:	1

Publications

Total	:	114
Published Research papers	:	96
Book chapters	:	15
Published Scientific Reviews	:	3

Name of the important periodicals/books in which research papers/book chapters have been published

Journal of Bacteriology
Journal of Biological Chemistry
Biochemistry
Proceeding of National Academy of Sciences (USA)
Gene
Molecular Microbiology
Methods in Enzymology
Journal of Infectious Diseases
Nucleic Acid Research
Nature Chemical Biology
Microbiology (U.K.)
European Journal of Biochemistry
Cancer Research

PLoS One
Biochemical Biophysical Research Communications
Achieves of Biochemistry and Biophysics
Biochemical Pharmacology
Physiology and genomics
Molecular Genetics for Mycobacteria, ASM Press, Washington DC
Advances in Polyamine Research, Raven Press, New York
Advances in Pharmacology and Chemotherapy, Academic Press, New York
The Mycobacteria Cell Envelope, ASM Press, Washington DC
Trends in Pharmacological Sciences
Journal of Applied Bacteriology
Federation Proceedings

Details of patents taken, if any.

1. **Title:** Mutants of mycobacteria and process thereof.
   Indian Patent Application No. 882/DEL/2003 dated 09.07.2003
   Investigators: Anil Tyagi *et al.*
   (Patent granted on 19th March 2014 (Patent No.259594).

2. **Title:** Mutants of mycobacteria and process thereof.
   PCT Application No. PCT/IN04/002003
   Investigators: Anil Tyagi *et al.*
   (*Patent granted by Singapore Patent Office, application pending in USA, Brazil and Japan*)

3. **Title:** Recombinant BCG-Ag85C based immunization against tuberculosis.
   Indian Patent Application No. 2639/DEL/2008 dated November 21, 2008
   Investigators: Anil Tyagi et al.

4. **Title:** Alpha-crystallin based immunization against *Mycobacterium* and methods thereof.
   Indian Patent Application No.473/DEL/2009 dated March 9, 2009
   Investigators: Anil Tyagi et al.

5. **Title:** A simple and fast process for evaluating promoter activity of persistent *M. tuberculosis* in hypoxic conditions using *M. smegmatis* as a surrogate host
   Indian Patent Application No. 981/DEL/2003
   Investigators: Jaya Tyagi *et al.*

PUBLICATIONS

1. Garima Khare, Praveen Kumar, **Anil K Tyagi**. (2013). Whole-Cell Screening-Based Identification of Inhibitors against the Intraphagosomal Survival of*Mycobacterium tuberculosis.* Antimicrobial Agents and Chemotherapy. doi:10.1128/AAC.01444-13.

2. Garima Khare, P. Vineel Reddy, Pragya Sidhwani & **Anil K. Tyagi** (2013). KefB inhibits phagosomal acidification but its role is unrelated to *M. tuberculosis* survival in host. Scientific Reports. 3 : 3527 | DOI: 10.1038/srep03527.

3. Priyanka Chauhan, P. Vineel Reddy, Ramandeep Singh, Neetika Jaisinghani, Sheetal Gandotra and **Anil K. Tyagi**. (2013). Secretory phosphatases deficient mutant of *Mycobacterium tuberculosis* imparts protection at the primary site of infection in guinea pigs. PloS ONE. 8(10): e77930. doi:10.1371.

4. Rupangi Verma Puri, Nisha Singh, Rakesh K. Gupta¤, **Anil K. Tyagi**. (2013). Endonuclease IV Is the Major Apurinic/Apyrimidinic Endonuclease in Mycobacterium tuberculosis and Is Important for Protection against Oxidative Damage. PLoS ONE 8(8): e71535. doi:10.1371/ journal.pone.0071535.

5. Rupangi Verma Puri, P. Vineel Reddy, **Anil K. Tyagi**. (2013). Secreted Acid Phosphatase (SapM) of Mycobacterium tuberculosis Is Indispensable for Arresting Phagosomal Maturation and Growth of the Pathogen in Guinea Pig Tissues. PLoS ONE 8(7): e70514. doi:10.1371/journal.pone.0070514.

6. P. Vineel Reddy, Rupangi Verma Puri, Priyanka Chauhan, Ritika Kar, Akshay Rohilla, Aparna Khera and **Anil K. Tyagi**. (2013). Disruption of mycobactin biosynthesis leads to attenuation of *Mycobacterium tuberculosis* for growth and virulence. Journal of Infectious Diseases. DOI: 10.1093/infdis/jit250.

7. Priyanka Chauhan, Ruchi Jain, Bappaditya Dey and **Anil K. Tyagi.** (2013). Adjunctive immunotherapy with α–crystallin based DNA vaccination reduces tuberculosis chemotherapy period in chronically infected mice. Scientific Reports. 3: 1821, DOI: 10.1038.

8. Garima Khare, Prachi Nangpal and **Anil K. Tyagi**. (2013). Unique residues at the 3-fold and 4-fold axis of mycobacterial ferritin are involved in oligomer switching. Biochemistry**,** 52(10) : 1694-1704.

9. Ruchi Jain, Bappaditya Dey and **Anil K. Tyagi**. (2012). Development of the first oligonucleotide microarray for global gene expression profiling in guinea pigs: defining the transcription signature of infectious diseases. BMC Genomics, 13: 520-530.

10. Vikram Saini, Saurabh Raghuvanshi, Jitendra P. Khurana, Niyaz Ahmed, Seyed E. Hasnain, Akhilesh K. Tyagi and **Anil K. Tyagi**. (2012). Massive gene acquisitions in

Mycobacterium indicus pranii provide a perspective on mycobacterial evolution. Nucleic Acids Research. 1-19, doi:10.1093/nar/gks793.

11. P. Vineel Reddy, Rupangi Verma Puri, Aparna Khera and **Anil K. Tyagi**. (2012). Iron Storage Proteins Are Essential for the Survival and Pathogenesis of *Mycobacterium tuberculosis* in THP-1 Macrophages and the Guinea Pig Model of Infection. *J. Bacteriol.* 194(3):567. DOI: 10.1128/JB.05553-11.

12. Ruchi Jain, Bappaditya Dey, Aparna Khera, Priyadarshani Srivastava, Umesh D. Gupta, V.M. Katoch, V.D. Ramanathan, **Anil K. Tyagi.** (2011). Over-expression of superoxide dismutase obliterates the protective effect of BCG against tuberculosis by modulating innate and adaptive immune responses. Vaccine. 29: 8118– 8125

13. Bappaditya Dey, Ruchi Jain, Umesh D. Gupta, V. M. Katoch, V. D. Ramanathan, **Anil K. Tyagi.** (2011). A Booster Vaccine Expressing a Latency-Associated Antigen Augments BCG Induced Immunity and Confers Enhanced Protection against Tuberculosis. PLoS ONE 6(8): e23360.

14. Garima Khare, Ritika Kar, **Anil K. Tyagi.** (2011). Identification of Inhibitors against Mycobacterium tuberculosis Thiamin Phosphate Synthase, an Important Target for the Development of Anti-TB Drugs. PLoS ONE 6(7): e22441.

15. Bappaditya Dey, Ruchi Jain, Aparna Khera, Umesh D. Gupta, V.M. Katoch, V.D. Ramanathan and **Anil K. Tyagi**. (2011). Latency antigen α–crystalin based vaccination imparts a robust protection against TB by modulating the dynamics of pulmonary cytokines. PLoSONE 6(4): e18773.

16. Garima Khare, Vibha Gupta, Prachi Nangpal, Rakesh K. Gupta, Nicholas K. Sauter and **Anil K. Tyagi**. (2011). Ferritin Structure from Mycobacterium tuberculosis: Comparative Study with Homologues identifies Extended C-terminus involved in Ferroxidase Activity. PLoSONE 6(4): e18570.

17. Purushothaman S, Annamalai K, **Tyagi AK**, Surolia A (2011). Diversity in Functional Organization of Class I and Class II Biotin Protein Ligase. PLoS ONE 6(3):e16850.

18. Nidhi Jatana, Sarvesh Jangid, Garima Khare, **Anil K. Tyagi** and Narayanan Latha. (2011). Molecular modeling studies of fatty acyl-CoA synthetase (FadD13) from *Mycobacterium tuberculosis* – a potential target for the development of antitubercular drugs. J. Mol. Model. 17(2) : 301-313.

19. Ashish Arora, Nagasuma R. Chandra, Amit Das, Balasubramanian Gopal, Shekhar C. Mande, Balaji Prakash, Ravishankar Ramachandran, Rajan Sankaranarayanan, K. Sekar, Kaza Suguna, **Anil K. Tyagi**, Mamannamana Vijayan. (2011). Structural biology of *Mycobacterium tuberculosis* proteins: The Indian efforts, Tuberculosis, doi:10.1016/j.tube.2011.03.004

20. **Anil K. Tyagi**, Prachi Nangpal, Vijaya Satchidanandam. (2011). Development of vaccines against tuberculosis. Tuberculosis. Doi:10.1016/j.tube.2011.01.003.

21. Anuj Kumar Gutpa, Vineel P. Reddy, Mallika Lavania, D.S. Chauhan, K. Venkatesan, V.D. Sharma, **A.K. Tyagi** and V.M. Katoch. (2010). *jefA* (Rv2459), a drug efflux gene in *Mycobacteirum tuberculosis* confers resistance to isoniazid and ethambutol. Indian J. Med. Res. 132: 176-188.

22. Vibha Gupta, Rakesh K. Gupta, Garima Khare, Dinakar M. Salunke, Avadhesha Surolia and **Anil K. Tyagi**. (2010). Structural ordering of disordered ligand-binding loops of biotin protein ligase into active conformations as a consequence of dehydration. PloS ONE 5(2): e9222.

23. Bappaditya Dey, Ruchi Jain, Aparna Khera, Vivek Rao, Neeraj Dhar, Umesh D. Gupta, V.M. Katoch, V.D. Ramanathan and **Anil K. Tyagi**. (2010). Boosting with a DNA vaccine expressing ESAT-6 (DNAE6) obliterates the protection imparted by recombinant BCG (rBCGE6) against aerosol *Mycobacterium tuberculosis* infection in guinea pigs. Vaccine. 28: 63-70.

24. Khare, G., Gupta, V., Gupta, R.K., Gupta, R, Bhat, R. and **Anil K. Tyagi**. (2009). Dissecting the role of critical residues and substrate preferene of a fatty Acyl-CoA synthetase (FadD13) of *Mycobacterium tuberculosis*. PLoS ONE 4(12): e8387,.

25. Vibha Gupta, Rakesh K. Gupta, Garima Khare, Dinakar M. Salunke and **Anil K. Tyagi**. (2009). Crystal structure of Bfr A from *Mycobacterium tuberculosis:* Incorporation of selenomethionine results in cleavage and demetallation of Haem. PLoS One. 4(11): e8028.

26. Preeti Sachdeva, Richa Misra, **Anil K. Tyagi** and Yogendra Singh. 2009. The sigma factors of *Mycobacterium tuberculosis:* regulation of the regulators. FEBS Journal. Doi:10.1111/j.1742-4658.2009.07479.x.

27. C.M. Santosh Kumar, Garima Khare, C.V. Srikanth, **Anil K. Tyagi**, Abhijit A. Sardesai and Shekhar C. Mande. (2009). Facilitated oligomericzation of mycobacterial GroEL: Evidence for phosphorylation-mediated oligomerization. J. Bacteriol. 191: 6525-6538.

28. Vikram Saini, S. Raghuvanshi, G.P. Talwar, N. Ahmed, J.P. Khurana, S.E. Hasnain, Akhilesh K. Tyagi, and **Anil K. Tyagi.** (2009). Polyphasic Taxonomic Analysis Establishes *Mycobacterium indicus pranii* as a Distinct Species. PLoS ONE 4(7): e6263.

29. D. Basu, Garima Khare, S. Singh, **Anil K. Tyagi,** S. Khosla, S.C. Mande. (2009). A novel nucleoid-associated protein of *Mycobacterium tuberculosis* is a sequence homolog of GrotL. Nucleic Acids Res. Doi:10.1093/nar/gkp502.

30. Pooja Arora, Aneesh Goyal, Vivek T. Natarajan, Eerappa Rajakumara, Priyanka Verma, Radhika Gupta, Malikmohamed Yousuf, Omkita A. Trivedi, Debasisa Mohanty, **Anil Tyagi**, Rajan Sankaranarayanan and Rajesh S. Gokhale. (2009). Mechanistic and functional insights into fatty acid activation in *Mycobacterium tuberculosis*. Nature Chemical Biology. 5, 166-173.

31. **Anil K. Tyagi,** Bappaditya Dey and Ruchi Jain (2009). Tuberculosis vaccine development: Current status and future expectations. In: Sharma, S.K., Mohan, A. (eds.). Tuberculosis, 2nd ed., New Delhi: Jaypee Brothers, Medical Publishers pg 918-946.

32. Ruchi Jain, Bappaditya Dey, Neeraj Dhar, Vivek Rao, Ramandeep Singh, Umesh D. Gupta, V.M. Katoch, V.D. Ramanathan and **Anil K. Tyagi**. (2008). Enhanced and Enduring Protection against Tuberculosis by Recombinant BCG-Ag85C and its Association with Modulation of Cytokine Profile in Lung. PLoS ONE. 3(12): 3869.

33. Mohd Akif, Garima Khare, **Anil K. Tyagi,** Shekhar C. Mande, and Abhijit A. Sardesai (2008). Functional Studies on Multiple Thioredoxins from *Mycobacterium tuberculosis.* J. Bacteriol. 190: 7087-7095.

34. Vibha Gupta, Rakesh K. Gupta, Garima Khare, Dinakar M. Salunke and **Anil K. Tyagi**. (2008). Cloning, expression, purification, crystallization and preliminary x-ray crystallographic analysis of bacterioferritin A from *Mycobacterium tuberculosis*. Acta Cryst. F 64: 398-401.

35. Vibha Gupta, Rakesh K. Gupta, Garima Khare, Avadhesha Surolia, Dinakar M. Salunke and **Anil K. Tyagi**. (2008). Crystallization and preliminary x-ray diffraction analhysis of biotin acetyl-CoA carboxylase ligare (BirA) from *Mycobacterium tuberculosis*. Acta Cryst. F 64: 524-527.

36. A. Farhana, S. Kumar, S.S. Rathore, P.C. Ghosh, N.Z. Ehtesham, **Anil K. Tyagi,** and S.E. Hasnain. (2008). Mechanistic insights into a novel export-import system of *Mycobacterium tuberculosis* unravel its role in trafficking of iron. PLoS ONE. 3(5): e2087.

37. Shruti Jain, Garima Khare, Pushplata Tripathi and **Anil K. Tyagi.** (2008). An inducible system for the identification of target genes for a regulator in mycobacteria. American Journal of Biochemistry and Biotechnology 4(3): 226-230.

38. Ruchi Jain, Bappaditya Dey and **Anil K. Tyagi**. (2008). Role of vaccines and immuno-modulation in tuberculosis. In: O.P. Sood and S.K. Sharma (eds.), Round Table Conference Series, Challenges of MDR/ XDR Tuberculosis in India, Ranbaxy Science Foundation, New Delhi, pg.93-102.

39. **Anil K. Tyagi,** Ramandeep Singh and Vibha Gupta. (2008). Role of Mycobacterial kinases and Phosphatases in Growth and Pathogenesis, in Reyrat, J.M. and Daffe, M. (Eds.): The Mycobacterial Cell Envelope, ASM Press, Washington DC, USA, pp.323-343.

40. Ahmed, N., Saini, V., Raghuvanshi, S., Khurana, J.P., Tyagi, Akhilesh K., **Tyagi, Anil K.** and Hasnain, S.E. (2007). Molecular analysis of a leprosy immunotherapeutic bacillus provides insights into *Mycobacterium* evolution. PLoS ONE 2(10): e968.

41. Azeet Narayan, Preeti Sachdeva, Kirti Sharma, Adesh K. Saini, **Anil K. Tyagi** and Yogendra Singh. (2007). Serine threonine protein kinases of mycobacterial genus: phylogeny to function. Physiol. Genomics 29: 66-75.

42. Nisheeth Agarwal and **Anil K. Tyagi.** (2006). Mycobacterial Transcriptional Signals: Requirements for Recognition by RNA polymerase and Optimal Transcriptional Activity. Nucleic Acid Research. 34: 4245-4257.

43. Ramandeep Singh, Amit Singh and **Anil K. Tyagi.** (2005). Deciphering the genes involved in pathogenesis of *Mycobacterium tuberculosis.* Tuberculosis 85:325-335.

44. Aparna Khera, Ramandeep Singh, H. Shakila, Vivek Rao, Neeraj Dhar, P.R. Narayanan, C.N. Parmasivan, V.D.Ramanathan and **Anil K.Tyagi**. (2005). Elicitation of efficient, protective immune responses by using DNA vaccines against tuberculosis. Vaccine 23:5655-5665.

45. Parampal, Deol, Reena Vohra, Adesh K. Saini, Amit Singh, Harish Chandra, Puneet Chopra, Taposh K. Das, **Anil K. Tyagi**, and Yogendra Singh. (2005). Role of *Mycobacterium tuberculosis* Ser/Thr kinase PknF: implications in glucose transport and cell division. J. Bacteriol. 187: 3415-3420.

46. Amit Singh, Radhika Gupta, R. A.Vishwakarma, P.R. Narayanan, C.N. Paramasivan, V. D. Ramanathan and **Anil K. Tyagi**. (2005). Requirement of *mymA* operon for appropriate cell wall ultrastructure and persistence of *Mycobacterium tuberculosis* in the spleens of guinea pigs. J. Bacteriol. 187: 4173-4186.

47. Vivek Rao, Neeraj Dhar, H. Shakila, Ramandeep Singh, Aparna Khera, M. Naseema, C. N. Paramasivan, P. R. Narayananan, V. D. Ramanthan and **Anil K. Tyagi.** (2005). Over-expression of the 19kDa lipoprotein of *Mycobacterium tuberculosis* obliterates the protective efficacy of BCG by polarizing the host immune responses to the Th2 phenotype. Scand. J. Immunol. 61: 410-417.

48. V.K. Chaudhary, Kulshresta Abhishek, Gupta Ghata, Verma Nitin, Sampati Kumari, S.K. Sharma, Gupta Amita, **A.K. Tyagi.** (2005). Expression and purification of 38-kDa and Mtb81 antigens of *Mycobacterium tuberculosis* for application in serodiagnosis. Protein Expr. Purif. 40: 169-176.

49. Ruchi Jain and **Anil K. Tyagi**. (2005). New technology forces microbes to reveal their secrets. In: T. Satyanarayana and B.N. Johri (eds.). Microbial Diversity: Current Perspectives and Potential Applications, I.K. International Pvt. Ltd., India, 1053-1074.

50. **Anil K. Tyagi,** Bappaditya Dey and Ruchi Jain. (2005). Development of vaccine against tuberculosis. In: N.K. Mehra and S.K. Sharma and O.P. Sood (eds.) "HIV and Tuberculosis: Co-infection" under "Round Table Conference Series", Ranbaxy Science Foundation, India, 15: 149-153.

51. Saini, A.K., Maithal, K., Chand, P., Chowdhury, S., Vohra, R., Goyal, A., Dubey, G.P., Chopra, P., Chandra, R., **Tyagi, A.K.**, Singh, Y., Tandon, V. (2004). Nuclear localization and in situ DNA damage by *Mycobacterium tuberculosis* nucleosidediphosphate kinase. J. Biol. Chem. 279: 50142-50149.

52. Chopra, P., Koduri, H., Singh, R., Koul, A., Ghildiyal, M., Sharma, K., **Tyagi, A.K.**, Singh, Y. (2004). Nucleoside diphosphate kinase of *Mycobacterium tuberculosis* acts as GTPase-activating protein for Rho-GTPases. FEBS Lett. 571: 212-216.

53. Neeraj Dhar, Vivek Rao and **Anil K. Tyagi**. (2004). Immunogenicity of recombinant BCG vaccine strains overexpressing components of the antigen 85 Complex of *M. tuberculosis*. Med Microbiol Immunol. 193: 19-25.

54. Ruchi Jain, Neeraj Dhar, Vivek Rao and **Anil K. Tyagi.** (2004). Development of candidate vaccines for protection against tuberculosis. In: Pawan Sharma (eds.). Tuberculosis: Current Research Trends, Allied Publishers Pvt. Ltd., India, 138-158.

55. **Anil K. Tyagi** and Aparna Khera. (2004). Protection against tuberculosis: How close are we to a perfect vaccine? Current Science. 86: 154-166.

56. **Anil K. Tyagi** and Neeraj Dhar. (2003). Recent Advances in Tuberculosis Research in India. In: T.K. Ghose and P. Ghosh (eds.): "Biotechnology in India" under the series "Advances in Biochemical Engineering/Biotechnology", Springer Verlag 84, 211-273.

57. Ramandeep Singh, Vivek Rao, H. Shakila, Radhika Gupta, Aparna Khera, Neeraj Dhar, Amit Singh, Anil Koul, Yogendra Singh, M. Naseema, P.R. Narayanan, C.N. Paramasivan, V.D. Ramanathan and **Anil K. Tyagi**. (2003). Disruption of *mptpB* impairs the ability of *Mycobacterium tuberculosis* to survive in guinea pigs. Molecular Microbiology 50(3): 751-762.

58. Chopra P, Singh B, Singh R, Vohra, R, Koul A, Meena LS, Koduri H, Ghildiyal M, Deol P, Das TK, **Tyagi AK**, Singh Y. (2003). Phosphoprotein phosphatase of Mycobacterium tuberculosis dephosphorylates serine-threonine kinases PknA and PknB. Biochem Biophys Res Commun. 311(1): 112-120.

59. Vivek Rao, Neeraj Dhar and **Anil K. Tyagi**. (2003). Modulation of host immune responses by over-expression of immunodominant antigens of *M. tuberculosis* in BCG. Scand J Immunol. 58(4): 449-461.

60. Amit Singh, Shruti Jain, Seema Gupta, Taposh Das and **Anil K. Tyagi.** (2003). *mymA* operon of *Mycobacterium tuberculosis* : its regulation and importance in the cell envelope. FEMS Microbiol. Lett. 227(1): 53-63.

61. Nisheeth Agarwal and **Anil K. Tyagi.** (2003). Role of 5`TGN3` Motif in the Interaction of Mycobacterial RNA Polymerase with a Promoter of "Extended –10" class. FEMS Microbiol. Lett. 225: 75-83.

62. Neeraj Dhar, Vivek Rao and **Anil K. Tyagi.** (2003). Skewing of the Th1/Th2 responses in mice due to variation in the level of expression of an antigen in a recombinant BCG system. Immunol Lett. 88(3): 175-184.

63. Puneet Chopra, Anubha Singh, Anil Koul, S. Ramachandran, Karl Drilica, **Anil K. Tyagi** and Yogendra Singh. (2003). Cytotoxic activity of nucleoside diphosphate kinase secreted from *Mycobacterium tuberculosis*. Eur. J. Biochem. 270, 625-634.

64. Koul, A., Choidas, A., **Tyagi, A.K.,** Drilca, K., Singh, Y. and Ullrich, A. 2001. Serine/threonine protein kinases PknF and PknG of *Mycobacterium tuberculosis*: Characterization and Localization. Microbiology (U.K.). 147, 2307-2314.

65. Dhar Neeraj, Rao, Vivek and **Tyagi, A.K.** 2000. Recombinant BCG approach for development of vaccines: cloning and expression of immunodominant antigens of *M.*tuberculosis. FEMS Microbiol. Lett., 190(2), 309-316.

66. Koul, Anil, Choidas, Axel, Treder, Martin, **Tyagi, A.K.**, Drlica, Karl, Singh, Y. and Ullrich, Axel. 2000. Cloning and characterization of secretory tyrosine phosphatases of *Mycobacterium tuberculosis*. J. Bacteriol. 182(19), 5425-5432.

67. **Anil K. Tyagi** (2000). A Perfect Vaccine Against Tuberculosis: Premises and Promises. In: Sushma Gupta and O.P. Sood (eds.) "Tuberculosis" under "Round Table Conference Series", Ranbaxy Science Foundation, India, 7: 135-139.

68. **Tyagi, A.K.**, Das Gupta, S.K. and Jain, S. 2000. Gene Expression: Reporter Technologies. In: Graham F. Hatfull and William R. Jacobs Jr. (eds.) : Molecular Genetics of Mycobacteria, ASM Press, pp.131-147.

69. **Tyagi, A.K.** and Jain, S. 1999. *Mycobacterium tuberculosis*: Studies on Gene Regulation, Pathogenesis and Development of Recombinant BCG Vaccines. In : R.C. Mahajan and Amu Therwath (eds.), Multidrug Resistance in Emerging and Re-emerging diseases. Narosa Press, pp.109-124.

70. Gupta, S., Jain, S. and **Tyagi, A.K**. 1999. Analysis, expression and prevalence of the *M.tuberculosis* homolog of bacterial virulence regulating proteins. FEMS. Microbiol. Lett. 172, 137-143.

71. Bashyam, M.D., and **Tyagi, A.K.** 1998. Identification and analysis of "Extended-10" promoters from Mycobacteria. J. Bacteriol. 180, 2568-2573.

72. DasGupta, S.K., Jain, S., Kaushal, D., and **Tyagi, A.K.** 1998. Expression systems for study of mycobacterial gene regulation and development of recombinant BCG vaccines. Biochem. Biophys. Res. Commun. 246, 797-804.

73. **Tyagi, A.K.** 1998. *Mycobacterium tuberculosis*: Studies on gene regulation, pathogenesis and development of recombinant BCG vaccines. In: Tuberculosis Research: Into the 21st Century, pp.29-42.

74. Jain, S., Kaushal, D., Dasgupta, S.K., and **Tyagi, A.K.** 1997. Construction of shuttle vectors for genetic manipulation and molecular analysis of mycobacteria. Gene 190, 37-44.

75. Bashyam, M.D., Kaushal, D., Dasgupta, S.K., and **Tyagi, A.K.** 1996. A study of the Mycobacterial transcriptional apparatus: Identification of novel features in promoter elements. J. Bacteriol. 178, 4847-4853.

76. Sarkar, N.K., Shankar, S., and **Tyagi, A.K.** 1995. Polyamines exert regulatory control on mycobacterial transcription - A study using RNA polymerase form *Mycobacterium phlei*. Biochemistry and Molecular Biology International 35, 1189-1198.

77. Bashyam, M.D. and **Tyagi, A.K.** 1994. An efficient and high-yielding method for isolation of RNA from mycobacteria. Biotechniques 17 : 834-836.

78. DasGupta, S.K., Bashyam, M.D., and **Tyagi, A.K.** 1993. Cloning and assessment of mycobacterial promoters by using a plasmid shuttle vector. J. Bacteriol. 175, 5186-5192.

79. Gupta, S., and **Tyagi, A.K.** 1993. Sequence of a newly identified *Mycobacterium tuberculosis* gene encoding a protein with sequence homology to virulence regulating proteins. Gene 126, 157-158.

80. Shankar, S., and **Tyagi, A.K.** 1993. Purification and Characterization of restriction endonuclease *Mgo*I from *Mycobacterium gordonae*, Gene 131, 153-154.

81. Shankar, S., and **Tyagi, A.K.** 1993. *Mch*AI and *Mch*AII, two class-II restriction endonucleases from *Mycobacterium chelonei*. Gene 132, 119-123.

82. Shankar, S., and **Tyagi, A.K.** 1992. *Mha*AI, a novel isoschizomer of *Pst*I from *Mycobacterium habana* recognizing 5'-CTGCA/G-3'. Nucleic Acids Res. 20, 2891.

83. Shankar, S.,and **Tyagi, A.K.** 1992. *Mfo*AI a novel isoschizomer of *Hae*III from *Mycobacterium fortuitum* recognizing 5'-GG/CC-3'. Nucleic Acids Res. 20, 2890.

84. Balasundaram, D. and **Tyagi, A.K.** 1991. Polyamine - DNA Nexus: Structural Ramifications and Biological Implications. Mol. Cell. Biochem. 100, 129-140.

85. Tyagi, J.S., **Tyagi, A.K.** and Bhargava, S. 1990. Transfer RNA genes in mycobacteria : organization and molecular cloning. Trop Med. Parasitol 41, 294-296.

86. Bhargava, S., **Tyagi, A.K.** and Tyagi, J.S. 1990. tRNA genes in mycobacteria: organization and molecular cloning. J. Bacteriol. 172, 2930-2934.

87. Balasundaram, D. and **Tyagi, A.K.**, 1989. Modulation of Arginine decarboxylase activity from *Mycobacterium smegmatis*: Evidence for pyridoxal -5'- phosphate mediated conformational changes in the enzyme. Eur. J. Biochem. 183, 339-345.

88. Balasundaram, D., and **Tyagi, A.K.** 1988. Regulation of ornithine decarboxylase from *Mycobacterium smegmatis.* Arch. Biochem. Biophys. 264, 288-294.

89. Jain, A., and **Tyagi, A.K.** 1987. Role of polyamines in the synthesis of RNA in mycobacteria. Mol. Cell. Biochem. 78, 3-8.

90. Bhutani, V., Kumar, V., **Tyagi, A.K.** and Misra, U.K. 1987. Changes of fatty acid composition in pancreas of rats fed inadequate dietary protein. Nutrition Research 7, 763-770.

91. **Tyagi, A.K.**, Wickner, R.B., Tabor, C.W. and Tabor, H. 1984. Specificity of polyamine requirements for the replication and maintenance of different double-stranded RNA plasmids in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA. 81, 1149-1153.

92. **Tyagi, A.K.** and Cooney, D.A. 1984. Biochemical pharmacology, metabolism, and mechanism of action of L-alanosine, a novel natural antitumor agent, in Caratini, S., Goldin, A., Howking, F., and Kopin, I.J. (Eds.): *Advances in Pharmacology and Chemotherapy* Vol. 20, Academic Press, New York, pp.69-121.

93. Tabor, C.W., Tabor, H. and **Tyagi, A.K.** 1983. Biochemical and genetic studies of polyamines in *Saccharomyces cerevisiae*. In Bachrach, U., Kaye. A. and Chayen, R. (Eds.) : Advances in Polyamine Research Vol. 4, Raven Press, New York, pp. 467-478.

94. **Tyagi A.K.**, Tabor, C.W. and Tabor, H. 1983. Ornithine decarboxylase (*Saccharomyces cerevisiae*), Tabor, H. and Tabor C.W. (eds.). Methods in Enzymology, Vol. 94, Academic Press, New York. pp. 135-139.

95. **Tyagi, A.K.** and Cooney, D.A. 1983. Prospects for the chemotherapy of cancer using analogs of L-aspartic acid. Trends Pharmacol Sci., 4, 299-304.

96. **Tyagi, A.K.**, Tabor, H. and Tabor, C.W. 1982. Inactivation of yeast ornithine decarboxylase by polyamines *in vivo* does not result from the incorporation of polyamines into enzyme protein. Biochem. Biophys. Res. Commun. 109, 533-540.

97. Tabor, C.W., Tabor, H., Tyagi**, A.K.** and Cohn, M.S. 1982. The biochemistry, genetics, and regulation of polyamine biosynthesis in *Saccharomyces cerevisiae*. Fed. Proc. 41 (review article), pp.3084-3088.

98. **Tyagi, A.K.**, Tabor, C.W. and Tabor, H. 1981. Ornithine decarboxylase from *Saccharomyces cerevisiae*: Purification, properties and regulation of activity. J. Biol. Chem. 256, 12156-12163.

99. **Tyagi, A.K.**, Thake, D.C., Mcgee, E. and Cooney, D.A. 1981. Determinants of the toxicity of L-alanosine to various organs of the mouse. Toxicology 21, 59-69.

100. Tyagi, J.S., **Tyagi, A.K.** and Venkitasubramanian, T.A. 1981. Some factors regulating [1-^{14}C] acetate incorporation into aflatoxins by spheroplast and spheroplasts lysates from *Aspergillus parasiticus*. Toxicon 19, 445-454.

101. Tyagi, J.S., **Tyagi, A.K.** and Venkitasubramanian, T.A. 1981. Preparation and properties of spheroplasts from *Aspergillus* parasiticus with special reference to the *denovo* synthesis of Aflatoxins. J. Appl. Bacteriol. 50, 481-491.

102. **Tyagi, A.K.**, Cooney, D.A., Jayaram, H.N., Swiniarski, N.K. and Johnson R.K. 1981. Studies on the mechanism of resistance of selected murine tumors to L-alanosine. Biochem. Pharmacol. 30, 915-924.

103. **Tyagi, A.K.** and Cooney, D.A. 1980. Identification of the antimetabolite of L-Alanosine, L-alanosyl-5-Amino-4-imidazolecarboxylic acid ribonucleotide in tumors and assessment of its inhibition of adenylosuccinate synthetase. Cancer Res. 40, 4390-4397.

104. Kensler, T.W., Erlichman, C., Jayaram, H.N., **Tyagi, A.K.**, Ardalan, B. and Cooney, D.A. 1980. Peripheral leukocytes as indicators of the enzymatic effects of N-(phosphonoacetyl)-L-aspartic acid (PALA) on human L-aspartate transcarbamoylase (ATCase) activity. Cancer Treatment Reports 64, 967-973.

105. Park, K.W., Tyagi **A.K.**, and Cooney, D.A. 1980. A radiometric technique for the measurement of adenylosuccinate lyase. J. Biochem. Biophys. Methods 2, 291-297.

106. Anandaraj, S.A., Jayaram, H.N., Cooney, D.A.; **Tyagi, A.K.** Han, N., Thomas, J.H., Chitnis, M., and Montgomery, J.A. 1980. Interaction of L-alanosine (NSC-153353) with enzymes metabolizing L-aspartic acid, L-glutamic acid and their amides. Biochem. Pharmacol. 29, 227-245.

107. **Tyagi, A.K.**, Cooney, D.A., Bledsoe, M. and Jayaram, H.N. 1980. Two complementary radiometric methods for the measurement of 5-amino-4-imidazole-N-succino-carboxamide ribonucleotide synthetase (SAICAR synthetase). J. Biochem. Biophys. Methods 2, 123-132.

108. Jayaram, H.N., **Tyagi, A.K.**, Anandaraj, S., Montgomery, J.A., Kelley, J.A., Kelley, J., Adamson, R.H. and Cooney, D.A. 1979. Metabolites of alanosine, an antitumor antibiotic. Biochem. Pharmacol. 28, 3551-3566.

109. **Tyagi, A.K.**, Jayaram, H.N., Anandaraj, S., Taylor, B. and Cooney D.A. 1979. Use of Automated chromatography on the amino acid analyzer with lithium citrate buffers to separate nucleic acid bases, nucleosides, nucleotides and their precursors. J. Biochem. Biophys. Methods. 1, 221-226.

110. Nizamuddin, A., **Tyagi, A.K.**, Padh, H. and Venkitasubramanian, T.A. 1978. Alkaline phosphatase activity in *Mycobacterium smegmatis*. Ind. J. Chest. Dis. 20, 11-20.

111. **Tyagi, A.K.**, Siddiqui, F.A. and Venkitasubramanian, T.A. 1977. Studies on the purification and characterization of malate dehydrogenase from *Mycobacterium phlei*. Biochem. Biophys. Acta 485, 255-267.

112. **Tyagi, A.K.** 1976. Sources of energy production in *Mycobacterium tuberculosis*. Ind. J. Chest Dis. 18, 250-255.

113. **Tyagi, A.K.**, Reddy, T.L.P. and Venkitasubramanian, T.A. 1976. Effect of oxygen tension on oxidative phosphorylation in *Mycobacterium phlei*. Ind. J. Biochem. Biophys. 13, 93-95.

114. **Tyagi, A.K.**, Reddy, T.L.P. and Venkitasubramanian, T.A. 1976. Oxidative phosphorylation in *Mycobacterium tuberculosis* BCG. Ind. J. Biochem. Biophys. 13, 43-45.

115. **Tyagi, A.K.** Reddy, T.L.P. and Venkitasubramanian, T.A. 1976. Site of action of nonheme iron in the malate (flavine adenine dinucleotide) pathway of *Mycobacterium phlei*. Can. J. Microb. 22, 1054-1057.

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1976	TYAGI AK	IND J BIOC BIOP	13	43	
	TYAGI AK	BIOCHIM BIOPHYS ACTA	485	255	1977
	RASTOGI N	INDIAN J BIOCHEM BIO	15	286	1978
1976	TYAGI AK	CAN J MICROB	22	1054	
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	RASTOGI N	INDIAN J BIOCHEM BIO	15	286	1978
	SALTVEIT ME	J AM SOC HORTIC SCI	105	252	1980
1977	TYAGI AK	BIOCHEM BIOPHYS ACTA	485	255	
	RASTOGI N	INDIAN J BIOCHEM BIO	15	286	1978
	STORER AC	BIOCHEM J	193	235	1981
	WALI AS	CAN J BIOCHEM CELL B	62	559	1984
	MASOOD R	J BIOSCIENCE	7	421	1985
	YUEH AY	BIOCHEM J	258	221	1989
	LAI YK	PHYTOCHEMISTRY	28	1579	1989
	MIKULASOVA D	FEMS MICROBIOL LETT	159	299	1998
	OIKAWA T	BIOSCI BIOTECH BIOCH	69	2146	2005
	EPRINTSEV AT	BIOCHEMISTRY-MOSCOW+	70	1027	2005
	WANG SY	J FOOD BIOCHEM	29	117	2005
	EPRINTSEV AT	BIOL BULL+	35	585	2008
1979	JAYARAM HN	BIOCHEM PHARMACOL	28	3551	
	ANANDARAJ SJ	BIOCHEM PHARMACOL	29	227	1980
	TYAGI AK	CANCER RES	40	4390	1980
	POWIS G	BIOCHEM PHARMACOL	30	771	1981
	TYAGI AK	BIOCHEM PHARMACOL	30	915	1981
	CADMAN E	BIOCHEM PHARMACOL	30	2469	1981
	POWIS G	CANCER CHEMOTH PHARM	6	1	1981
	BENZ C	CANCER RES	41	994	1981
	TYAGI AK	TOXICOLOGY	21	59	1981
	ALSTON TA	BIOCHEM BIOPH RES CO	105	560	1982
	ALSTON TA	ACCOUNTS CHEM RES	16	418	1983
	PORTER DJT	ARCH BIOCHEM BIOPHYS	225	157	1983
	POWIS G	DRUG METAB REV	14	1145	1983
	TYAGI AK	ADV PHARMACOL CHEMOT	20	69	1984
	REJ R	CRC CR REV CL LAB SC	21	99	1984
	STRAZZOLINI P	J MED CHEM	27	1295	1984
	GALLIANI G	CANCER CHEMOTH PHARM	14	74	1985

	ALSTON TA	J BIOL CHEM	260	4069	1985
	DAMON LE	PHARMACOL THERAPEUT	38	73	1988
	AHLUWALIA GS	PHARMACOL THERAPEUT	46	243	1990
1979	TYAGI AK	J BIOCH BIOP METH	1	221	
	ANANDARAJ SJ	BIOCHEM PHARMACOL	29	227	1980
	ARDALAN B	CANCER RES	40	1431	1980
	TYAGI AK	CANCER RES	40	4390	1980
	KENSLER TW	CANCER TREAT REP	64	967	1980
	KENSLER TW	J BIOCHEM BIOPH METH	2	29	1980
	TYAGI AK	J BIOCHEM BIOPH METH	2	123	1980
	TYAGI AK	BIOCHEM PHARMACOL	30	915	1981
	ARDALAN B	CANCER RES	41	150	1981
	KENSLER TW	CANCER RES	41	894	1981
	JAYARAM HN	CANCER TREAT REP	65	491	1981
	TYAGI AK	TOXICOLOGY	21	59	1981
	ARDALAN B	CANCER RES	43	1598	1983
	TYAGI AK	ADV PHARMACOL CHEMOT	20	69	1984
1980	TYAGI AK	CANCER RES	40	4390	
	TYAGI AK	BIOCHEM PHARMACOL	30	915	1981
	TYAGI AK	TOXICOLOGY	21	59	1981
	HEIMER R	BIOCHEM PHARMACOL	32	199	1983
	STAYTON MM	CURR TOP CELL REGUL	22	103	1983
	TYAGI AK	TRENDS PHARMACOL SCI	4	299	1983
	TYAGI AK	ADV PHARMACOL CHEMOT	20	69	1984
	WANG CC	BIOCHEM PHARMACOL	33	1323	1984
	WEBSTER HK	BIOCHEM PHARMACOL	33	1555	1984
	MISTRELLO G	J IMMUNOPHARMACOL	6	25	1984
	STRAZZOLINI P	J MED CHEM	27	1295	1984
	CASEY PJ	J BIOL CHEM	261	3637	1986
	AHLUWALIA G	BIOCHEM PHARMACOL	36	3797	1987
	ARONOW B	J BIOL CHEM	262	5106	1987
	CASEY PJ	BIOCHEM PHARMACOL	36	705	1987
	JOHNSON MA	J BIOL CHEM	263	15354	1988
	BENNETT LL	BIOCHEM PHARMACOL	37	1233	1988
	DAMON LE	PHARMACOL THERAPEUT	38	73	1988
	FRIDLAND A	ANN NY ACAD SCI	616	205	1990
	ALENIN VV	VOP MED KHIM	36	59	1990
	LOWENSTEIN JM	INT J SPORTS MED	11	S37	1990
	VANDENBERGHE G	PROG NEUROBIOL	39	547	1992
	MEREDITH M	BBA-MOL CELL RES	1266	16	1995
	HOU ZL	J BIOL CHEM	274	17505	1999
	BATOVA A	CANCER RES	59	1492	1999
	HARASAWA H	LEUKEMIA	16	1799	2002
	IANCU CV	J BIOL CHEM	277	26779	2002
	EFFERTH T	BIOCHEM PHARMACOL	66	613	2003
	NELSON SW	BIOCHEMISTRY-US	44	766	2005
	GINDER ND	J BIOL CHEM	281	20680	2006
	MARCE S	CLIN CANCER RES	12	3754	2006
	BATOVA A	BLOOD	107	898	2006
	BRAND J	CHEM-EUR J	12	499	2006
1980	KENSLER TW	CANCER TREATMENT REP	64	967	
	KARLE JM	CANCER RES	41	4952	1981
	WEISS GR	CANCER CHEMOTH PHARM	8	301	1982
	PAVLIK EJ	GYNECOL ONCOL	14	243	1982
	ERLICHMAN C	J NATL CANCER I	68	227	1982
	MARTIN DS	CANCER RES	43	2317	1983
	MONKS A	J BIOL CHEM	258	3564	1983
	WHITE JC	BIOCHEM PHARMACOL	33	3645	1984

	WHITE JC	CANCER RES	44	507	1984
	OCONNELL MJ	J CLIN ONCOL	2	1133	1984
	MARTIN DS	CANCER TREAT REP	69	421	1985
	CHAN TCK	CANCER RES	46	3168	1986
	LEYLANDJONES B	CANCER TREAT REP	70	219	1986
	GREM JL	CANCER RES	48	4441	1988
	ARDALAN B	J CLIN ONCOL	6	1053	1988
	PETERS GJ	CANCER RES	50	4644	1990
	ODWYER PJ	PHARMACOL THERAPEUT	48	371	1990
	BLIJHAM GH	ANTI-CANCER DRUG	2	233	1991
	KEMENY N	J CLIN ONCOL	10	747	1992
	PIZZORNO G	CANCER RES	52	1660	1992
	RAGNHAMMAR P	MED ONCOL	12	187	1995
	FLEMING RA	CLIN CANCER RES	2	1107	1996
	JIN L	PROTEINS	37	729	1999
	GAGNARD V	EUR J MED CHEM	38	883	2003
	WILS J	EUR J CANCER	39	346	2003
	WHITEHEAD RP	INVEST NEW DRUG	22	467	2004
1980	PARK KW	J BIOCHEM BIOPHYS METH	2	291	
	TYAGI AK	BIOCHEM PHARMACOL	30	915	1981
	TYAGI AK	TOXICOLOGY	21	59	1981
	TYAGI AK	ADV PHARMACOL CHEMOT	20	69	1984
1980	ANANDARAJ SA	BIOCHEM PHARMACOL	29	227	
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	TYAGI AK	BIOCHEM PHARMACOL	30	915	1981
	TYAGI AK	TOXICOLOGY	21	59	1981
	ALSTON TA	BIOCHEM BIOPH RES CO	105	560	1982
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	TYAGI AK	TRENDS PHARMACOL SCI	4	299	1983
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	STRAZZOLINI P	J MED CHEM	27	1295	1984
	ALSTON TA	J BIOL CHEM	260	4069	1985
	CASEY PJ	J BIOL CHEM	261	3637	1986
	JALAL MAF	ACTA CRYSTALLOGR C	42	733	1986
	CASEY PJ	BIOCHEM PHARMACOL	36	705	1987
	HONG SS	JPN J CANCER RES	80	592	1989
	AHLUWALIA GS	PHARMACOL THERAPEUT	46	243	1990
	PEETERS MA	ANN GENET-PARIS	34	219	1991
	RAMACHANDRAN B	J BIOL CHEM	268	23891	1993
	PALOS TP	MOL BRAIN RES	37	297	1996
	BATOVA A	CANCER RES	59	1492	1999
	EISENBERG D	BBA-PROTEIN STRUCT M	1477	122	2000
	HARASAWA H	LEUKEMIA	16	1799	2002
	STRAZZOLINI P	EUR J ORG CHEM	47	10	2004
	MARCE S	CLIN CANCER RES	12	3754	2006
	BATOVA A	BLOOD	107	898	2006
	HUANG JW	BIOCHEMISTRY-US	45	346	2006
	BERLICKI L	MINI-REV MED CHEM	8	869	2008
1980	TYAGI AK	J BIOC BIOP METH	2	123	
	TYAGI AK	CANCER RES	40	4390	1980
	TYAGI AK	BIOCHEM PHARMACOL	30	915	1981
	TYAGI AK	TOXICOLOGY	21	59	1981
	TYAGI AK	ADV PHARMACOL CHEMOT	20	69	1984
	CASEY PJ	J BIOL CHEM	261	3637	1986
	CASEY PJ	BIOCHEM J	246	263	1987
	CASEY PJ	BIOCHEM PHARMACOL	36	705	1987
	ALENIN VV	ZH OBSHCH KHIM+	57	692	1987
	SANT ME	ANAL BIOCHEM	182	121	1989

	ALENIN VV	BIOCHEMISTRY-MOSCOW+	57	572	1992
	TRETYAKOV OY	BIOCHEMISTRY-MOSCOW+	60	1535	1995
1981	TYAGI AK	J BIOL CHEM	256	12156	
	TABOR CW	MED BIOL	59	272	1981
	POSO H	ADV POLYAMINE RES	4	603	1982
	KUEHN GD	ADV POLYAMINE RES	4	615	1982
	TYAGI AK	BIOCHEM BIOPH RES CO	109	533	1982
	MITCHELL JLA	BIOCHEM J	205	551	1982
	SEELY JE	BIOCHEMISTRY-US	21	3394	1982
	PRITCHARD ML	J BIOL CHEM	257	5892	1982
	SEELY JE	J BIOL CHEM	257	7549	1982
	TABOR CW	ADV POLYAMINE RES	4	467	1982
	LAPOINTE DS	ARCH BIOCHEM BIOPHYS	224	515	1983
	ERWIN BG	BIOCHEMISTRY-US	22	3027	1983
	POSO H	BIOCHIM BIOPHYS ACTA	747	209	1983
	SEELY JE	J BIOL CHEM	258	2496	1983
	CHOI JH	J BIOL CHEM	258	2601	1983
	TYAGI AK	METHOD ENZYMOL	94	135	1983
	ZAGON IS	METHOD ENZYMOL	94	169	1983
	TABOR CW	ANNU REV BIOCHEM	53	749	1984
	FLAMIGNI F	BIOCHIM BIOPHYS ACTA	802	245	1984
	KAYE AM	CELL BIOCHEM FUNCT	2	2	1984
	BACHRACH U	CELL BIOCHEM FUNCT	2	6	1984
	BARNETT GR	J BIOL CHEM	259	179	1984
	SKLAVIADIS TK	BIOCHIM BIOPHYS ACTA	831	288	1985
	TABOR CW	MICROBIOL REV	49	81	1985
	FONZI WA	MOL CELL BIOL	5	161	1985
	DAVIS RH	P NATL ACAD SCI USA	82	4105	1985
	HIATT AC	J BIOL CHEM	261	1293	1986
	BELLOFATTO V	MOL BIOCHEM PARASIT	25	227	1987
	DORAZI D	PHYSIOL PLANTARUM	71	177	1987
	FONZI WA	J BIOL CHEM	262	10127	1987
	DIGANGI JJ	J BIOL CHEM	262	7889	1987
	PANDIT M	PHYTOCHEMISTRY	27	1609	1988
	EICHLER W	J PROTOZOOL	36	577	1989
	FONZI WA	J BIOL CHEM	264	18110	1989
	FONZI WA	BIOCHEM BIOPH RES CO	162	1409	1989
	BALASUNDARAM D	EUR J BIOCHEM	183	339	1989
	SCHAEFFER JM	BIOCHEM J	270	599	1990
	MATSUFUJI S	J BIOCHEM-TOKYO	108	365	1990
	PANDIT M	J BIOSCIENCE	15	83	1990
	COONS T	MOL BIOCHEM PARASIT	39	77	1990
	SMITH TA	PHYTOCHEMISTRY	29	1759	1990
	JOSEPH K	J EXP ZOOL	258	158	1991
	BABY TG	BIOCHIM BIOPHYS ACTA	1092	161	1991
	ROSENBERGHASSON Y	EUR J BIOCHEM	196	647	1991
	SMITH TA	MYCOL RES	96	395	1992
	HANSON S	J BIOL CHEM	267	2350	1992
	RAJAM MV	CURR SCI INDIA	65	461	1993
	YARLETT N	BIOCHEM J	293	487	1993
	SCHIPPER RG	J IMMUNOL METHODS	161	205	1993
	BALASUNDARAM D	J BACTERIOL	176	7126	1994
	NIEMANN G	BIOCHEM J	317	135	1996
	BALASUNDARAM D	J BACTERIOL	178	2721	1996
	KAOUASS M	MOL CELL BIOL	17	2994	1997
	HAMASAKIKATAGIRI N	GENE	187	35	1997
	KAOUASS M	J BIOL CHEM	273	2109	1998
	TOTH C	J BIOL CHEM	274	25921	1999
	PANTAZAKI AA	MOL CELL BIOCHEM	195	55	1999

	KRAUSE T	BIOCHEM J	352	287	2000
	ZHU C	BIOINFORMATICS	16	478	2000
	LEE YS	J BIOCHEM MOL BIOL	34	478	2001
	GUPTA R	P NATL ACAD SCI USA	98	10620	2001
	CHATTOPADHYAY MK	J BIOL CHEM	276	21235	2001
	COFFINO P	BIOCHIMIE	83	319	2001
	MOREHEAD TA	VIROLOGY	301	165	2002
	ARTEAGA-NIETO P	EXP PARASITOL	101	215	2002
	GANDRE S	BIOCHEM BIOPH RES CO	293	139	2002
	BAIS HP	PLANT CELL TISS ORG	69	1	2002
	POULIN R	EUR COMMISS SCI RES		3	2002
	BACHMANN AS	PHYSIOL MOL PLANT P	63	57	2003
	HOYT MA	J BIOL CHEM	278	12135	2003
	ZAVADA MS	NORTHEAST NAT	11	33	2004
	AOUIDA M	J BIOL CHEM	280	24267	2005
	SMIT AY	S AFR J ENOL VITIC	29	109	2008
	FOGLE EJ	BBA-PROTEINS PROTEOM	1814	1113	2011
1981	TYAGI AK	TOXICOLOGY	21	59	
	TYAGI AK	ADV PHARMACOL CHEMOT	20	69	1984
	GALLIANI G	CANCER CHEMOTH PHARM	14	74	1985
	AHLUWALIA GS	PHARMACOL THERAPEUT	46	243	1990
	BRAND J	CHEM-EUR J	12	499	2006
1981	TYAGI JS	TOXICON	19	445	
	ZAIKA LL	J FOOD PROTECT	50	691	1987
	KHAN SN	J SCI IND RES INDIA	47	130	1988
1981	TYAGI JS	J APPL BACTERIOL	50	481	
	DLUGONSKI J	CAN J MICROBIOL	30	57	1984
	LARROYA S	IRCS MED SCI-BIOCHEM	12	1064	1984
	KAWULA TH	J INVERTEBR PATHOL	43	282	1984
	LYNCH PT	T BRIT MYCOL SOC	85	135	1985
	BHATNAGAR RK	J APPL BACTERIOL	60	135	1986
	CLEVELAND TE	CAN J MICROBIOL	33	1108	1987
	CLEVELAND TE	APPL ENVIRON MICROB	53	1711	1987
	PFEIFER TA	APPL MICROBIOL BIOT	26	248	1987
	PEBERDY JF	MYCOL RES	93	1	1989
	DLUGONSKI J	J BASIC MICROB	31	347	1991
	AZIZ NH	MICROBIOS	89	47	1997
1981	TYAGI AK	BIOCHEM PHARMACOL	30	915	
	TYAGI AK	TOXICOLOGY	21	59	1981
	TYAGI AK	TRENDS PHARMACOL SCI	4	299	1983
	TYAGI AK	ADV PHARMACOL CHEMOT	20	69	1984
	AHLUWALIA GS	BIOCHEM PHARMACOL	33	1195	1984
	MISTRELLO G	J IMMUNOPHARMACOL	6	25	1984
	STRAZZOLINI P	J MED CHEM	27	1295	1984
	GALLIANI G	CANCER CHEMOTH PHARM	14	74	1985
	CASEY PJ	J BIOL CHEM	261	3637	1986
	CASEY PJ	BIOCHEM PHARMACOL	36	705	1987
	AHLUWALIA GS	PHARMACOL THERAPEUT	46	243	1990
	STRAZZOLINI P	EUR J ORG CHEM	47	10	2004
	MANJUNATH K	ACTA CRYSTALLOGR F	66	180	2010
1982	TYAGI AK	BIOC BIOP RES COMM	109	533	
	MITCHELL JLA	BIOCHEM J	214	345	1983
	ERWIN BG	BIOCHEMISTRY-US	22	3027	1983
	TABOR CW	ANNU REV BIOCHEM	53	749	1984
	PERSSON L	BIOCHEMISTRY-US	23	3777	1984
	DIENEL GA	J NEUROCHEM	42	1053	1984

	SEKAR V	METHOD ENZYMOL	107	154	1984
	LORAND L	MOL CELL BIOCHEM	58	9	1984
	GRILLO MA	INT J BIOCHEM	17	943	1985
	TABOR CW	MICROBIOL REV	49	81	1985
	FONZI WA	MOL CELL BIOL	5	161	1985
	HOLTTA E	J BIOL CHEM	261	9502	1986
	FONZI WA	J BIOL CHEM	262	10127	1987
	BALASUNDARAM D	ARCH BIOCHEM BIOPHYS	264	288	1988
	FONZI WA	J BIOL CHEM	264	18110	1989
	FONZI WA	BIOCHEM BIOPH RES CO	162	1409	1989
	MIYAMOTO K	J BIOCHEM-TOKYO	106	167	1989
	AL-SHABANAH OA	PHARMACOL RES	40	75	1999
1982	TABOR CW	FED PROC	41	3084	
	TABOR CW	ADV POLYAMINE RES	4	467	1982
	LIN PPC	PLANT PHYSIOL	74	975	1984
1983	TABOR CW	ADV POLYAM RES	4	467	
	TABOR CW	ANNU REV BIOCHEM	53	749	1984
	KAYE AM	CELL BIOCHEM FUNCT	2	2	1984
	LUK GD	WESTERN J MED	142	88	1985
	LUK GD	GASTROENTEROLOGY	90	1261	1986
	JAIN A	MOL CELL BIOCHEM	78	3	1987
	BALASUNDARAM D	MOL CELL BIOCHEM	100	129	1991
1983	TYAGI AK	METH ENZYMOLOGY	94	135	
	TABOR CW	ANNU REV BIOCHEM	53	749	1984
	TABOR CW	MICROBIOL REV	49	81	1985
	BIRECKA H	PLANT PHYSIOL	80	798	1986
	BALASUNDARAM D	ARCH BIOCHEM BIOPHYS	264	288	1988
	YAMAMOTO S	MICROBIOL IMMUNOL	32	675	1988
	NAKAO H	J GEN MICROBIOL	135	345	1989
	KLEIN RD	EXP PARASITOL	87	171	1997
	KLEIN RD	MICROBIOL-UK	145	301	1999
	GUPTA R	P NATL ACAD SCI USA	98	10620	2001
	SUBHI AL	J BIOL CHEM	278	49868	2003
	CHATTOPADHYAY MK	P NATL ACAD SCI USA	102	16158	2005
1983	TYAGI AK	TRENDS PHARMACOL SCI	4	299	
	TYAGI AK	ADV PHARMACOL CHEMOT	20	69	1984
	CASEY PJ	J BIOL CHEM	261	3637	1986
	AHLUWALIA GS	PHARMACOL THERAPEUT	46	243	1990
	BRAND J	CHEM-EUR J	12	499	2006
1984	TYAGI AK	P NAT ACAD SCI	81	1149	
	TABOR CW	ANNU REV BIOCHEM	53	749	1984
	SLOCUM RD	ARCH BIOCHEM BIOPHYS	235	283	1984
	TIPPER DJ	MICROBIOL REV	48	125	1984
	ESCRIBANO MI	ENDOCYT CELL RES	2	239	1985
	TABOR CW	MICROBIOL REV	49	81	1985
	HANNIG EM	NUCLEIC ACIDS RES	13	4379	1985
	LEE M	J VIROL	58	402	1986
	FUJIMURA T	MOL CELL BIOL	6	404	1986
	WICKNER RB	ANNU REV BIOCHEM	55	373	1986
	BENDOVA O	FOLIA MICROBIOL	31	422	1986
	JAIN A	MOL CELL BIOCHEM	78	3	1987
	UEMURA H	MOL CELL BIOL	8	938	1988
	ICHO T	J BIOL CHEM	263	1467	1988
	FUJIMURA T	J BIOL CHEM	263	454	1988
	NESTEROVA GF	GENETIKA+	24	1141	1988
	BROWN GG	INT REV CYTOL	117	1	1989

	BALASUNDARAM D	MOL CELL BIOCHEM	100	129	1991
	TERCERO JC	J BIOL CHEM	267	20270	1992
	VANVUUREN HJJ	AM J ENOL VITICULT	43	119	1992
	WICKNER RB	ANNU REV MICROBIOL	46	347	1992
	SINGH V	DNA REPAIR	12	450	2013
1984	TYAGI AK	ADV PHARMA CHEM	20	69	
	CASEY PJ	BIOCHEM PHARMACOL	36	705	1987
	HONG SS	JPN J CANCER RES	80	592	1989
	AHLUWALIA GS	PHARMACOL THERAPEUT	46	243	1990
	PEETERS MA	ANN GENET-PARIS	34	219	1991
	ALENIN VV	BIOCHEMISTRY-MOSCOW+	57	572	1992
	RAMACHANDRAN B	J BIOL CHEM	268	23891	1993
	CARRERA CJ	HEMATOL ONCOL CLIN N	8	357	1994
	GUICHERIT OM	J BIOL CHEM	269	4488	1994
	GUICHERIT OM	ADV EXP MED BIOL	370	585	1994
	HORI H	CANCER RES	56	5653	1996
	BATOVA A	BLOOD	88	3083	1996
	PALOS TP	MOL BRAIN RES	37	297	1996
	BATOVA A	CANCER RES	59	1492	1999
	BATIUK TD	AM J PHYSIOL-CELL PH	281	C1776	2001
	HARASAWA H	LEUKEMIA	16	1799	2002
	HRABIE JA	CHEM REV	102	1135	2002
	ARULSAMY N	TETRAHEDRON LETT	44	4267	2003
	STRAZZOLINI P	EUR J ORG CHEM	47	10	2004
	BATOVA A	BLOOD	107	898	2006
	LI XM	MOL CANCER THER	5	337	2006
	BRAND J	CHEM-EUR J	12	499	2006
	BILODEAU-GOESEELS S	MOL REPROD DEV	74	1021	2007
1987	JAIN A	MOL CELL BIOCHEM	78	3	
	OLLER AR	BIOCHEMISTRY-US	30	2543	1991
	BALASUNDARAM D	MOL CELL BIOCHEM	100	129	1991
	SCHWARTZ B	BIOCHEM J	312	83	1995
1987	BHUTANI V	NUTRITION RES	7	763	
	BHUTANI V	INT J VITAM NUTR RES	58	452	1988
	BHUTANI V	NUTR RES	9	465	1989
1988	BALASUNDARAM D	ARCH BIOC BIOP	264	288	
	BALASUNDARAM D	EUR J BIOCHEM	183	339	1989
	SARKAR NK	BIOCHEM MOL BIOL INT	35	1189	1995
	TALAUE MT	J BACTERIOL	188	4830	2006
1989	BALASUNDARAM D	EUR J BIOCHEM	183	339	
	SANCHEZ CP	BIOCHEM BIOPH RES CO	212	396	1995
	SARKAR NK	BIOCHEM MOL BIOL INT	35	1189	1995
	SETH A	J BACTERIOL	182	919	2000
	COLEMAN CS	BIOCHEM J	379	849	2004
1990	TYAGI JS	TROP MED PARASITOL	41	294	
	VERMA A	INDIAN J BIOCHEM BIO	32	429	1995
1990	BHARGAVA S	J BACTERIOL	172	2930	
	LEE MH	P NATL ACAD SCI USA	88	3111	1991
	KEMPSELL KE	J GEN MICROBIOL	138	1717	1992
	TYAGI JS	NUCLEIC ACIDS RES	20	138	1992
	KINGER AK	GENE	131	113	1993
	GUPTA S	GENE	126	157	1993
	VERMA A	GENE	148	113	1994
	VERMA A	INDIAN J BIOCHEM BIO	31	288	1994

	VERMA A	INDIAN J BIOCHEM BIO	32	429	1995
	MISRA N	INT J LEPROSY	63	35	1995
	VASANTHAKRISHNA M	MICROBIOL-UK	143	3591	1997
	PENA CEA	J MOL BIOL	266	76	1997
	VASANTHAKRISHNA M	J BIOSCIENCE	23	101	1998
	DASTUR A	ARCH MICROBIOL	178	288	2002
	LI AH	MICROBIOL-SGM	154	2291	2008
	KOSER CU	INFECT GENET EVOL	12	807	2012
1991	BALASUNDARAM D	MOL CELL BIOCHEM	100	129	
	BLACHIER F	BIOCHIM BIOPHYS ACTA	1175	21	1992
	RAJAM MV	CURR SCI INDIA	65	461	1993
	WING LYC	J PHARMACOL EXP THER	266	179	1993
	MCCORMACK SA	AM J PHYSIOL	264	G367	1993
	BALASUNDARAM D	P NATL ACAD SCI USA	91	172	1994
	HUANG H	BIOGENIC AMINES	10	259	1994
	LINARES PN	BIOGENIC AMINES	10	365	1994
	SHINOZAKI T	J RHEUMATOL	22	1907	1995
	SARKAR NK	BIOCHEM MOL BIOL INT	35	1189	1995
	HUBER M	CANCER RES	55	934	1995
	AUCHTER RM	ARCH OTOLARYNGOL	122	977	1996
	YOUNOSZAI MK	P SOC EXP BIOL MED	211	339	1996
	MADESH M	BBA-LIPID LIPID MET	1348	324	1997
	MURLEY JS	CELL PROLIFERAT	30	283	1997
	BERLAIMONT V	ANTICANCER RES	17	2057	1997
	CORRALIZA IM	BBA-GEN SUBJECTS	1334	123	1997
	MITCHELL JLA	BIOCHEM J	335	329	1998
	LEVEQUE J	ANTICANCER RES	18	2663	1998
	SARAN S	CELL BIOL INT	22	575	1998
	BOOTH VK	RADIAT RES	153	813	2000
	PENDEVILLE H	MOL CELL BIOL	21	6549	2001
	MCCORMACK SA	J PHYSIOL PHARMACOL	52	327	2001
	SCORCIONI F	BIOCHEM J	354	217	2001
	HAHM HA	CLIN CANCER RES	7	391	2001
	BAIS HP	PLANT CELL TISS ORG	69	1	2002
	BLACHIER F	AMINO ACIDS	33	547	2007
	WORTHAM BW	ADV EXP MED BIOL	603	106	2007
	DAIGLE ND	J CELL PHYSIOL	220	680	2009
	WORTHAM BW	ENVIRON MICROBIOL	12	2034	2010
	PLEDGIE-TRACY A	CANCER CHEMOTH PHARM	65	1067	2010
	LEFEVRE PLC	ENDOCR REV	32	694	2011
	CERRADA-GIMENEZ M	AMINO ACIDS	42	451	2012
	MURRAY-STEWART T	MOL CANCER THER	12	2088	2013
1992	SHANKAR S	NUCLEIC ACIDS RES	20	2891	
	SHANKAR S	GENE	132	119	1993
	SHANKAR S	GENE	131	153	1993
	ROBERTS RJ	NUCLEIC ACIDS RES	21	3125	1993
	VANSOOLINGEN D	J BACTERIOL	178	78	1996
	MANDAL P	J BIOCHEM MOL BIOL	39	140	2006
1992	SHANKAR S	NUCLEIC ACID RES	20	2890	
	SHANKAR S	GENE	132	119	1993
	SHANKAR S	GENE	131	153	1993
	ROBERTS RJ	NUCLEIC ACIDS RES	21	3125	1993
	VANSOOLINGEN D	J BACTERIOL	178	78	1996
1993	DASGUPTA SK	J BACTERIOL	175	5186	
	BASHYAM MD	BIOTECHNIQUES	17	834	1994
	TIMM JL	J BACTERIOL	176	6749	1994
	VERMA A	GENE	148	113	1994

TIMM J	MOL MICROBIOL	12	491	1994
RAMESH GR	INDIAN J BIOCHEM BIO	32	361	1995
KREMER L	MOL MICROBIOL	17	913	1995
NESBIT CE	MOL MICROBIOL	17	1045	1995
DELLAGOSTIN OA	MICROBIOL-UK	141	1785	1995
WINTER N	MOL MICROBIOL	16	865	1995
SARKAR NK	BIOCHEM MOL BIOL INT	35	1189	1995
KREMER L	J BACTERIOL	177	642	1995
PAGET E	J BACTERIOL	178	6357	1996
TYAGI JS	GENE	177	59	1996
BEGGS ML	GENE	174	285	1996
BASHYAM MD	J BACTERIOL	178	4847	1996
HATFULL GF	CURR TOP MICROBIOL	215	29	1996
VASANTHAKRISHNA M	MICROBIOL-UK	143	3591	1997
PARISH T	MICROBIOL-UK	143	2267	1997
MOVAHEDZADEH F	J BACTERIOL	179	3509	1997
JAIN S	GENE	190	37	1997
BANNANTINE JP	MICROBIOL-UK	143	921	1997
MULDER MA	TUBERCLE LUNG DIS	78	211	1997
BATONI G	FEMS MICROBIOL LETT	169	117	1998
KNIPFER N	GENE	217	69	1998
BARKER LP	MOL MICROBIOL	29	1167	1998
RAYCHAUDHURI S	MICROBIOL-UK	144	2131	1998
CHUBB AJ	MICROBIOL-UK	144	1619	1998
DASGUPTA SK	BIOCHEM BIOPH RES CO	246	797	1998
BASHYAM MD	J BACTERIOL	180	2568	1998
PARISH A	MOL BIOTECHNOL	13	191	1999
UNNIRAMAN S	GENES CELLS	4	697	1999
CARBONELLI DL	FEMS MICROBIOL LETT	177	75	1999
VERMA A	J BACTERIOL	181	4326	1999
BARKER LP	FEMS MICROBIOL LETT	175	79	1999
GUPTA S	FEMS MICROBIOL LETT	172	137	1999
CHAWLA M	PLASMID	41	135	1999
RUBIN EJ	P NATL ACAD SCI USA	96	1645	1999
HATFULL GF	METHOD MICROBIOL	29	251	1999
DHAR N	FEMS MICROBIOL LETT	190	309	2000
TYAGI AK	MULTI-DRUG RESISTANCE IN EMERG	10	9	2000
DASGUPTA N	TUBERCLE LUNG DIS	80	141	2000
UNNIRAMAN S	J BIOL CHEM	276	41850	2001
TRICCAS JA	MICROBIOL-SGM	147	1253	2001
COWLEY SC	GENE	264	225	2001
UNNIRAMAN S	NUCLEIC ACIDS RES	30	5376	2002
SIRAKOVA TD	J BACTERIOL	184	6796	2002
DASTUR A	ARCH MICROBIOL	178	288	2002
UNNIRAMAN S	J BACTERIOL	184	5449	2002
MEDEIROS MA	MICROBIOL-SGM	148	1999	2002
KAMALAKANNAN V	FEMS MICROBIOL LETT	209	261	2002
BASU A	J BACTERIOL	184	2204	2002
CHATTOPADHYAY C	J BIOCHEM MOL BIOL	36	586	2003
RAO V	SCAND J IMMUNOL	58	449	2003
AGARWAL N	FEMS MICROBIOL LETT	225	75	2003
UNNIRAMAN S	BIOTECHNIQUES	35	256	2003
SMITH I	CLIN MICROBIOL REV	16	463	2003
SATCHIDANANDAM V	FEMS MICROBIOL LETT	218	365	2003
SOHASKEY CD	FEMS MICROBIOL LETT	240	187	2004
SAU S	J BIOCHEM MOL BIOL	37	254	2004
BASU A	J BACTERIOL	186	335	2004
BAGCHI G	MICROBIOL-SGM	151	4045	2005
ZHU JC	THERMOCHIM ACTA	439	52	2005
DEOL P	J BACTERIOL	187	3415	2005
RAO V	SCAND J IMMUNOL	61	410	2005

	DATTA I	J BIOCHEM MOL BIOL	38	89	2005
	MACHOWSKI EE	INT J BIOCHEM CELL B	37	54	2005
	AGARWAL N	NUCLEIC ACIDS RES	34	4245	2006
	GUPTA R	BIOCHEM BIOPH RES CO	343	1141	2006
	GALL K	FEMS MICROBIOL LETT	255	301	2006
	TOBIAS NJ	PLOS NEGLECT TROP D	3	e553	2009
	JOON M	BMC MICROBIOL	10	128	2010
	TYAGI AK	TUBERCULOSIS	91	469	2011
	BANDYOPADHYAY B	J BACTERIOL	194	4688	2012
	REDDY PV	J BACTERIOL	194	567	2012
	BARTASUN P	J GEN APPL MICROBIOL	58	387	2012
	ROY S	MICROB BIOTECHNOL	5	98	2012
	PURI RV	PLOS ONE	8	e71535	2013
	PURI RV	PLOS ONE	8	e70514	2013
	NEWTON-FOOT M	TUBERCULOSIS	93	60	2013
1993	GUPTA S	GENE	126	157	
	SHANKAR S	GENE	132	119	1993
	SHANKAR S	GENE	131	153	1993
	RAMESH GR	INDIAN J BIOCHEM BIO	32	361	1995
	YOUNG DB	ANNU REV MICROBIOL	49	641	1995
	COLLINS DM	TRENDS MICROBIOL	4	426	1996
	GORDON S	J APPL BACTERIOL	81	S10	1996
	QUINN FD	CURR TOP MICROBIOL	215	131	1996
	GALLEGOS MT	MICROBIOL MOL BIOL R	61	393	1997
	MATSUSAKI H	J BACTERIOL	180	6459	1998
	RIVERA-MARRERO CA	MICROB PATHOGENESIS	25	307	1998
	GERRITSE G	J BIOTECHNOL	64	23	1998
	GUPTA S	FEMS MICROBIOL LETT	172	137	1999
	AV-GAY Y	TRENDS MICROBIOL	8	238	2000
	TYAGI AK	MULTI-DRUG RESISTANCE IN EMERG	10	9	2000
	MONAHAN IM	MICROBIOL-UK	147	459	2001
	SINGH A	FEMS MICROBIOL LETT	227	53	2003
	RECCHI C	J BIOL CHEM	278	33763	2003
	PETTINARI MJ	PLASMID	50	36	2003
	SINGH R	TUBERCULOSIS	85	325	2005
	SINGH A	J BACTERIOL	187	4173	2005
	PAWARIA S	APPL ENVIRON MICROB	74	3512	2008
	NDE CW	APPL MICROBIOL BIOT	90	277	2011
1993	SHANKAR S	GENE	131	153	
	ROBERTS RJ	NUCLEIC ACIDS RES	22	3628	1994
	VANSOOLINGEN D	J BACTERIOL	178	78	1996
	MANDAL P	J BIOCHEM MOL BIOL	39	140	2006
	VOSSOUGHI M	MA COMPUT SCI ENG		7	2007
	JACOBS-SERA D	VIROLOGY	434	187	2012
1993	SHANKAR S	GENE	132	119	
	ROBERTS RJ	NUCLEIC ACIDS RES	22	3628	1994
	VANSOOLINGEN D	J BACTERIOL	178	78	1996
	MANDAL P	J BIOCHEM MOL BIOL	39	140	2006
	JACOBS-SERA D	VIROLOGY	434	187	2012
1994	BASHYAM MD	BIOTECHNIQUES	17	834	
	TYAGI JS	GENE	177	59	1996
	JACKSON M	MICROBIOL-UK	142	2439	1996
	BASHYAM MD	J BACTERIOL	178	4847	1996
	FALKINHAM JO	CLIN MICROBIOL REV	9	177	1996
	CACERES NE	J BACTERIOL	179	5046	1997
	NAGY I	LETT APPL MICROBIOL	25	75	1997
	PEIRS P	EUR J BIOCHEM	244	604	1997

	BANNANTINE JP	MICROBIOL-UK	143	921	1997
	MANGAN JA	NUCLEIC ACIDS RES	25	675	1997
	BERTHET FX	MICROBIOL-UK	144	3195	1998
	VERMA A	J BACTERIOL	181	4326	1999
	GUPTA S	FEMS MICROBIOL LETT	172	137	1999
	HATFULL GF	METHOD MICROBIOL	29	251	1999
	ALONSO G	FEMS MICROBIOL LETT	192	257	2000
	GILOT P	J MED MICROBIOL	49	887	2000
	YAO YF	J MICROBIOL METH	51	191	2002
	FENG ZY	J BACTERIOL	184	5001	2002
	MANGAN JA	METHOD MICROBIOL	33	137	2002
	SUNG K	FEMS MICROBIOL LETT	229	97	2003
	SINGH A	FEMS MICROBIOL LETT	227	53	2003
	FENG ZY	ANTIMICROB AGENTS CH	47	283	2003
	STEPHAN J	BMC MICROBIOL	4	45	2004
	SHARBATI-TEHRANI S	INT J MED MICROBIOL	294	235	2004
	SHARBATI-TEHRANI S	MICROBIOL-SGM	151	2403	2005
	KIM BH	J PHYCOL	42	1137	2006
	JAHN CE	J MICROBIOL METH	75	318	2008
	SHARBATI S	BMC MICROBIOL	9	31	2009
	AKHTAR S	ANAL BIOCHEM	417	286	2011
	KIM BH	PLOS ONE	7	e37770	2012
1995	SARKAR NK	BIOCH MOL BIOL INT	35	1189	
	BERGER BJ	BMC MICROBIOL	3	12	2003
	KHEDKAR SA	J MOL GRAPH MODEL	23	355	2005
1996	BASHYAM MD	J BACTERIOL	178	4847	
	PLUM G	INFECT IMMUN	65	4548	1997
	MENENDEZ MC	J BACTERIOL	179	6880	1997
	GONZALEZYMERCHAND JA	J BACTERIOL	179	6949	1997
	BARNES MR	J BACTERIOL	179	6145	1997
	SPOHN G	MOL MICROBIOL	26	361	1997
	CACERES NE	J BACTERIOL	179	5046	1997
	NAGY I	LETT APPL MICROBIOL	25	75	1997
	MOVAHEDZADEH F	J BACTERIOL	179	3509	1997
	WU QL	J BACTERIOL	179	2922	1997
	JAIN S	GENE	190	37	1997
	BANNANTINE JP	MICROBIOL-UK	143	921	1997
	GOMEZ JE	TUBERCLE LUNG DIS	78	175	1997
	MULDER MA	TUBERCLE LUNG DIS	78	211	1997
	BOSHOFF HIM	J BACTERIOL	180	5809	1998
	BERTHET FX	MICROBIOL-UK	144	3195	1998
	KNIPFER N	GENE	217	69	1998
	RAYCHAUDHURI S	MICROBIOL-UK	144	2131	1998
	DHANDAYUTHAPANI S	GENE	215	213	1998
	LARKIN MJ	ANTON LEEUW INT J G	74	133	1998
	GOMEZ M	MOL MICROBIOL	29	617	1998
	DASGUPTA SK	BIOCHEM BIOPH RES CO	246	797	1998
	FORD ME	J MOL BIOL	279	143	1998
	BASHYAM MD	J BACTERIOL	180	2568	1998
	PLIKAYTIS BB	J BACTERIOL	180	1037	1998
	MATSUMOTO S	MICROBIOL IMMUNOL	42	15	1998
	UNNIRAMAN S	GENES CELLS	4	697	1999
	MULDER MA	MICROBIOL-UK	145	2507	1999
	FERNANDES ND	J BACTERIOL	181	4266	1999
	VERMA A	J BACTERIOL	181	4326	1999
	BARKER LP	FEMS MICROBIOL LETT	175	79	1999
	DUSSURGET O	J BACTERIOL	181	3402	1999
	HU YM	J BACTERIOL	181	3486	1999
	HU YM	J BACTERIOL	181	1380	1999

RUBIN EJ	P NATL ACAD SCI USA	96	1645	1999
STOLT P	NUCLEIC ACIDS RES	27	396	1999
HU JM	J BACTERIOL	181	469	1999
HATFULL GF	METHOD MICROBIOL	29	251	1999
DHAR N	FEMS MICROBIOL LETT	190	309	2000
ALLAND D	J BACTERIOL	182	1802	2000
BIGI F	MICROBIOL-UK	146	1011	2000
PARKER AE	MICROB PATHOGENESIS	28	135	2000
RAMASWAMY SV	ANTIMICROB AGENTS CH	44	326	2000
TYAGI AK	MULTI-DRUG RESISTANCE IN EMERG	10	9	2000
DASGUPTA N	TUBERCLE LUNG DIS	80	141	2000
TULLIUS MV	INFECT IMMUN	69	6348	2001
HARRIS NB	CLIN MICROBIOL REV	14	489	2001
INGLIS NF	MICROBIOL-SGM	147	1557	2001
TORRES A	MICROB PATHOGENESIS	30	289	2001
DASTUR A	TUBERCULOSIS	81	267	2001
SIRAKOVA TD	J BACTERIOL	184	6796	2002
DASTUR A	ARCH MICROBIOL	178	288	2002
UNNIRAMAN S	J BACTERIOL	184	5449	2002
KALATE RN	BIOPHYS CHEM	99	77	2002
MAYURI	FEMS MICROBIOL LETT	211	231	2002
TYAGI JS	TRENDS MICROBIOL	10	68	2002
MUSATOVOVA O	FEMS MICROBIOL LETT	229	73	2003
KALATE RN	COMPUT BIOL CHEM	27	555	2003
CHATTOPADHYAY C	J BIOCHEM MOL BIOL	36	586	2003
GOPAUL KK	J BACTERIOL	185	6005	2003
RECCHI C	J BIOL CHEM	278	33763	2003
PATEK M	J BIOTECHNOL	104	325	2003
SALA C	J BACTERIOL	185	5357	2003
BAGCHI G	MICROBIOL-SGM	149	2303	2003
AGARWAL N	FEMS MICROBIOL LETT	225	75	2003
UNNIRAMAN S	BIOTECHNIQUES	35	256	2003
SAVIOLA B	INFECT IMMUN	71	1379	2003
SATCHIDANANDAM V	FEMS MICROBIOL LETT	218	365	2003
ROY S	RES MICROBIOL	155	817	2004
SOHASKEY CD	FEMS MICROBIOL LETT	240	187	2004
SHARBATI-TEHRANI S	INT J MED MICROBIOL	294	235	2004
ROBERTS EA	J BACTERIOL	186	5410	2004
SAFI H	MOL MICROBIOL	52	999	2004
LEE BR	BIOTECHNOL LETT	26	589	2004
SAU S	J BIOCHEM MOL BIOL	37	254	2004
BAGCHI G	MICROBIOL-SGM	151	4045	2005
JAIN V	GENE	351	149	2005
BURONI S	ANTIMICROB AGENTS CH	50	4044	2006
HERNANDEZ-ABANTO SM	ARCH MICROBIOL	186	459	2006
AGARWAL N	NUCLEIC ACIDS RES	34	4245	2006
EHRT S	FUTURE MICROBIOL	1	177	2006
GUPTA R	BIOCHEM BIOPH RES CO	343	1141	2006
FABOZZI G	MICROB PATHOGENESIS	40	211	2006
GONZALEZ-DIAZ H	BIOORG MED CHEM LETT	16	547	2006
JAIN V	J MICROBIOL	44	1	2006
CHOWDHURY RP	J BACTERIOL	189	8973	2007
SEO JG	MICROBIOL-SGM	153	4174	2007
HALBEDEL S	J MOL BIOL	371	596	2007
BYRNE GA	J BACTERIOL	189	5082	2007
RICHTER L	GENE	395	22	2007
SUBBIAN S	CAN J MICROBIOL	53	599	2007
GONZALEZ-DIAZ H	CHEMOMETR INTELL LAB	85	20	2007
CHURCHILL PF	J ENVIRON SCI HEAL B	43	698	2008
GONZALES M	MOL BIOL REP	36	1225	2009
SHARMA D	NUCLEIC ACIDS RES	37	W193	2009

	KAUR P	PLOS ONE	4	e5923	2009
	NASH KA	ANTIMICROB AGENTS CH	53	1367	2009
	VALLECILLO AJ	MICROB PATHOGENESIS	46	119	2009
	JOON M	BMC MICROBIOL	10	128	2010
	SACHDEVA P	FEBS J	277	605	2010
	TYAGI AK	TUBERCULOSIS	91	469	2011
	BHARATI BK	GENE	528	99	2013
	NEWTON-FOOT M	TUBERCULOSIS	93	60	2013
1997	JAIN S	GENE	190	37	
	BARKER LP	MOL MICROBIOL	29	1167	1998
	DASGUPTA SK	BIOCHEM BIOPH RES CO	246	797	1998
	ROWLAND B	FEMS MICROBIOL LETT	179	317	1999
	VERMA A	J BACTERIOL	181	4326	1999
	GUPTA S	FEMS MICROBIOL LETT	172	137	1999
	HATFULL GF	METHOD MICROBIOL	29	251	1999
	JAIN S	MOL MICROBIOL	38	971	2000
	PINEIRO SA	CURR MICROBIOL	40	302	2000
	TYAGI AK	MULTI-DRUG RESISTANCE IN EMERG	10	9	2000
	DASGUPTA N	TUBERCLE LUNG DIS	80	141	2000
	CHATTOPADHYAY C	J BIOCHEM MOL BIOL	36	586	2003
	SINGH A	FEMS MICROBIOL LETT	227	53	2003
	KIM AI	MOL MICROBIOL	50	463	2003
	BAGCHI G	MICROBIOL-SGM	149	2303	2003
	AGARWAL N	FEMS MICROBIOL LETT	225	75	2003
	GANGULY T	J BIOCHEM MOL BIOL	37	709	2004
	SAU S	J BIOCHEM MOL BIOL	37	254	2004
	SINGH R	TUBERCULOSIS	85	325	2005
	JAIN V	GENE	351	149	2005
	RAGHUNAND TR	MICROBIOL-SGM	152	2735	2006
	AGARWAL N	MICROBIOL-SGM	152	2749	2006
	AGARWAL N	NUCLEIC ACIDS RES	34	4245	2006
	GANGULY T	PROTEIN PEPTIDE LETT	13	793	2006
	CHOWDHURY RP	J BACTERIOL	189	8973	2007
	MANDAL S	MICROBIOL-SGM	153	80	2007
	JAIN R	PLOS ONE	3	e3869	2008
	MALHOTRA M	ENVIRON MICROBIOL	10	1365	2008
	DEY B	VACCINE	28	63	2009
	DAM B	APPL ENVIRON MICROB	75	4362	2009
	MORTON MJ	J BIOL CHEM	285	33737	2010
	RAO T	FEMS MICROBIOL LETT	310	24	2010
	JOON M	BMC MICROBIOL	10	128	2010
	PARUA PK	ARCH BIOCHEM BIOPHYS	493	175	2010
	PARUA PK	J GEN VIROL	91	306	2010
	JAIN R	VACCINE	29	8118	2011
	TYAGI AK	TUBERCULOSIS	91	469	2011
	DEY B	PLOS ONE	6	e18773	2011
	DAM B	PLASMID	65	185	2011
	TARE P	PLOS ONE	7	e43900	2012
	BANDYOPADHYAY B	J BACTERIOL	194	4688	2012
	MANDAL S	ARCH MICROBIOL	194	737	2012
	MANDAL S	CURR MICROBIOL	64	259	2012
	BHARATI BK	GENE	528	99	2013
	RATHOR N	TUBERCULOSIS	93	389	2013
1998	BASHYAM MD	J BACTERIOL	180	2568	
	PARISH A	MOL BIOTECHNOL	13	191	1999
	FERNANDES ND	J BACTERIOL	181	4266	1999
	BARKER LP	FEMS MICROBIOL LETT	175	79	1999
	BURNS HD	NUCLEIC ACIDS RES	27	2051	1999
	MADSEN SM	MOL MICROBIOL	32	75	1999

	BOWN JA	J BIOL CHEM	274	2263	1999
	STOLT P	NUCLEIC ACIDS RES	27	396	1999
	NARAYANAN S	FEMS MICROBIOL LETT	192	263	2000
	GIARD JC	J BACTERIOL	182	4512	2000
	TYAGI AK	MULTI-DRUG RESISTANCE IN EMERG	10	9	2000
	DASGUPTA N	TUBERCLE LUNG DIS	80	141	2000
	LI MS	MICROBIOL-SGM	147	2293	2001
	HARRIS NB	CLIN MICROBIOL REV	14	489	2001
	INGLIS NF	MICROBIOL-SGM	147	1557	2001
	GAL-MOR O	J BACTERIOL	184	3823	2002
	CHATTOPADHYAY C	J BIOCHEM MOL BIOL	36	586	2003
	RECCHI C	J BIOL CHEM	278	33763	2003
	MITCHELL JE	NUCLEIC ACIDS RES	31	4689	2003
	AGARWAL N	FEMS MICROBIOL LETT	225	75	2003
	UNNIRAMAN S	BIOTECHNIQUES	35	256	2003
	HAYASHI K	PLANT CELL PHYSIOL	44	334	2003
	SATCHIDANANDAM V	FEMS MICROBIOL LETT	218	365	2003
	MENENDEZ MD	J BACTERIOL	187	534	2005
	PASHLEY CA	MICROBIOL-SGM	152	2727	2006
	AGARWAL N	NUCLEIC ACIDS RES	34	4245	2006
	EHRT S	FUTURE MICROBIOL	1	177	2006
	DOHERTY N	J BACTERIOL	188	2885	2006
	RICHTER L	GENE	395	22	2007
	DANILCHANKA O	ANTIMICROB AGENTS CH	52	2503	2008
	MICK V	J ANTIMICROB CHEMOTH	61	39	2008
	KOO BM	MOL MICROBIOL	72	815	2009
	SACHDEVA P	FEBS J	277	605	2010
	JEONG DW	J BACTERIOL	193	4672	2011
	SCHUESSLER DL	PLOS ONE	7	e34471	2012
	LIGON LS	J BACTERIOL	195	4456	2013
	NEWTON-FOOT M	TUBERCULOSIS	93	60	2013
1998	DASGUPTA SK	BIOC BIOP RES COMM	246	797	
	VERMA A	J BACTERIOL	181	4326	1999
	CHAWLA M	PLASMID	41	135	1999
	JAIN S	MOL MICROBIOL	38	971	2000
	DHAR N	FEMS MICROBIOL LETT	190	309	2000
	OJHA AK	INFECT IMMUN	68	4084	2000
	TYAGI AK	MULTI-DRUG RESISTANCE IN EMERG	10	9	2000
	UNNIRAMAN S	J BACTERIOL	184	5449	2002
	MEDEIROS MA	MICROBIOL-SGM	148	1999	2002
	BASU A	J BACTERIOL	184	2204	2002
	AL-ZAROUNI M	TUBERCULOSIS	82	283	2002
	SINGH R	MOL MICROBIOL	50	751	2003
	SINGH A	FEMS MICROBIOL LETT	227	53	2003
	RAO V	SCAND J IMMUNOL	58	449	2003
	DHAR N	IMMUNOL LETT	88	175	2003
	VENKATESH J	J BIOL CHEM	278	24350	2003
	SATCHIDANANDAM V	FEMS MICROBIOL LETT	218	365	2003
	BASU A	J BACTERIOL	186	335	2004
	SHENOY AR	BIOCHEMISTRY-US	44	15695	2005
	MATHEW R	J BACTERIOL	187	6565	2005
	SINGH A	J BACTERIOL	187	4173	2005
	RAO V	SCAND J IMMUNOL	61	410	2005
	DENNEHY M	VACCINE	23	1209	2005
	YU JS	CLIN VACCINE IMMUNOL	13	1204	2006
	RAO A	APPL ENVIRON MICROB	73	1320	2007
	SALLAM KI	GENE	386	173	2007
	JAIN R	PLOS ONE	3	e3869	2008
	DEY B	VACCINE	28	63	2009
	SESHADRI A	TUBERCULOSIS	89	453	2009

	LU L	VACCINE	27	972	2009
	FAN XY	PLASMID	61	39	2009
	ZHANG H	SCAND J IMMUNOL	72	349	2010
	GUPTA AK	INDIAN J MED RES	132	176	2010
	TYAGI AK	TUBERCULOSIS	91	469	2011
	BANDYOPADHYAY B	J BACTERIOL	194	4688	2012
	ZHAO SM	PLOS ONE	7	e31908	2012
	LIN CW	APMIS	120	72	2012
	ROY S	MICROB BIOTECHNOL	5	98	2012
	REDDY PV	J INFECT DIS	208	1255	2013
1999	GUPTA S	FEMS MICROB LETT	172	137	
	TYAGI AK	MULTI-DRUG RESISTANCE IN EMERG	10	9	2000
	MEHROTRA J	INT J MED MICROBIOL	291	171	2001
	COLLINS DM	TUBERCULOSIS	81	97	2001
	SINGH A	FEMS MICROBIOL LETT	227	53	2003
	RECCHI C	J BIOL CHEM	278	33763	2003
	FROTA CC	INFECT IMMUN	72	5483	2004
	MOSTOWY S	J BACTERIOL	186	104	2004
	MARRI PR	FEMS MICROBIOL REV	30	906	2006
	TALAAT AM	J BACTERIOL	189	4265	2007
	HOMOLKA S	PLOS PATHOG	6	e1000988	2010
	NDE CW	APPL MICROBIOL BIOT	90	277	2011
2000	DHAR N	FEMS MICROBIOL LETT	190	309	
	CHOUDHARY RK	INFECT IMMUN	71	6338	2003
	SINGH A	FEMS MICROBIOL LETT	227	53	2003
	RAO V	SCAND J IMMUNOL	58	449	2003
	DHAR N	IMMUNOL LETT	88	175	2003
	DHAR N	MED MICROBIOL IMMUN	193	19	2004
	KHERA A	VACCINE	23	5655	2005
	RAO V	SCAND J IMMUNOL	61	410	2005
	DENNEHY M	VACCINE	23	1209	2005
	JOSEPH J	EXPERT REV VACCINES	5	827	2006
	FAN XL	ACTA BIOCH BIOPH SIN	38	683	2006
	SHARMA K	J BACTERIOL	188	2936	2006
	WANG LM	CHINESE MED J-PEKING	120	1220	2007
	JAIN R	PLOS ONE	3	e3869	2008
	FARHANA A	PLOS ONE	3	e2087	2008
	DEY B	VACCINE	28	63	2009
	SHI C	SCAND J IMMUNOL	69	140	2009
	FAN XY	PLASMID	61	39	2009
	LORENZI JCC	BMC BIOTECHNOL	10	77	2010
	LORENZI JCC	REC ADV BIOL BIOMED	42	6	2010
	DENG YH	MICROBIOL IMMUNOL	55	798	2011
	JAIN R	VACCINE	29	8118	2011
	DEY B	PLOS ONE	6	e18773	2011
	MOUSTAFA D	VACCINE	29	784	2011
2000	KOUL A	J BACTERIOL	182	5425	
	KENNELLY PJ	CHEM REV	101	2291	2001
	KOUL A	MICROBIOL-SGM	147	2307	2001
	BARRY CE	TRENDS MICROBIOL	9	237	2001
	VAN HUIJSDUIJNEN RH	DRUG DISCOV TODAY	7	1013	2002
	BATONI G	SCAND J IMMUNOL	56	43	2002
	COWLEY SC	RES MICROBIOL	153	233	2002
	PRENETA R	COMP BIOCHEM PHYS B	131	103	2002
	LI RH	J BACTERIOL	185	6780	2003
	CHOPRA P	BIOCHEM BIOPH RES CO	311	112	2003
	SINGH R	MOL MICROBIOL	50	751	2003
	SINHA I	FEMS MICROBIOL LETT	227	141	2003

BOITEL B	MOL MICROBIOL	49	1493	2003
ANAYA-RUIZ M	INT J PARASITOL	33	663	2003
CHOPRA P	INDIAN J MED RES	117	1	2003
DEWANG PM	CURR ORG CHEM	8	947	2004
PRENETA R	MICROBIOL-SGM	150	2135	2004
SHARMA K	EXPERT OPIN THER TAR	8	79	2004
COZZONE AJ	ARCH MICROBIOL	181	171	2004
KOUL A	NAT REV MICROBIOL	2	189	2004
TYAGI JS	CURR SCI INDIA	86	93	2004
CASTANDET J	RES MICROBIOL	156	1005	2005
GRUNDNER C	STRUCTURE	13	1625	2005
MANGER M	CHEMBIOCHEM	6	1749	2005
SAXENA K	J BIOMOL NMR	33	136	2005
LEI JQ	CURR MICROBIOL	51	141	2005
SINGH R	TUBERCULOSIS	85	325	2005
VERGNE I	P NATL ACAD SCI USA	102	4033	2005
MADHURANTAKAM C	J BACTERIOL	187	2175	2005
PRABHAKAR S	J IMMUNOL	174	1003	2005
BIALY L	ANGEW CHEM INT EDIT	44	3814	2005
DEWANG PM	CURR MED CHEM	12	1	2005
GREENSTEIN AE	J MOL MICROB BIOTECH	9	167	2005
COZZONE AJ	J MOL MICROB BIOTECH	9	198	2005
BACH H	INFECT IMMUN	74	6540	2006
LESCOP E	J BIOL CHEM	281	19570	2006
XU HM	J BACTERIOL	188	1509	2006
WEIDE T	BIOORG MED CHEM LETT	16	59	2006
POOK SH	ONCOL REP	18	1315	2007
BERESFORD N	BIOCHEM J	406	13	2007
DEGHMANE AE	J CELL SCI	120	2796	2007
SOELLNER MB	J AM CHEM SOC	129	9613	2007
HOLTON SJ	CURR PROTEIN PEPT SC	8	365	2007
AGUIRRE-GARCIA MM	PARASITOL RES	101	85	2007
JANIN YL	BIOORGAN MED CHEM	15	2479	2007
O'SHEA DJ	ANAL CHIM ACTA	583	349	2007
GRANGEASSE C	TRENDS BIOCHEM SCI	32	86	2007
CORREA IR	CHEM-ASIAN J	2	1109	2007
OKU T	J BIOL CHEM	283	28918	2008
GRUNDNER C	FEMS MICROBIOL LETT	287	181	2008
BACH H	CELL HOST MICROBE	3	316	2008
MADHURANTAKAM C	PROTEINS	71	706	2008
WEHENKEL A	BBA-PROTEINS PROTEOM	1784	193	2008
RAWLS KA	BIOORG MED CHEM LETT	19	6851	2009
MUKHERJEE S	INT J BIOL MACROMOL	45	463	2009
COZZONE AJ	TRENDS MICROBIOL	17	536	2009
AMLABU E	PARASITOL INT	58	238	2009
BLOBEL J	FEBS J	276	4346	2009
BACH H	BIOCHEM J	420	155	2009
BERESFORD NJ	J ANTIMICROB CHEMOTH	63	928	2009
IRANDOUST M	ANTI-CANCER AGENT ME	9	212	2009
HENEBERG P	CURR MED CHEM	16	706	2009
HE RJ	CHEMMEDCHEM	5	2051	2010
CHANDRA K	BIOORGAN MED CHEM	18	8365	2010
BERESFORD NJ	BMC GENOMICS	11	457	2010
SILVA APG	FUTURE MED CHEM	2	1325	2010
MASCARELLO A	BIOORGAN MED CHEM	18	3783	2010
MEENA LS	FEBS J	277	2416	2010
CHAO J	BBA-PROTEINS PROTEOM	1804	620	2010
ECCO G	CHEM COMMUN	46	7501	2010
LI W	J CELL BIOCHEM	112	2688	2011
VINTONYAK VV	TETRAHEDRON	67	6713	2011
SHAPLAND EB	J BACTERIOL	193	4361	2011

	MUKHOPADHYAY A	J BIOCHEM	149	551	2011
	PEREIRA SFF	MICROBIOL MOL BIOL R	75	192	2011
	STEHLE T	J BIOL CHEM	287	34569	2012
	EITSON JL	APPL ENVIRON MICROB	78	6829	2012
	JAYACHANDRAN R	EXPERT REV ANTI-INFE	10	1007	2012
	DONG LH	J MOL MODEL	18	3847	2012
	RAHMAT JN	UROLOGY	79	1411.e15	2012
	HENEBERG P	CURR MED CHEM	19	1530	2012
	WHITMORE SE	INT J ORAL SCI	4	1	2012
	CHIARADIA LD	J MED CHEM	55	390	2012
	NIR-PAZ R	FEMS MICROBIOL LETT	326	151	2012
	WONG D	TRENDS MICROBIOL	21	100	2013
	RAI R	PARASITOL RES	112	147	2013
	HE RJ	CHEM COMMUN	49	2064	2013
	MATIOLLO C	BBA-PROTEINS PROTEOM	1834	191	2013
	FORRELLAD MA	VIRULENCE	4	3	2013
2000	TYAGI AK	MOL GEN MYCOBACTERIA	131		
	HOTTER GS	FEMS MICROBIOL LETT	200	151	2001
	HOBSON RJ	MICROBIOL-SGM	148	1571	2002
	SMITH I	CLIN MICROBIOL REV	16	463	2003
	CLARK-CURTISS JE	ANNU REV MICROBIOL	57	517	2003
	DELOGU G	MOL MICROBIOL	52	725	2004
	MACHOWSKI EE	INT J BIOCHEM CELL B	37	54	2005
2001	KOUL A	MICROBIOLOGY	147	2307	
	PALLEN M	TRENDS MICROBIOL	10	556	2002
	MADEC E	MOL MICROBIOL	46	571	2002
	CHABA R	EUR J BIOCHEM	269	1078	2002
	MOLLE V	BIOCHEMISTRY-US	42	15300	2003
	CHOPRA P	BIOCHEM BIOPH RES CO	311	112	2003
	SINGH R	MOL MICROBIOL	50	751	2003
	SINHA I	FEMS MICROBIOL LETT	227	141	2003
	VERMA A	INFECT IMMUN	71	5772	2003
	MOLLE V	BIOCHEM BIOPH RES CO	308	820	2003
	BOITEL B	MOL MICROBIOL	49	1493	2003
	ORTIZ-LOMBARDIA M	J BIOL CHEM	278	13094	2003
	YOUNG TA	NAT STRUCT BIOL	10	168	2003
	CHOPRA P	INDIAN J MED RES	117	1	2003
	PULLEN KE	STRUCTURE	12	1947	2004
	PRENETA R	MICROBIOL-SGM	150	2135	2004
	GOPALASWAMY R	PROTEIN EXPRES PURIF	36	82	2004
	WALBURGER A	SCIENCE	304	1800	2004
	KUMARI S	DRUGS TODAY	40	487	2004
	COWLEY S	MOL MICROBIOL	52	1691	2004
	GOOD MC	J MOL BIOL	339	459	2004
	MOLLE V	FEMS MICROBIOL LETT	234	215	2004
	SHARMA K	EXPERT OPIN THER TAR	8	79	2004
	SHARMA K	FEMS MICROBIOL LETT	233	107	2004
	KOUL A	NAT REV MICROBIOL	2	189	2004
	TYAGI JS	CURR SCI INDIA	86	93	2004
	FONTAN PA	CURR SCI INDIA	86	122	2004
	DURAN R	BIOCHEM BIOPH RES CO	333	858	2005
	CURRY JM	INFECT IMMUN	73	4471	2005
	PAPAVINASASUNDARAM KG	J BACTERIOL	187	5751	2005
	KANG CM	GENE DEV	19	1692	2005
	DEOL P	J BACTERIOL	187	3415	2005
	GREENSTEIN AE	J MOL MICROB BIOTECH	9	167	2005
	MOLLE V	J BIOL CHEM	281	30094	2006
	MOLLE V	PROTEOMICS	6	3754	2006
	SHARMA K	FEBS J	273	2711	2006

	NIEBISCH A	J BIOL CHEM	281	12300	2006
	DASGUPTA A	MICROBIOL-SGM	152	493	2006
	SINGH A	TUBERCULOSIS	86	28	2006
	BOKAS D	APPL MICROBIOL BIOT	76	773	2007
	COX RA	CURR MOL MED	7	231	2007
	DOVER LG	CURR MOL MED	7	247	2007
	ZHENG XJ	BIOCHEM BIOPH RES CO	355	162	2007
	NARAYAN A	PHYSIOL GENOMICS	29	66	2007
	O'HARE HM	MOL MICROBIOL	70	1408	2008
	HEGYMEGI-BARAKONYI B	CURR MED CHEM	15	2760	2008
	FIUZA M	J BIOL CHEM	283	18099	2008
	THAKUR M	J BIOL CHEM	283	8023	2008
	HETT EC	MICROBIOL MOL BIOL R	72	126	2008
	CANOVA MJ	PROTEOMICS	8	521	2008
	WEHENKEL A	BBA-PROTEINS PROTEOM	1784	193	2008
	TIWARI D	J BIOL CHEM	284	27467	2009
	WOLFF KA	ANTIMICROB AGENTS CH	53	3515	2009
	SCHERR N	J BACTERIOL	191	4546	2009
	GUPTA MK	J PROTEOME RES	8	2319	2009
	SILVESTRONI A	J PROTEOME RES	8	2563	2009
	KUMAR P	J BIOL CHEM	284	11090	2009
	LIN WJ	MOL MICROBIOL	71	1477	2009
	MEENA LS	FEBS J	277	2416	2010
	JANG J	MICROBIOL-SGM	156	1619	2010
	ARORA G	PLOS ONE	5	e10772	2010
	BURNSIDE K	J BIOL CHEM	286	44197	2011
	CHAKRABORTI PK	TUBERCULOSIS	91	432	2011
	ARORA G	J BIOL CHEM	287	26749	2012
	FORRELLAD MA	VIRULENCE	4	3	2013
2003	TYAGI AK	SR ADV BIOCHEM ENGG	84	211	
	TYAGI AK	TUBERCULOSIS	91	469	2011
2003	SINGH R	MOLECUL MICROBIOL	50	751	
	SAINI AK	J BIOL CHEM	279	50142	2004
	ALZARI PM	STRUCTURE	12	1923	2004
	SHARMA K	EXPERT OPIN THER TAR	8	79	2004
	KOUL A	NAT REV MICROBIOL	2	189	2004
	CASTANDET J	RES MICROBIOL	156	1005	2005
	GRUNDNER C	STRUCTURE	13	1625	2005
	MANGER M	CHEMBIOCHEM	6	1749	2005
	SINGH R	TUBERCULOSIS	85	325	2005
	VILLARINO A	J MOL BIOL	350	953	2005
	SINGH A	J BACTERIOL	187	4173	2005
	RAO V	SCAND J IMMUNOL	61	410	2005
	MADHURANTAKAM C	J BACTERIOL	187	2175	2005
	GREENSTEIN AE	J MOL MICROB BIOTECH	9	167	2005
	COZZONE AJ	J MOL MICROB BIOTECH	9	198	2005
	MUSTELIN T	NAT REV IMMUNOL	5	43	2005
	SZOOR B	J CELL BIOL	175	293	2006
	MULLER D	J MED CHEM	49	4871	2006
	TAUTZ L	EXPERT OPIN THER TAR	10	157	2006
	MUSTELIN T	ADV EXP MED BIOL	584	53	2006
	SEIBERT SF	ORG BIOMOL CHEM	4	2233	2006
	BRENCHLEY R	BMC GENOMICS	8	434	2007
	BERESFORD N	BIOCHEM J	406	13	2007
	SOELLNER MB	J AM CHEM SOC	129	9613	2007
	GRUNDNER C	STRUCTURE	15	499	2007
	PRUIJSSERS AJ	J VIROL	81	1209	2007
	CORREA IR	CHEM-ASIAN J	2	1109	2007
	JAIN R	PLOS ONE	3	e3869	2008

	OKU T	J BIOL CHEM	283	28918	2008
	GRUNDNER C	FEMS MICROBIOL LETT	287	181	2008
	WALTHER T	ORG LETT	10	3199	2008
	SHI M	VIRUS GENES	36	595	2008
	NOREN-MULLER A	ANGEW CHEM INT EDIT	47	5973	2008
	WEHENKEL A	BBA-PROTEINS PROTEOM	1784	193	2008
	VINTONYAK VV	CURR OPIN CHEM BIOL	13	272	2009
	BERESFORD NJ	J ANTIMICROB CHEMOTH	63	928	2009
	LILIENKAMPF A	J MED CHEM	52	2109	2009
	MUSA TL	ADV PROTEIN CHEM STR	77	41	2009
	HE RJ	CHEMMEDCHEM	5	2051	2010
	CHEN L	ACS MED CHEM LETT	1	355	2010
	EUM JH	INSECT BIOCHEM MOLEC	40	690	2010
	BERESFORD NJ	BMC GENOMICS	11	457	2010
	SILVA APG	FUTURE MED CHEM	2	1325	2010
	FLYNN EM	J AM CHEM SOC	132	4772	2010
	ZHOU B	P NATL ACAD SCI USA	107	4573	2010
	CHAO J	BBA-PROTEINS PROTEOM	1804	620	2010
	VINTONYAK VV	ANGEW CHEM INT EDIT	49	5902	2010
	ASHFORTH EJ	NAT PROD REP	27	1709	2010
	RAWLS KA	ORG BIOMOL CHEM	8	4066	2010
	LI W	J CELL BIOCHEM	112	2688	2011
	VINTONYAK VV	TETRAHEDRON	67	6713	2011
	SHAPLAND EB	J BACTERIOL	193	4361	2011
	DE OLIVEIRA KN	MEDCHEMCOMM	2	500	2011
	VINTONYAK VV	BIOORGAN MED CHEM	19	2145	2011
	JAIN R	BMC GENOMICS	13	520	2012
	EITSON JL	APPL ENVIRON MICROB	78	6829	2012
	LIU XT	ANTON LEEUW INT J G	102	447	2012
	CHAWLA M	MOL MICROBIOL	85	1148	2012
	DONG LH	J MOL MODEL	18	3847	2012
	HE YT	BIOORGAN MED CHEM	20	1940	2012
	ARORA N	MINI-REV MED CHEM	12	187	2012
	CHIARADIA LD	J MED CHEM	55	390	2012
	GISING J	ORG BIOMOL CHEM	10	2713	2012
	BALLA T	PHYSIOL REV	93	1019	2013
	GAO JM	CHEM REV	113	4755	2013
	ZENG LF	CHEMMEDCHEM	8	904	2013
	HUANG XS	ORG LETT	15	721	2013
	HE YT	J MED CHEM	56	832	2013
	WONG D	TRENDS MICROBIOL	21	100	2013
	LUGO-CABALLERO C	BIOMED RES INT	493	525	2013
	HE RJ	CHEM COMMUN	49	2064	2013
	BOHMER F	FEBS J	280	413	2013
	HE RJ	FEBS J	280	731	2013
2003	CHOPRA P	BIOCH BIOP RES COMMUN	311	112	
	ALZARI PM	STRUCTURE	12	1923	2004
	PULLEN KE	STRUCTURE	12	1947	2004
	SHARMA K	EXPERT OPIN THER TAR	8	79	2004
	DURAN R	BIOCHEM BIOPH RES CO	333	858	2005
	LAI SM	MICROBIOL-SGM	151	1159	2005
	GREENSTEIN AE	J MOL MICROB BIOTECH	9	167	2005
	THAKUR M	J BIOL CHEM	281	40107	2006
	MOLLE V	J BIOL CHEM	281	30094	2006
	MITIC N	CHEM REV	106	3338	2006
	SHARMA K	FEBS J	273	2711	2006
	SHARMA K	J BACTERIOL	188	2936	2006
	ALDERWICK LJ	P NATL ACAD SCI USA	103	2558	2006
	DASGUPTA A	MICROBIOL-SGM	152	493	2006
	DOVER LG	CURR MOL MED	7	247	2007

	YOOSEPH S	PLOS BIOL	5	432	2007
	SACHDEVA P	FEBS J	275	6295	2008
	HETT EC	MICROBIOL MOL BIOL R	72	126	2008
	WEHENKEL A	BBA-PROTEINS PROTEOM	1784	193	2008
	SILVA APG	FUTURE MED CHEM	2	1325	2010
	ARORA G	PLOS ONE	5	e10772	2010
	YOUNG M	J BACTERIOL	192	841	2010
	CHAKRABORTI PK	TUBERCULOSIS	91	432	2011
	SAJID A	PLOS ONE	6	e17871	2011
	PEREIRA SFF	MICROBIOL MOL BIOL R	75	192	2011
	JAYACHANDRAN R	EXPERT REV ANTI-INFE	10	1007	2012
	PUNIYA BL	MOL BIOSYST	9	2798	2013
2003	RAO V	SCAND J IMMUNOL	58	449	
	DOHERTY TM	CLIN MICROBIOL REV	18	687	2005
	ANDERSEN P	MICROBES INFECT	7	911	2005
	RAO V	SCAND J IMMUNOL	61	410	2005
	DOHERTY TM	VACCINE	23	2109	2005
	KAUFMANN SHE	INT J TUBERC LUNG D	10	1068	2006
	MEHTA A	CURR SCI INDIA	93	1501	2007
	HWANG SA	VACCINE	25	6730	2007
	HERNANDEZ-PANDO R	CURR MOL MED	7	365	2007
	GUPTA UD	VACCINE	25	3742	2007
	SHI CH	ACTA BIOCH BIOPH SIN	39	290	2007
	NAGY G	INT J MED MICROBIOL	298	379	2008
	ZVI A	BMC MED GENOMICS	1	18	2008
	DEY B	VACCINE	28	63	2009
	BASTOS RG	VACCINE	27	6495	2009
	FAN XY	PLASMID	61	39	2009
	CHAPMAN R	CURR HIV RES	8	282	2010
	RODRIGUEZ-ALVAREZ M	VACCINE	28	3997	2010
	TYAGI AK	TUBERCULOSIS	91	469	2011
	KOVACS-SIMON A	INFECT IMMUN	79	548	2011
	MOUSTAFA D	VACCINE	29	784	2011
	KADAM K	PROTEIN PEPTIDE LETT	19	1155	2012
	YOU Q	SCAND J IMMUNOL	75	77	2012
2003	SINGH A	FEMS MICROBIOL LETT	227	53	
	DANIEL J	J BACTERIOL	186	5017	2004
	RAHMAN MT	VET MICROBIOL	110	131	2005
	SINGH R	TUBERCULOSIS	85	325	2005
	SINGH A	J BACTERIOL	187	4173	2005
	GOLBY P	MICROBIOL-SGM	153	3323	2007
	ROBACK P	NUCLEIC ACIDS RES	35	5085	2007
	KING A	PLANTA	226	381	2007
	RICHTER L	GENE	395	22	2007
	NARAYAN A	PHYSIOL GENOMICS	29	66	2007
	CHERUVU M	TUBERCULOSIS	87	12	2007
	LAM THJ	MICROB PATHOGENESIS	45	12	2008
	GOUDE R	FUTURE MICROBIOL	3	299	2008
	IBARRA JA	GENETICA	133	65	2008
	FONTAN P	INFECT IMMUN	76	717	2008
	KHARE G	PLOS ONE	4	e8387	2009
	GONZALES M	MOL BIOL REP	36	1225	2009
	KUMAR P	J BIOL CHEM	284	11090	2009
	SHELINE KD	TUBERCULOSIS	89	114	2009
	NGUYEN L	ANNU REV PHARMACOL	49	427	2009
	MALHOTRA V	MICROBIOL-SGM	156	2829	2010
	HOMOLKA S	PLOS PATHOG	6	e1000988	2010
	TANEJA NK	PLOS ONE	5	e10860	2010
	MOLLE V	MOL MICROBIOL	75	1064	2010

	ANDERSSON CS	STRUCTURE	20	1062	2012
	REDDY PV	J INFECT DIS	208	1255	2013
	GOPINATH K	OPEN BIOL	3	120175	2013
	KUMARI R	MOL CELL BIOCHEM	374	149	2013
	FORRELLAD MA	VIRULENCE	4	3	2013
2003	AGARWAL N	FEMS MICROBIOL LETT	225	75	
	SHARMA K	EXPERT OPIN THER TAR	8	79	2004
	SHARMA K	FEMS MICROBIOL LETT	233	107	2004
	PASHLEY CA	MICROBIOL-SGM	152	2727	2006
	AGARWAL N	NUCLEIC ACIDS RES	34	4245	2006
	GALL K	FEMS MICROBIOL LETT	255	301	2006
	SCHOEP TD	MICROBIOL-SGM	153	3071	2007
	RICHTER L	GENE	395	22	2007
	JEAMTON W	J APPL PHYCOL	23	83	2011
	SCHUESSLER DL	PLOS ONE	7	e34471	2012
	NEWTON-FOOT M	TUBERCULOSIS	93	60	2013
2003	DHAR N	IMMUNOL LETT	88	175	
	RAO V	SCAND J IMMUNOL	61	410	2005
	KABBESH M	DIAGN MICR INFEC DIS	51	251	2005
	STORNI T	ADV DRUG DELIVER REV	57	333	2005
	RAPEAH S	VACCINE	24	3646	2006
	KLEIN AB	J IMMUNOASS IMMUNOCH	27	61	2006
	HERNANDEZ-PANDO R	CURR MOL MED	7	365	2007
	SHI CH	ACTA BIOCH BIOPH SIN	39	290	2007
	JAIN R	PLOS ONE	3	e3869	2008
	TANG C	J INFECT DIS	197	1263	2008
	ZEINALI M	IMMUNOL LETT	126	48	2009
	HO PY	J LEUKOCYTE BIOL	88	1073	2010
	COUTINHO-ABREU IV	J MED ENTOMOL	47	1146	2010
	CHAPMAN R	CURR HIV RES	8	282	2010
	SPERANZA V	MICROB PATHOGENESIS	48	150	2010
2003	CHOPRA P	EUR J BIOCHEM	270	625	
	DORION S	ANAL BIOCHEM	323	188	2003
	MEENA LS	BIOTECHNOL APPL BIOC	38	169	2003
	SAINI AK	J BIOL CHEM	279	50142	2004
	KUMAR P	DNA REPAIR	3	1483	2004
	TIWARI S	J BIOL CHEM	279	43595	2004
	CHOPRA P	FEBS LETT	571	212	2004
	SHAH YM	MOL CELL ENDOCRINOL	219	127	2004
	SHARMA K	EXPERT OPIN THER TAR	8	79	2004
	TOMIOKA H	CURR PHARM DESIGN	10	3297	2004
	HAVLASOVA J	PROTEOMICS	5	2090	2005
	KUMAR P	NUCLEIC ACIDS RES	33	2707	2005
	DE OLIVEIRA AHC	COMP BIOCHEM PHYS D	1	300	2006
	MUKHOPADHYAY S	INFECT IMMUN	74	3853	2006
	SHARMA K	FEBS J	273	2711	2006
	RUMJAHN SM	BRIT J CANCER	97	1372	2007
	ZHOU QH	BIOCHEM BIOPH RES CO	356	348	2007
	COUTINHO-SILVA R	PURINERG SIGNAL	3	83	2007
	MATTOO AR	FEBS J	275	6237	2008
	SANSOM FM	MICROBIOL MOL BIOL R	72	765	2008
	KOLLI BK	MOL BIOCHEM PARASIT	158	163	2008
	MATTOO AR	FEBS J	275	739	2008
	KREHENBRINK M	BMC GENOMICS	9	55	2008
	SILVA MT	LANCET INFECT DIS	9	699	2009
	RUMJAHN SM	BRIT J CANCER	100	1465	2009
	SUN J	PLOS ONE	5	e8769	2010
	MITTAL P	PLOS ONE	6	e27398	2011

	DAR HH	MICROBIOL-SGM	157	3024	2011
	VILLELA AD	CURR MED CHEM	18	1286	2011
	PALANIYANDI K	MICROBIOL RES	167	520	2012
	SUN J	PLOS PATHOG	9	e1003499	2013
	SANTAREM N	J PROTEOMICS	84	106	2013
	GEORGESCAULD F	PLOS ONE	8	e57867	2013
	FORRELLAD MA	VIRULENCE	4	3	2013
2004	SAINI AK	J BIOL CHEM	279	50142	
	RICH RL	J MOL RECOGNIT	18	431	2005
	KUMAR P	NUCLEIC ACIDS RES	33	2707	2005
	DE OLIVEIRA AHC	COMP BIOCHEM PHYS D	1	300	2006
	MISRA G	ACTA CRYSTALLOGR F	63	1084	2007
	MIRANDA MR	PARASITOLOGY	135	1661	2008
	UENO PM	MICROBIOL-SGM	154	3033	2008
	DUBEY GP	ARCH MICROBIOL	191	241	2009
	DAR HH	BIOCHEM J	430	539	2010
	FALAGAS ME	QJM-INT J MED	103	461	2010
	SUN J	PLOS ONE	5	e8769	2010
	GEORGESCAULD F	PLOS ONE	8	e57867	2013
2004	CHOPRA P	FEBS LETT	571	212	
	FISCHBACH MA	METHOD ENZYMOL	407	33	2006
	SUN J	J LEUKOCYTE BIOL	82	1437	2007
	GARCIA-PEREZ BE	MICROB PATHOGENESIS	45	1	2008
	KOUMANDOU VL	BMC GENOMICS	9	298	2008
	VAN DER SAR AM	MOL IMMUNOL	46	2317	2009
	SUN J	PLOS ONE	5	e8769	2010
	STEEG PS	N-S ARCH PHARMACOL	384	331	2011
	SUN J	PLOS PATHOG	9	e1003499	2013
	GEORGESCAULD F	PLOS ONE	8	e57867	2013
2004	DHAR N	MED MICROBIOL IMMUN	193	19	
	SKEIKY YAW	VACCINE	23	3937	2005
	TSENOVA L	INFECT IMMUN	74	2392	2006
	GUPTA UD	VACCINE	25	3742	2007
	ZHANG M	FEMS IMMUNOL MED MIC	49	68	2007
	JAIN R	PLOS ONE	3	e3869	2008
	TANG C	J INFECT DIS	197	1263	2008
	DEY B	VACCINE	28	63	2009
	BASTOS RG	VACCINE	27	6495	2009
	WANG JL	MED MICROBIOL IMMUN	198	5	2009
	WANG DA	VACCINE	28	3134	2010
	JAIN R	VACCINE	29	8118	2011
	TYAGI AK	TUBERCULOSIS	91	469	2011
	ZHANG P	INT IMMUNOPHARMACOL	14	252	2012
2005	SINGH R	TUBERCULOSIS	85	325	
	RANJAN S	BMC BIOINFORMATICS	7	S9	2006
	SOELLNER MB	J AM CHEM SOC	129	9613	2007
	STINEAR TP	GENOME RES	18	729	2008
	KUMAR P	J BIOL CHEM	284	11090	2009
	ARORA A	TUBERCULOSIS	91	456	2011
	ANDERSSON CS	STRUCTURE	20	1062	2012
	PELOSI A	PLOS ONE	7	e31788	2012
	ZENG LF	CHEMMEDCHEM	8	904	2013
	HE YT	J MED CHEM	56	832	2013
	HE RJ	FEBS J	280	731	2013
	FORRELLAD MA	VIRULENCE	4	3	2013
2005	KHERA A	VACCINE	23	5655	

	HUYGEN K	FUTURE MICROBIOL	1	63	2006
	LI H	VACCINE	24	1315	2006
	MEHER AK	VACCINE	25	6098	2007
	MITSUYAMA M	TUBERCULOSIS	87	S10	2007
	WALKER KB	CURR MOL MED	7	339	2007
	GUPTA UD	VACCINE	25	3742	2007
	ZVI A	BMC MED GENOMICS	1	18	2008
	LIU SG	IMMUNOL LETT	117	136	2008
	DEY B	VACCINE	28	63	2009
	DEY A	VACCINE	27	5152	2009
	GUMBER S	VET MICROBIOL	137	290	2009
	HUANG JM	VACCINE	28	7523	2010
	SHI CW	VACCINE	28	5237	2010
	OKADA M	HUM VACCINES	6	297	2010
	DEENADAYALAN A	MOL CELL PROTEOMICS	9	538	2010
	JAIN R	VACCINE	29	8118	2011
	TYAGI AK	TUBERCULOSIS	91	469	2011
	DEY B	PLOS ONE	6	e23360	2011
	DEY B	PLOS ONE	6	e18773	2011
	JEON BY	MICROBES INFECT	13	284	2011
	YOU Q	SCAND J IMMUNOL	75	77	2012
	CHAUHAN P	SCI REP-UK	3	1821	2013
2005	DEOL P	J BACTERIOL	187	3415	
	GREENSTEIN AE	J MOL MICROB BIOTECH	9	167	2005
	COZZONE AJ	J MOL MICROB BIOTECH	9	198	2005
	FERNANDEZ P	J BACTERIOL	188	7778	2006
	PEREZ J	BIOCHEM BIOPH RES CO	348	6	2006
	RAGHUNAND TR	MICROBIOL-SGM	152	2735	2006
	CASHIN P	FEMS MICROBIOL LETT	261	155	2006
	MOLLE V	PROTEOMICS	6	3754	2006
	SHARMA K	FEBS J	273	2711	2006
	ALDERWICK LJ	P NATL ACAD SCI USA	103	2558	2006
	DOVER LG	CURR MOL MED	7	247	2007
	NARAYAN A	PHYSIOL GENOMICS	29	66	2007
	RAO A	APPL ENVIRON MICROB	73	1320	2007
	PIMENTEL-SCHMITT EF	J MOL MICROB BIOTECH	12	75	2007
	LEWIN A	BMC MICROBIOL	8	91	2008
	LAKSHMINARAYAN H	PROTEIN EXPRES PURIF	58	309	2008
	HETT EC	MICROBIOL MOL BIOL R	72	126	2008
	CANOVA MJ	PROTEOMICS	8	521	2008
	WEHENKEL A	BBA-PROTEINS PROTEOM	1784	193	2008
	GOPALASWAMY R	FEMS MICROBIOL LETT	278	121	2008
	TIWARI D	J BIOL CHEM	284	27467	2009
	GUPTA MK	J PROTEOME RES	8	2319	2009
	KUMAR P	J BIOL CHEM	284	11090	2009
	CANOVA MJ	J BACTERIOL	191	2876	2009
	VEYRON-CHURLET R	J BIOL CHEM	284	6414	2009
	PARIKH A	J MOL BIOL	386	451	2009
	SHARBATI S	BMC MICROBIOL	9	31	2009
	COOK GM	ADV MICROB PHYSIOL	55	81	2009
	SATHEKGE M	Q J NUCL MED MOL IM	54	698	2010
	KHAN S	J BIOL CHEM	285	37860	2010
	MALHOTRA V	MICROBIOL-SGM	156	2829	2010
	JANG J	MICROBIOL-SGM	156	1619	2010
	TYAGI N	PLOS ONE	5	e10608	2010
	ARORA G	PLOS ONE	5	e10772	2010
	SATHEKGE M	NUKLEARMED-NUCL MED	49	35	2010
	CHAKRABORTI PK	TUBERCULOSIS	91	432	2011
	SPIVEY VL	J BIOL CHEM	286	26198	2011
	MESZAROS B	PLOS COMPUT BIOL	7	e1002118	2011

	DANILENKO VN	CURR TOP MED CHEM	11	1352	2011
	KUMARI R	MOL CELL BIOCHEM	369	67	2012
	KUNISCH R	BMC MICROBIOL	12	165	2012
	KIRSEBOM LA	ADV APPL MICROBIOL	80	81	2012
	SPIVEY VL	FEMS MICROBIOL LETT	347	107	2013
	KUMARI R	MOL CELL BIOCHEM	374	149	2013
	KHATRI B	PLOS ONE	8	e52673	2013
	FORRELLAD MA	VIRULENCE	4	3	2013
	KUMAR D	ARCH MICROBIOL	195	75	2013
2005	SINGH A	J BACTERIOL	187	4173	
	RICHTER L	GENE	395	22	2007
	CHERUVU M	TUBERCULOSIS	87	12	2007
	KRUH NA	J BIOL CHEM	283	31719	2008
	RUSSELL-GOLDMAN E	INFECT IMMUN	76	4269	2008
	LAM THJ	MICROB PATHOGENESIS	45	12	2008
	GOUDE R	FUTURE MICROBIOL	3	299	2008
	TOBIN DM	CELL MICROBIOL	10	1027	2008
	IBARRA JA	GENETICA	133	65	2008
	FONTAN P	INFECT IMMUN	76	717	2008
	KHARE G	PLOS ONE	4	e8387	2009
	NOMOTO M	MICROBIOL IMMUNOL	53	550	2009
	GONZALES M	MOL BIOL REP	36	1225	2009
	DEB C	PLOS ONE	4	e6077	2009
	KUMAR P	J BIOL CHEM	284	11090	2009
	SHELINE KD	TUBERCULOSIS	89	114	2009
	NGUYEN L	ANNU REV PHARMACOL	49	427	2009
	BEAULIEU AM	PLOS ONE	5	e15120	2010
	STALLINGS CL	MICROBES INFECT	12	1091	2010
	MALHOTRA V	MICROBIOL-SGM	156	2829	2010
	HOMOLKA S	PLOS PATHOG	6	e1000988	2010
	VEYRON-CHURLET R	J BIOL CHEM	285	12714	2010
	DUTTA NK	PLOS ONE	5	e10069	2010
	MOLLE V	MOL MICROBIOL	75	1064	2010
	JATANA N	J MOL MODEL	17	301	2011
	LAMRABET O	TUBERCULOSIS	92	365	2012
	ANDERSSON CS	STRUCTURE	20	1062	2012
	SIKRI K	CURR SCI INDIA	105	607	2013
	GOPINATH K	OPEN BIOL	3	120175	2013
	FORRELLAD MA	VIRULENCE	4	3	2013
2005	RAO V	SCAND J IMMUNOL	61	410	
	ARAVINDHAN V	FEMS IMMUNOL MED MIC	47	45	2006
	HOVAV AH	MICROBES INFECT	8	1750	2006
	JUNG SB	INFECT IMMUN	74	2686	2006
	HENAO-TAMAYO M	VACCINE	25	7153	2007
	DA FONSECA DM	IMMUNOLOGY	121	508	2007
	HERNANDEZ-PANDO R	CURR MOL MED	7	365	2007
	HUNG CY	ANN NY ACAD SCI	1111	225	2007
	ZVI A	BMC MED GENOMICS	1	18	2008
	BASTIAN M	J IMMUNOL	180	3436	2008
	SALI M	INFECT IMMUN	78	5202	2010
	AL-ATTIYAH R	FEMS IMMUNOL MED MIC	59	177	2010
	TYAGI AK	TUBERCULOSIS	91	469	2011
	KOVACS-SIMON A	INFECT IMMUN	79	548	2011
	WANG C	CLIN DEV IMMUNOL	563	838	2012
	SAKTHI S	MICROBIOL RES	168	407	2013
2006	AGARWAL N	NUCL ACID RES	34	4245	
	CHOWDHURY RP	J BACTERIOL	189	8973	2007
	FIELDS CJ	J BACTERIOL	189	6236	2007

	HALBEDEL S	J MOL BIOL	371	596	2007
	CHEN SC	J BACTERIOL	189	5108	2007
	PIMENTEL-SCHMITT EF	J MOL MICROB BIOTECH	12	75	2007
	GEBHARD S	MICROBIOL-SGM	154	2786	2008
	PAWARIA S	APPL ENVIRON MICROB	74	3512	2008
	CHAUHAN S	J BACTERIOL	190	4301	2008
	GEBHARD S	J BACTERIOL	190	1335	2008
	TOUZAIN F	BMC BIOINFORMATICS	9	73	2008
	DAVIS SL	PLOS ONE	4	e6297	2009
	NASH KA	ANTIMICROB AGENTS CH	53	1367	2009
	SONG T	MICROBIOL-SGM	156	999	2010
	SACHDEVA P	FEBS J	277	605	2010
	CHAUHAN S	FEMS MICROBIOL LETT	303	190	2010
	DICHIARA JM	NUCLEIC ACIDS RES	38	4067	2010
	BHATTACHARYA M	BIOCHEM BIOPH RES CO	415	17	2011
	TYAGI AK	TUBERCULOSIS	91	469	2011
	GUPTA RK	FEBS J	278	2131	2011
	HUNT DM	J BACTERIOL	194	2307	2012
	NEWTON-FOOT M	TUBERCULOSIS	93	60	2013
2007	AHMED N	PLOS ONE	2	E968	
	AHMED N	NAT REV MICROBIOL	6	387	2008
	SALAH IB	CLIN MICROBIOL INFEC	15	894	2009
	SAINI V	PLOS ONE	4	e6263	2009
	AHMED N	PLOS ONE	4	e5831	2009
	STAVRUM R	PLOS ONE	4	e4540	2009
	AHMED N	INFECT GENET EVOL	9	142	2009
	RANI PS	GUT PATHOG	2	1	2010
	AHMED N	TUBERCULOSIS	91	407	2011
	DJELOUADJI Z	LANCET INFECT DIS	11	641	2011
	TALWAR GP	AM J REPROD IMMUNOL	66	26	2011
	MEDIE FM	PLOS ONE	6	e20499	2011
	SAINI V	NUCLEIC ACIDS RES	40	10832	2012
	LAMRABET O	PLOS ONE	7	e34754	2012
	MEENA JK	JAMA DERMATOL	149	237	2013
2007	NARAYAN A	PHYSIOL GENOM	29	66	
	BOKAS D	APPL MICROBIOL BIOT	76	773	2007
	MATTOO AR	FEBS J	275	6237	2008
	O'HARE HM	MOL MICROBIOL	70	1408	2008
	CANOVA MJ	PLASMID	60	149	2008
	FIUZA M	J BIOL CHEM	283	18099	2008
	WOLUCKA BA	FEBS J	275	2691	2008
	MOLLE V	BIOCHEM J	410	309	2008
	HETT EC	MICROBIOL MOL BIOL R	72	126	2008
	MATTOO AR	FEBS J	275	739	2008
	CANOVA MJ	PROTEOMICS	8	521	2008
	WEHENKEL A	BBA-PROTEINS PROTEOM	1784	193	2008
	KUMAR CMS	J BACTERIOL	191	6525	2009
	COHEN-GONSAUD M	J BIOL CHEM	284	19290	2009
	SCHERR N	INDIAN J EXP BIOL	47	401	2009
	KUMAR P	J BIOL CHEM	284	11090	2009
	DUBEY GP	ARCH MICROBIOL	191	241	2009
	KATEETE DP	BMC MICROBIOL	10	272	2010
	MALHOTRA V	MICROBIOL-SGM	156	2829	2010
	JANG J	MICROBIOL-SGM	156	1619	2010
	ARORA G	PLOS ONE	5	e10772	2010
	MOLLE V	MOL MICROBIOL	75	1064	2010
	SUREKA K	PLOS ONE	5	e8590	2010
	CHAKRABORTI PK	TUBERCULOSIS	91	432	2011
	LOUGHEED KEA	TUBERCULOSIS	91	277	2011

	DANILENKO VN	CURR TOP MED CHEM	11	1352	2011
	ELIZAROV SM	BIOCHEMISTRY-MOSCOW+	77	1258	2012
	KUMARI R	MOL CELL BIOCHEM	369	67	2012
	CHAPMAN TM	BIOORG MED CHEM LETT	22	3349	2012
	HEGDE SR	PLOS ONE	7	e33893	2012
	ZAKHAREVICH NV	PROTEINS	80	1363	2012
	COX RA	PLOS ONE	8	e59883	2013
	FORRELLAD MA	VIRULENCE	4	3	2013
2008	JAIN R	PLOS ONE	3	3869	
	DEY B	VACCINE	28	63	2009
	BASTOS RG	VACCINE	27	6495	2009
	SALI M	INFECT IMMUN	78	5202	2010
	SHI CW	VACCINE	28	5237	2010
	SHI CH	SCAND J INFECT DIS	43	848	2011
	JAIN R	VACCINE	29	8118	2011
	TYAGI AK	TUBERCULOSIS	91	469	2011
	DEY B	PLOS ONE	6	e23360	2011
	DEY B	PLOS ONE	6	e18773	2011
	JAIN R	BMC GENOMICS	13	520	2012
	GUPTA A	VACCINE	30	6198	2012
	GUPTA A	PLOS ONE	7	e39215	2012
	CHRISTY AJ	VACCINE	30	1364	2012
	JUAREZ-RODRIGUEZ MD	INFECT IMMUN	80	815	2012
	REDDY PV	J BACTERIOL	194	567	2012
	WANG C	CLIN DEV IMMUNOL	563	838	2012
	REDDY PV	J INFECT DIS	208	1255	2013
	PURI RV	PLOS ONE	8	e70514	2013
	CHAUHAN P	SCI REP-UK	3	1821	2013
	ZHANG L	DNA Cell Biol.	2	179	31
2008	AKIF M	J BACTERIOL	190	7087	
	BASU D	NUCLEIC ACIDS RES	37	4944	2009
	CHU H	J MICROBIOL	48	124	2010
	LIN TY	MOL BIOSYST	6	1454	2010
	KUMAR A	EXPERT REV MOL MED	13	e39	2011
	HALL G	PROTEIN SCI	20	210	2011
	SERATA M	MICROBIOL-SGM	158	953	2012
	TRIVEDI A	ADV MICROB PHYSIOL	60	263	2012
	HANSCHMANN EM	ANTIOXID REDOX SIGN	19	1539	2013
	PHULERA S	BIOCHEMISTRY-US	52	4056	2013
	DAVEY L	J BIOL CHEM	288	16416	2013
	OLSON AL	PROTEINS	81	675	2013
	VAN LAER K	J BIOL CHEM	288	7942	2013
2008	GUPTA V	ACTA CRYST	64	398	
	GUPTA V	PLOS ONE	4	e8028	2009
	MCMATH LM	ACTA CRYSTALLOGR F	66	1657	2010
	AGARWAL R	J PROTEOMICS	73	976	2010
2008	GUPTA V	ACTA CRYST	64	524	
	GUPTA V	PLOS ONE	5	e9222	2010
2008	FARHANA A	PLOS ONE	3	E2087	
	SIEGRIST MS	P NATL ACAD SCI USA	106	18792	2009
	DOMENECH P	J BACTERIOL	191	477	2009
	COOK GM	ADV MICROB PHYSIOL	55	81	2009
	PATEL P	BIOCHEMISTRY-US	49	8033	2010
	YETERIAN E	ENV MICROBIOL REP	2	412	2010
	RAJAGOPALAN M	J BIOL CHEM	285	15816	2010
	RYNDAK MB	J BACTERIOL	192	861	2010

	BANERJEE S	INFECT GENET EVOL	11	825	2011
	EITINGER T	FEMS MICROBIOL REV	35	3	2011
	MOHAREER K	J MOL MICROB BIOTECH	21	97	2011
	SANTHANAGOPALAN SM	TUBERCULOSIS	92	60	2012
	SAHA R	J BASIC MICROB	53	303	2013
	LI W	CELL PHYSIOL BIOCHEM	31	1	2013
	FORRELLAD MA	VIRULENCE	4	3	2013
2008	TYAGI AK	MYCOBACTERIAL CELL ENVELOP	3	23	
	REYNOLDS RC	TUBERCULOSIS	92	72	2012
2009	KHARE G	PLOS ONE	4	E8387	
	GRIMES KD	ANAL BIOCHEM	417	264	2011
	JATANA N	J MOL MODEL	17	301	2011
	MOHN WW	J BACTERIOL	194	6712	2012
	ANDERSSON CS	STRUCTURE	20	1062	2012
	ANAND S	BMC STRUCT BIOL	12	10	2012
	DUCKWORTH BP	CURR TOP MED CHEM	12	766	2012
	XIONG XM	CURR DRUG TARGETS	14	676	2013
	NAMBI S	J BIOL CHEM	288	14114	2013
2009	GUPTA V	PLOS ONE	4	E8028	
	MCMATH LM	ACTA CRYSTALLOGR F	66	1657	2010
	LE BRUN NE	BBA-GEN SUBJECTS	1800	732	2010
	ARORA A	TUBERCULOSIS	91	456	2011
	TAKATSUKA M	PLOS ONE	6	e20985	2011
	ARDEJANI MS	BIOCHEMISTRY-US	50	4029	2011
	KHARE G	PLOS ONE	6	e18570	2011
	MOURA DF	EUR J IMMUNOL	42	2925	2012
	WAHLGREN WY	PLOS ONE	7	e46992	2012
	PANDEY R	INFECT IMMUN	80	3650	2012
	REDDY PV	J BACTERIOL	194	567	2012
	MCMATH LM	J PORPHYR PHTHALOCYA	17	229	2013
2009	SACHDEVA P	FEBS J	277	605	
	STALLINGS CL	MICROBES INFECT	12	1091	2010
	THAKUR KG	PROTEIN EXPRES PURIF	74	223	2010
	SCHRODER J	FEMS MICROBIOL REV	34	685	2010
	GUARIGLIA-OROPEZA V	J BACTERIOL	193	6223	2011
	SUBBIAN S	PLOS PATHOG	7	e1002262	2011
	PATEK M	J BIOTECHNOL	154	101	2011
	ROY S	CURR MICROBIOL	62	1581	2011
	ZHAO QJ	CRIT REV EUKAR GENE	21	347	2011
	MOHAREER K	J MOL MICROB BIOTECH	21	97	2011
	MIOTTO P	PLOS ONE	7	e51950	2012
	SAINI V	NUCLEIC ACIDS RES	40	10832	2012
	GIOVANNINI D	MICROB PATHOGENESIS	53	135	2012
	VASHISHT R	PLOS ONE	7	e39808	2012
	KIM MS	MOL MICROBIOL	85	326	2012
	ZHANG B	FUTURE MED CHEM	4	1273	2012
	PELLY S	GENE	500	85	2012
	HUNT DM	J BACTERIOL	194	2307	2012
	HARTKOORN RC	J BACTERIOL	194	2001	2012
	LEE JH	NAT COMMUN	3	753	2012
	BASU A	J BACTERIOL	194	1331	2012
	BURIAN J	J BIOL CHEM	287	299	2012
	KIRSEBOM LA	ADV APPL MICROBIOL	80	81	2012
	GAUDION A	TUBERCULOSIS	93	482	2013
	SHELL SS	PLOS PATHOG	9	e1003419	2013
	NAMBU T	ARCH ORAL BIOL	58	681	2013
	GHOSH P	INFECT IMMUN	81	2242	2013

	PETTERSSON BMF	FEMS MICROBIOL LETT	342	98	2013
	JAISWAL RK	NUCLEIC ACIDS RES	41	3414	2013
	FORRELLAD MA	VIRULENCE	4	3	2013
	LI SK	RNA	19	74	2013
2009	KUMAR CMS	J BACTERIOL	191	6525	
	HUQ S	BIOSCI BIOTECH BIOCH	74	2273	2010
	CEHOVIN A	INFECT IMMUN	78	3196	2010
	SURAGANI M	BIOCHEM BIOPH RES CO	414	390	2011
	LUO HB	BIOCHEM BIOPH RES CO	413	389	2011
	SHAHAR A	J MOL BIOL	412	192	2011
	HENDERSON B	INFECT IMMUN	79	3476	2011
	KUMAR CMS	CURR SCI INDIA	100	1646	2011
	ZORINA A	DNA RES	18	137	2011
	GE RG	PROTEOMICS	11	1449	2011
	NOENS EE	BMC BIOTECHNOL	11	27	2011
	SIELAFF B	J MOL BIOL	405	831	2011
	YAMAUCHI S	EXTREMOPHILES	16	871	2012
	FAN MQ	MOL MICROBIOL	85	934	2012
	JEONG J	PROTEOMICS	12	1452	2012
	WANG Y	PLOS GENET	9	e1003306	2013
2009	SAINI V	PLOS ONE	4	E6263	
	PURSWANI S	CURR SCI INDIA	99	169	2010
	PARIDA SK	CURR OPIN IMMUNOL	22	374	2010
	RANI PS	GUT PATHOG	2	1	2010
	AHMAD F	PLOS ONE	6	e25424	2011
	PURSWANI S	J REPROD IMMUNOL	91	24	2011
	TALWAR GP	AM J REPROD IMMUNOL	66	26	2011
	PURSWANI S	VACCINE	29	2341	2011
	PANDEY RK	PLOS ONE	6	e17093	2011
	SAINI V	NUCLEIC ACIDS RES	40	10832	2012
	GUPTA A	PLOS ONE	7	e39215	2012
	PANDEY RK	MICROBES INFECT	14	348	2012
	RAKSHIT S	INT J CANCER	130	865	2012
	GONZALEZ-PEREZ M	INFECT IMMUN	81	4001	2013
	MOLLICA A	CURR DRUG TARGETS	14	938	2013
	TALWAR GP	CONTRACEPTION	87	280	2013
	TALWAR GP	ANN NY ACAD SCI	1283	50	2013
	KIM BJ	INT J SYST EVOL MICR	63	192	2013
2009	BASU D	NUCLEIC ACID RES	DOI 10.1093		
	BROWNING DF	CURR OPIN MICROBIOL	13	773	2010
	HENDERSON B	J LEUKOCYTE BIOL	88	445	2010
	SIELAFF B	ACTA CRYSTALLOGR F	66	418	2010
	HENDERSON B	TUBERCULOSIS	90	119	2010
	HENDERSON B	INFECT IMMUN	79	3476	2011
	PILAK O	ENVIRON MICROBIOL	13	2232	2011
	KUMAR CMS	CURR SCI INDIA	100	1646	2011
	NOENS EE	BMC BIOTECHNOL	11	27	2011
	GHATAK P	PLOS ONE	6	e16019	2011
	SIELAFF B	J MOL BIOL	405	831	2011
	GOYAL M	NUCLEIC ACIDS RES	40	1174	2012
	HENDERSON B	BIOL REV	88	955	2013
	MISHRA A	PLOS ONE	8	e69985	2013
	WEIGOLDT M	MICROBIOL-SGM	159	380	2013
	DELMAS S	MOL MICROBIOL	87	168	2013
2009	ARORA P	NAT CHEM BIOL	5	166	
	KHARE G	PLOS ONE	4	e8387	2009
	SCHMELZ S	CURR OPIN STRUC BIOL	19	666	2009
	GULICK AM	ACS CHEM BIOL	4	811	2009

	BARKAN D	CHEM BIOL	16	499	2009
	WILSON DJ	ANAL BIOCHEM	404	56	2010
	ZIEBART KT	J MED CHEM	53	3718	2010
	LU XQ	J AM CHEM SOC	132	1748	2010
	KHURANA P	BMC BIOINFORMATICS	11	57	2010
	LEE TV	J BIOL CHEM	285	2415	2010
	GRIMES KD	ANAL BIOCHEM	417	264	2011
	SELVI BR	ACS CHEM BIOL	6	982	2011
	HAYASHI T	CHEMBIOCHEM	12	2166	2011
	MOHANTY D	TUBERCULOSIS	91	448	2011
	BHARDWAJ A	TUBERCULOSIS	91	479	2011
	HOTTER GS	VET MICROBIOL	151	91	2011
	ZHANG ZN	J MOL BIOL	406	313	2011
	NAKAMURA H	J AM CHEM SOC	134	18518	2012
	ANAND S	FEBS J	279	3214	2012
	VATS A	J BIOL CHEM	287	30677	2012
	VASHISHT R	PLOS ONE	7	e39808	2012
	REDWAN IN	EUR J ORG CHEM	36	65	2012
	ANDERSSON CS	STRUCTURE	20	1062	2012
	HAMILTON JJ	PLOS ONE	7	e34670	2012
	CHHABRA A	P NATL ACAD SCI USA	109	5681	2012
	DUCKWORTH BP	CURR TOP MED CHEM	12	766	2012
	GOYAL A	J MOL BIOL	416	221	2012
	LU XQ	CHEMBIOCHEM	13	129	2012
	ANAND S	MOL BIOSYST	8	1157	2012
	STANLEY SA	P NATL ACAD SCI USA	110	11565	2013
	LIU Z	J BIOL CHEM	288	18473	2013
	HERBST DA	J BIOL CHEM	288	1991	2013
	CASABON I	MOL MICROBIOL	87	269	2013
2010	GUPTA AK	INDIAN J MED RES	132	176	
	DA SILVA PEA	FEMS IMMUNOL MED MIC	63	1	2011
	GUPTA AK	INFECT GENET EVOL	12	853	2012
	MACHADO D	PLOS ONE	7	e34538	2012
2010	GUPTA V	PLOS ONE	5	E9222	
	DUCKWORTH BP	CHEM BIOL	18	1432	2011
	ARORA A	TUBERCULOSIS	91	456	2011
	LOMBARD J	BMC EVOL BIOL	11	232	2011
	RUSSI S	J STRUCT BIOL	175	236	2011
	FENG J	PLASMID	68	105	2012
	ADIKARAM PR	J MOL BIOL	419	223	2012
	DA COSTA TPS	J BIOL CHEM	287	17823	2012
	PETERS-WENDISCH P	APPL MICROBIOL BIOT	93	2493	2012
	WHEELER MJ	ACTA CRYSTALLOGR F	68	111	2012
	PENDINI NR	PROTEIN SCI	22	762	2013
	TIEU W	CHEM SCI	4	3533	2013
2010	DEY B	VACCINE	28	63	
	TYAGI AK	TUBERCULOSIS	91	469	2011
2011	JAIN R	VACCINE	29	8118	
	KERNODLE DS	VACCINE	30	6013	2012
	JAIN R	VACCINE	30	6015	2012
	LEVERSEN NA	SCAND J IMMUNOL	75	489	2012
	KERNODLE DS	J INFECT DIS	205	1186	2012
	OZTURK P	J DERMATOL	40	114	2013
2011	DEY B	PLOS ONE	6	E23360	
	TAYLOR JL	IMMUNOL CELL BIOL	90	945	2012

	BILLESKOV R	PLOS ONE	7	e39909	2012
	KAO FF	PLOS ONE	7	e34991	2012
	LEUNG CC	RESPIROLOGY	18	1047	2013
	TYNE AS	VACCINE	31	4322	2013
	CHAUHAN P	SCI REP-UK	3	1821	2013
	VAN HELDEN PD	COMP IMMUNOL MICROB	36	287	2013
	WANG CC	RESPIROLOGY	18	412	2013
	LAKSHMI PS	PLOS ONE	8	e54708	2013
	ZHAI YZ	CELL IMMUNOL	281	1	2013
2011	KHARE G	PLOS ONE	6	E22441	
	NODWELL MB	CHEMBIOCHEM	13	1439	2012
	GUPTA A	J ANTIMICROB CHEMOTH	67	1380	2012
	ANUSUYA S	EXPERT OPIN DRUG DIS	8	1239	2013
	SHAPIRO S	J ANTIBIOT	66	371	2013
2011	DEY B	PLOS ONE	6	E18773	
	REECE ST	VACCINE	29	8740	2011
	DEY B	PLOS ONE	6	e23360	2011
	DALMIA N	EXPERT REV VACCINES	11	1221	2012
	GUPTA A	VACCINE	30	6198	2012
	CHAUHAN P	SCI REP-UK	3	1821	2013
	VAN HELDEN PD	COMP IMMUNOL MICROB	36	287	2013
	PINTO R	J INFECT DIS	207	778	2013
2011	KHARE G	PLOS ONE	6	E18570	
	MCDEVITT ME	BBA-PROTEINS PROTEOM	1814	1854	2011
	LI CH	PLOS ONE	7	e51428	2012
	PANDEY R	INFECT IMMUN	80	3650	2012
	CABAN-HERNANDEZ K	MOL BIOCHEM PARASIT	182	54	2012
	REDDY PV	J BACTERIOL	194	567	2012
	KHARE G	BIOCHEMISTRY-US	52	1694	2013
2011	PURUSHOTHAMAN S	PLOS ONE	6	E16850	
	FISHER DJ	PLOS ONE	7	e46052	2012
	PETERS-WENDISCH P	APPL MICROBIOL BIOT	93	2493	2012
2011	JATANA N	J MOL MODEL	17	301	
	EKINS S	TRENDS MICROBIOL	19	65	2011
	SANCHITA	J BIOMOL STRUCT DYN	31	874	2013
	NAMBI S	J BIOL CHEM	288	14114	2013
2011	TYAGI AK	TUBERCULOSIS	91	469	
	BEENA	MED RES REV	33	693	2013
	LAKSHMI PS	PLOS ONE	8	e54708	2013
2012	REDDY PV	J BACTERIOL	194	567	
	PANDEY R	INFECT IMMUN	80	3650	2012
	YAO HL	J AM CHEM SOC	134	13470	2012
	BAHR A	REPROD DOMEST ANIM	47	59	2012
	REDDY PV	J INFECT DIS	208	1255	2013
	PURI RV	PLOS ONE	8	e71535	2013
	PURI RV	PLOS ONE	8	e70514	2013
	KHARE G	BIOCHEMISTRY-US	52	1694	2013
	MCMATH LM	J PORPHYR PHTHALOCYA	17	229	2013
	CHAUDHARY VK	PROT EXP PURIF	40	169	
	KULSHRESTHA A	PROTEIN EXPRES PURIF	44	75	2005
	ACHKAR JM	CLIN VACCINE IMMUNOL	13	1291	2006
	BENABDESSELEM C	J CLIN MICROBIOL	44	3086	2006
	ABEBE F	SCAND J IMMUNOL	66	176	2007
	ZHANG HM	CLIN MICROBIOL INFEC	13	139	2007

	LEE JS	RESPIROLOGY	13	432	2008
	STEINGART KR	CLIN VACCINE IMMUNOL	16	260	2009
	BISEN PS	REC ADV BIOL BIOMED	3	81	2010
	ROTHERHAM LS	PLOS ONE	7	e46862	2012

PAPERS WITHOUT CITATION:

1976	TYAGI AK	J CHEST DIS	18	250
1976	TYAGI AK	IND J BIOC BIOP	13	93
1978	NIZAMUDDIN A	IND J CHEST DIS	20	11
1998	TYAGI AK	TUBERCULOSIS RES		29
1999	TYAGI AK	MULTIDRUG RESIST....		109
2000	TYAGI AK	R T CONF SR.	7	135
2004	JAIN R	TUBERCULOSIS: CURR RES TREND		138
2004	TYAGI AK	CURR SCI	86	154
2005	JAIN R	MICROBIAL DIVERSITY.....		1053
2005	TYAGI AK	R T CONF SER	15	149
2008	JAIN S	AMER J BIOC BIOTECH	4	226
2008	JAIN R	ROUND TABLE CONF SR		93
2009	TYAGI AK	TUBERCULOSIS		918
2011	ARORA A	TUBERCULOSIS	DOI	10.1016
2012	JAIN R	BMC GENOMICS	13	520
2012	SAINI V	NUCL ACID RES	DOI	10.1093
2013	KHARE G	BIOCHEMISTRY	52	1694

Important Research Contributions

Summary of Important Research Contributions

Broadly, Dr. Tyagi's laboratory, for the last 20 years, has focused on the following two important areas related to tuberculosis

(1) Vaccine development
(2) Novel targets in *M. tuberculosis* and drug discovery.

The research efforts of his group have been focused on developing the strategies, tools and knowledge related to these two aspects for the control and amelioration of tuberculosis.

In addition, Dr. Tyagi and colleagues have also carried out studies on *mycobacterium indicus pranii*. The collaborative work on this mycobacterial species, with Dr. Tyagi as the Principal Investigator, was responsible for the publication of the first completed genome of a new bacterial species from India.

The summary of the important research contributions is given below:

1. Work on the development of TB vaccines and related aspects

Dr. Tyagi and colleagues have worked in this area for the last twenty years. For this, they first studied the expression signals especially the promoters of mycobacteria and then employed them for the development of expression vectors which they later used for the expression of mycobacterial genes and development of candidate TB vaccines. The brief summary of these efforts is as follows:

A. Studies on the transcriptional signals of mycobacteria

Dr. Tyagi's group has contributed significantly to the understanding of transcriptional machinery and gene expression in mycobacteria. By isolating and characterizing, a large number of transcriptional signals from the slow growing *Mycobacterium tuberculosis* and the fast growing *Mycobacterium smegmatis*, it was demonstrated that most of the mycobacterial promoter elements function poorly in *E.coli.* His work has also provided evidence that RNA polymerases of *M.smegmatis, M.tuberculosis* and *M.bovis* BCG recognize mycobacterial promoter elements with comparable efficiencies and that mycobacterial transcriptional signals differ from their counterparts in *E.coli* with respect to their -35 regions and the corresponding recognition domain of sigma factor of RNA polymerase. These studies have shed significant light on the divergence of mycobacterial transcriptional machinery from those of other bacteria. Also, these studies have provided a better understanding of the molecular basis of slow growth rate of *M.tuberculosis* and an explanation for the poor expression of mycobacterial genes in *E.coli*.

B. Development of tools for genetic manipulations in mycobacteria

Dr. Tyagi's laboratory has developed a repertoire of vectors, which have proved to be extremely useful to several investigators in genetic manipulations of mycobacteria for the basic understanding of these organisms at a molecular level. Besides developing several vectors, for the isolation of promoters, for construction of expression libraries and for trapping the promoters of structural genes under the control of a transcriptional regulator, Dr. Tyagi and colleagues have also developed an integration-proficient vector system for stable expression of genes in mycobacteria. This recombinant BCG system has been very useful for a large number of investigators for expression of mycobacterial genes as well as antigen genes from several other pathogens for the development of BCG into a multipurpose vaccine vehicle.

C. Development of candidate vaccines against tuberculosis

With the aim of modifying BCG into a more potent vaccine against TB, a generic approach was developed by Dr. Tyagi's laboratory for expression of genes in mycobacteria which provides a desired level of expression of an antigen based upon the choice of mycobacterial promoter. Dr. Tyagi's group has expressed several antigens of *M. tuberculosis* by using this expression system to develop a number of candidate vaccines against TB. The evaluation of these candidate vaccines for immune responses in mice and for protective efficacy in guinea pigs has shown that two of the recombinant BCG vaccines provide more efficient protection than BCG itself against a sub-cutaneous challenge of *M. tuberculosis* in guinea pigs. In a parallel approach, Dr. Tyagi and colleagues have also developed several candidate DNA vaccines. Based on reduction in the bacillary load in lung and spleen of guinea pigs as well as associated histopathological changes, some of these candidate DNA vaccines imparted significant protection against the subcutaneous challenge of *M. tuberculosis*.

Till this point of time, no aerosol challenge facility was available in India. Hence, evaluation of the candidate vaccines was carried out by using subcutaneous infection of guinea pigs. However, as the aerosol infection facility at the National JALMA Institute of Leprosy and Other Mycobacterial Diseases, Agra became available, the promising candidate vaccines were evaluated against the aerosol challenge of *M. tuberculosis* in guinea pigs by using heterologous prime boost approach. In this study, three regimens comprising of (i) recombinant BCG overexpressing 85C, (ii) recombinant BCG overexpressing α–crystallin as the priming agent followed by boosting with a DNA vaccine expressing the same antigen and (iii) BCG as priming agent followed by boosting with DNA vaccine expressing α–crystallin showed extremely good results and proved their superiority in comparison to the present BCG vaccine both on the basis of reduction in the bacillary load in lung and spleen as well as histopathological changes. The Tuberculosis Vaccine Clinical Trial Expert Group (TVCTEG) of the Department of Biotechnology, Government of India, has approved these vaccine regimens for human clinical trials. Currently, pre-clinical work on these candidate vaccines is in progress so that the human clinical trials can be initiated.

By employing modified Cornell model, Dr. Tyagi and colleagues have also evaluated the potential of adjunctive immunotherapy with DNA vaccines to shorten the tuberculosis

chemotherapy period and reduce disease reactivation and demonstrated that α-crystallin based DNA vaccine (DNAacr) significantly reduced the chemotherapy period from 12 weeks to 8 weeks when compared with the chemotherapy alone. Hence, ?-crystallin based DNA vaccine holds a significant promise for its use both as a prophylactic vaccine as well as in the therapeutic approach.

D. Development of first oligonucleotidew microarray for global gene expression profiling in guinea pigs: defining the transcription signature of infectious diseases

The Guinea pig (Cavia porcellus) is one of the most extensively used animal models to study infectious diseases. However, despite its tremendous contribution towards understanding the establishment, progression and control of a number of diseases in general and tuberculosis in particular, the lack of fully annotated guinea pig genome sequence as well as appropriate molecular reagents has severely hampered detailed genetic and immunological analysis in this animal model. Dr. Tyagi and colleagues developed the first comprehensive microarray (44K) for studying the global gene expression profile in guinea pigs and validation of its usefulness with tuberculosis as a case study. This study by Dr. Tyagi and colleagues addressed an important gap in the area of infectious diseases and vaccine development and provided a valuable molecular tool to optimally harness the potential of guinea pig model to develop better vaccines and therapies against human diseases.

Since, fully annotated guinea pig genome sequence was not available, Dr. Tyagi and colleagues employed cross-species hybridization technology to develop a 44 K microarray platform to study gene expression profile in guinea pigs. In their study, the pulmonary transcriptional profiling of M. tuberculosis infected guinea pigs revealed a significant regulation of 3200 unique targets. While, 1344 unique genes exhibited a marked up regulation, 1856 genes were significantly down regulated. Differentially regulated genes were further classified into different categories based on their direct or indirect involvement in various biological processes or pathways. A massive re-alignment of metabolic pathways, mostly associated with catabolism, emerged as one of the interesting themes from their analysis. The most prominent observation related to the repression of numerous genes related to MAPK, Wnt and calcium signaling pathways. MAPK signaling is known to be crucial for the anti-bacterial response of the host and it also represents a strategic target for bacterial subversion tactics. Thus, dampening of the MAPK signaling has emerged as a key to achieve alteration in the antibacterial phenotype of macrophages. Recently, Wnt signaling pathway has been implicated in the generation of long-lived multi-potent memory T cells and in the modulation of inflammatory response of macrophages to M. tuberculosis infection, thus repression of Wnt signaling pathway observed by Dr. Tyagi and colleagues suggested a possible mechanism by which, M. tuberculosis inhibits effective Tcell memory response.

The transcriptional profiling of M. tuberculosis infected guinea pig lungs developed by Dr. Tyagi and colleagues not only revealed modulation of key immunologically relevant genes but also demonstrated involvement of novel metabolic and signaling pathways in TB pathogenesis. Moreover, their analysis revealed a higher resemblance of guinea pigs to humans in terms of transcriptional response to M. tuberculosis infection when compared to

mouse and non-human primates. Development of the 44 K GPOM thus has been a critical step towards characterization of the guinea pig model, which will greatly aid in improving our understanding of host responses to a number of infectious diseases.

2. Novel targets in *M. tuberculosis* and drug discovery

In a comprehensive approach, Dr. Tyagi and colleagues have worked on several aspects related to this broad area of drug discovery which include study of *M. tuberculosis* genes essential for the pathogenesis of *M. tuberculosis* and validation of their essentiality in animal models, crystallization and structure determination of important *M. tuberculosis* proteins, characterization of important *M. tuberculosis* targets and finally use these targets for the identification of mycobacterial inhibitors by target based virtual screening in addition to whole cell based screens. The summary of these efforts is provided below:

A. Study of genes that are essential for the pathogenesis of *M. tuberculosis* – identification of new drug targets

(i) mymA operon

Dr. Tyagi's laboratory identified a new gene (*virS*) from *M .tuberculosis*. The 7 genes (*Rv3083-Rv3089*), which were present divergently to *virS (Rv3082c)* constitute an operon designated as the *mymA* operon. Dr. Tyagi's group showed that transcription of the *mymA* operon is dependent on the presence of VirS protein. A 4-fold induction of the *mymA* operon promoter occurs specifically in the wild type *M. tuberculosis* and not in the *virS* mutant of *M. tuberculosis* (Mtb∆*virS*) when exposed to acidic pH. Dr. Tyagi's group showed that the expression of the *mymA* operon was also induced by 10-folds in infected macrophages. Based on further studies, his group proposed the involvement of these proteins in the modification of fatty acids required for cell envelope under acetic environment. This was supported by altered colony morphology and cell envelope ultra structure displayed by the *virS* mutant of *M. tuberculosis* (Mtb∆*virS*). Dr. Tyagi and colleagues showed that disruption of *virS* and *mymA* genes impairs the ability of *M. tuberculosis* to survive in the activated macrophages, but not in resting macrophages, suggesting the importance of *mymA* operon in protecting the bacterium against harsher conditions. Infection of guinea pigs with Mtb∆*virS*, Mtb*mym:hyg* and the parental strain resulted in ~800-fold reduced bacillary load of the mutant strains as compared with the parental strain in the spleens of animals at 20 weeks post infection. These observations by Dr. Tyagi's laboratory demonstrated important role of *mymA* operon in the pathogenesis of *M. tuberculosis* at later stages of progression of the disease.

(ii) Tyrosine phosphatases of *M. tuberculosis*

Two tyrosine phosphatases namely MptpA and MptpB have been identified and characterized from *Mycobacterium tuberculosis*. To determine the role of MptpB in the pathogenesis of *M. tuberculosis* Dr. Tyagi and colleagues constructed an *mptpB* mutant strain and showed that disruption of the *mptpB* gene specifically impairs the ability of the mutant strain to survive in guinea pigs but not *in vitro* or in a macrophage cell line suggesting the importance of its role in the host-pathogen interaction. Infection of guinea

pigs with the mutant strain resulted in a 70-fold reduction in the bacillary load of spleens in infected animals as compared to the bacillary load in the animals infected with the parental strain along with the commensurate pathological damage in the organs.

Dr. Tyagi and colleagues also showed that disruption of *mptpA* gene impairs the ability of *M. tuberculosis* to survive in IFN-γ activated macrophages as well as in guinea pigs. Infection of activated macrophages with *M. tuberculosis*, or *mptipA* mutant resulted in an approximately 14-fold reduction in the survival of intracellular *mptpA* mutant in comparison to the intracellular parental strain. Dr. Tyagi and colleagues also demonstrated that on infection of guinea pigs the bacillary load in guinea pigs infected with the *mptpA* mutant strain was reduced by 80 and 90 folds in spleens and lungs, respectively, in comparison to bacillary load in guinea pigs infected with the parental strain. Commensurate with these observations, infection of animals with the *mptpA* mutant strain showed a significantly reduced histopathological damage to lungs in comparison to infection with the parental strain. These studies by Dr. Tyagi and colleagues established the importance of *mptpB* and *mptpA* operon in the intracellular survival of *M. tuberculosis*. These studies have provided a better understanding of the importance of tyrosine phosphatases in the survival of *M. tuberculosis* in the host tissue and led to the identification of these two tyrosine phosphatases as attractive targets for the development of new anti-tubercular drugs.

(iii) Iron storage proteins and their importance in the pathogenesis and survival of *Mycobacterium tuberculosis* in the host

Iron is an essential nutrient for almost all microbes, including pathogens such as *Mycobacterium tuberculosis*. It is an indispensable cofactor for proteins involved in critical cellular processes, such as electron transfer, oxygen transport, DNA synthesis, etc. Although iron is essential, excess free iron is potentially toxic for the cells because it catalyzes the production of reactive oxygen radicals by a Fenton reaction, leading to oxidative damage. Thus, all living organisms tightly regulate the cellular levels of iron by employing efficient iron acquisition and storage mechanisms. The sequencing of the *M. tuberculosis* H37Rv genome revealed the presence of two putative iron storage proteins, namely, BfrA (Rv1876), a bacterioferritin, and BfrB (Rv3841), a ferritin-like protein.

However, the biological significance of these iron-storing proteins for *M. tuberculosis* has not been genetically proven. Hence, Dr. Tyagi and colleagues generated mutants of *M. tuberculosis* lacking *bfrA* (Rv1876) and *bfrB* (Rv3841) that encode the iron storage proteins and showed that the mutant of *M. tuberculosis*, H37Rv Δ*bfrA*, Δ*bfrB*, which lacks the function of both *bfrA* and *bfrB*, has significantly reduced growth under iron-deprived conditions, is markedly vulnerable to oxidative stress, and exhibits the attenuation of growth in human macrophages. Moreover, reduced bacillary load in lung and spleen of H37Rv Δ*bfrA* Δ*bfrB*-infected guinea pigs, resulting in a significant reduction in pathology, clearly implied that these proteins play a crucial role in the pathogenesis of *M. tuberculosis.* Mycobacteria are continuously exposed to oxidative stress generated by the activated macrophages that they inhabit. Dr. Tyagi and colleagues evaluated the ability of *M. tuberculosis* mutants lacking the function of *bfrA* and *bfrB* to resist oxidative stress and observed that simultaneous mutations in *bfrA* and *bfrB* in *M. tuberculosis* (H37Rv Δ*bfrA* Δ*bfrB*) tremendously reduced its ability to withstand oxidative stress, implying the role of

these iron storage proteins in restricting oxidative damage. These observations by Dr. Tyagi's laboratory clearly demonstrated the importance of these iron storage proteins in the mycobacterial response to oxidative stress.

Thus, Dr. Tyagi and colleagues demonstrated that BfrA and BfrB proteins play a crucial role in protecting the pathogen against oxidative stress encountered during infection. In addition, they showed that BfrA and BfrB proteins are important for the survival and hematogenous spread of the pathogen. Their studies established these proteins as attractive drug targets for the development of new therapeutic molecules against mycobacterial infections.

(iv) Importance of mycobactin biosynthesis in the physiology, growth and pathogenesis of *M. tuberculosis*

M.tuberculosis has developed an efficient mechanism to sequester iron from the host by secreting siderophores known as mycobactins. Mycobactins bind to iron more strongly than the iron storage proteins of the host and play a crucial role of scavenging iron from the iron limiting host environment. *M.tuberculosis*, *mbt* cluster is induced under low iron conditions. No studies have been carried out to evaluate the importance of mycobactin biosynthesis during the survival of *M.tuberculosis* in the host.

Dr. Tyagi and colleagues disrupted the *mbtE* gene (Rv2380c) of *M.tuberculosis* that encodes a non ribosomal peptide synthetase in the *mbt* cluster. Disruption of this gene renders *M.tuberculosis* incapable of synthesizing mycobactins. The Mtb*ΔmbtE* mutant displayed an altered colony morphology and was drastically affected in its ability to grow on agar medium and in broth culture as compared to the parental strain. Supplementation of agar and broth medium with Fe3+CMBT or Fe3+MBT restored the growth of Mtb*ΔmbtE* to levels similar to that of the parental strain. Genetic complementation of Mtb*ΔmbtE* with *mbtE* gene restored the in vitro growth phenotype of the mutant similar to that of the parental strain. From these observations by Dr. Tyagi and colleagues, it was evident that mycobactin mediated iron acquisition is important for the normal growth of the pathogen. Transmission electron microscopy studies demonstrated an altered cell wall permeability of Mtb*ΔmbtE*. Supplementation of growth medium with Fe3+CMBT restored the staining of Mtb*ΔmbtE* similar to that of the parental strain. The altered colony morphology, cell wall permeability and growth characteristics of Mtb*ΔmbtE* suggested that in the absence of mycobactins, several iron requiring systems of Mtb*ΔmbtE* might have been affected (emanating as a consequence of inability of the mutant to synthesize mycobactins). The restoration of normal growth, cell wall permeability as well as colony morphology resulting from the addition of mycobactins in the media suggested that due to its essential role in procuring iron, mycobactin biosynthesis plays an important role in the biology of the pathogen.

Dr. Tyagi and colleagues also demonstrated that Mtb*ΔmbtE* mutant displayed a significantly reduced ability to infect and grow inside the human THP-1 macrophages in comparison to the parental strain, emphasizing that mycobactins are vital for mycobacterial growth. Their studies in guinea pigs provided further evidence that Mtb*ΔmbtE* is highly attenuated for its growth and ability to cause pathology. In the case of infection with the

parental strain, a substantial number of CFU was recovered from the lungs and spleen of animals, at 4 as well as 10 weeks post infection, while no CFU was obtained from the animals infected with Mtb∆*mbtE* at both the time points. These observations demonstrated that the mutant strain could survive in the host only for a limited period of time. These observations demonstrated a severe attenuation in the ability of the mutant to grow in the host and cause disease. Thus, this study Dr. Tyagi and colleagues highlighted the importance of mycobactins for the normal physiology of *M.tuberculosis*, in vitro as well as in the host.

(v) Secreted acid phosphatase (SapM) of *Mycobacterium tuberculosis*

Phagosomal maturation arrest is an important strategy employed by *Mycobacterium tuberculosis* to evade the host immune system. Secretory acid phosphatase (SapM) of *M.tuberculosis* is known to dephosphorylate phosphotidylinositol 3-phosphate (PI3P) present on phagosomes. However, there have been divergent reports on the involvement of SapM in phagosomal maturation arrest in mycobacteria. Dr Tyagi and colleagues conducted a study to reascertain the involvement of SapM in phagosomal maturation arrest in *M.tuberculosis*. Further, for the first time, they also studied whether SapM is essential for the pathogenesis of *M.tuberculosis*. By deleting the *sapM* gene of *M.tuberculosis*, Dr Tyagi and colleagues demonstrated that SapM mediates an important role in the protection of *M.tuberculosis* against the host defense by subverting the phagosomal maturation pathway. Moreover, the disruption of *sapM* in *M.tuberculosis* resulted in a highly attenuated strain with an impaired ability to grow in the THP-1 macrophages. Dr Tyagi et al further showed that Mtb∆*sapM* is severely attenuated for growth in the lungs and spleen of guinea pigs and has a significantly reduced ability to cause pathological damage in the host when compared with the parental strain. Also, the guinea pigs infected with Mtb∆*sapM* exhibited a significantly enhanced survival when compared with *M.tuberculosis* infected animals. The importance of SapM in phagosomal maturation arrest as well as in the pathogenesis of *M.tuberculosis* established it as an important target for the designing of anti-tubercular molecules.The fact that there are no known human analogues of SapM makes it even more important target for the development of new therapeutic molecules against TB. In addition, the secretory nature of SapM presents a unique opportunity in order to avoid the drug permeability issue due to thick hydrophobic cell envelope of *M.tuberculosis*.

(vi) Apurinic / Apyrimidinic endonucleases of *Mycobacterium tuberculosis*

In host cells, *Mycobacterium tuberculosis* encounters an array of reactive molecules capable of damaging its genome. Non-bulky DNA lesions are the most common damages produced on exposure to reactive species and base excision repair (BER) pathway is involved in the removal of such damage. During BER, apurinic / apyrimidinic (AP) endonuclease enzymes repair the abasic sites that are generated after spontaneous DNA base loss or by the action of DNA glycosylases, which if left unrepaired lead to inhibition of replication and transcription. However, the role of AP endonucleases in the growth and pathogenesis of *M.tuberculosis* has not yet been elucidated. To demonstrate the biological significance of these enzymes in *M.tuberculosis*, Dr Tyagi and colleagues generated *M.tuberculosis* mutants of the base excision repair (BER) system, disrupted in either one (Mtb∆*end* or Mtb∆*xthA*) or both (Mtb∆*end*∆*xthA*) the AP endonucleases and demonstrate that these genes are crucial

for bacteria to withstand alkylation and oxidative stress *in vitro*. In addition, the mutant disrupted in both the AP endonucleases (Mtb∆*end*∆*xthA*) was shown to exhibit a significant reduction in its ability to survive inside human macrophages. However, infection of guinea pigs with either Mtb∆*end* or Mtb∆*xthA* or Mtb∆*end*∆*xthA* resulted in the similar bacillary load and pathological damage in the organs as observed in the case of infection with *M.tuberculosis*indicating that the pathogen must have alternate repair machinery for the repair of the damaged DNA to safeguard its genome during its survival in the host.

B. Crystallization of *M. tuberculosis* proteins and structure determination

Dr. Tyagi and colleagues determined the crystal structure of several important *M. tuberculosis* proteins such as BfrA, BfrB and BirA.

(i) BfrA

Dr. Tyagi et al. determined the crystal structure of the selenomethionyl analog of bacterioferritin A (SeMet-BfrA) from *Mycobacterium tuberculosis* (*Mtb*) at 2.5 A^{o} resolution. Unexpectedly, electron density observed in the crystals of SeMet-BfrA analogous to haem location in bacterioferritins, showed a demetallated and degraded product of haem. They showed that this unanticipated observation was a consequence of the altered spatial electronic environment around the axial ligands of haem (in lieu of Met52 modification to SeMet52). Furthermore, the structure of *Mtb* SeMet-BfrA displayed a possible lost protein interaction with haem propionates due to formation of a salt bridge between Arg53-Glu57, which appeared to be unique to *Mtb* BfrA, resulting in slight modulation of haem binding pocket in this organism. Determination of the crystal structure of *Mtb* SeMet-BfrA by Dr. Tyagi and colleagues provided novel leads to the physiological function of haem in Bfrs. It may also serve as a scaffold for designing specific inhibitors. In addition, this study provided evidence against the general belief that a selenium derivative of a protein represents its true physiological native structure.

(ii) BfrB

Dr. Tyagi and colleagues also determined a 3.0 A° crystal structure of BfrB from *Mycobacterium tuberculosis* (Mtb). The Mtb BfrB subunit exhibited the characteristic fold of a four-helical bundle that possesses the ferroxidase catalytic centre. Dr. Tyagi et al. compared the structure of Mtb BfrB with representatives of the ferritin family belonging to the archaea, eubacteria and eukarya. Unlike most other ferritins, Mtb BfrB has an extended C-terminus. To dissect the role of this extended C-terminus, truncated Mtb BfrB was purified and biochemical studies carried out by Dr. Tyagi and colleagues implicate this region in ferroxidase activity and iron release in addition to providing stability to the protein.

(iii) BirA

The first committed step in lipid biosynthesis is the biotinylation of Acetyl Coenzyme A Carboxylase (ACC) mediated by biotin acetyl-CoA carboxylase ligase / biotin protein ligase (BirA). BirA appears to be an attractive target for the development of broad spectrum therapeutic agents against multiple infections. The apo BirA crystal structure developed by

Dr. Tyagi et al. (at 2.69 A^{o} resolution) revealed the presence of disordered flexible loops, which undergo a conformational transition upon biotin and biotinyl-59-AMP binding. These loops are known to participate in either dimer interface or ligandbinding or both. Dr. Tyagi and colleagues showed that dehydration of *Mtb*-BirA crystals traps both the apo and active conformations in its asymmetric unit, and for the first time provided structural evidence of such transformation. In addition, crystal dehydration resulted in a shift of 3.5 A° in the flexible loop L6, a proline-rich loop unique to *Mtb* complex as well as around the L11 region. The shift in loop L11 in the C-terminal domain on dehydration emulates the action responsible for the complex formation with its protein ligand biotin carboxyl carrier protein (BCCP) domain of ACCA3. This is contrary to the involvement of loop L14 observed in Pyrococcus horikoshii BirA-BCCP complex. This dehydrated crystal structure not only provided key leads to the understanding of the structure/function relationships in the protein in the absence of any ligand-bound structure, but also demonstrated the merit of dehydration of crystals as an inimitable technique to have a glance at proteins in action.

C. Characterization of Drug Target Proteins

(i) Characterization of FaD13 and identification of important residues

To gain further insight into the functioning of *mymA* operon, a potential target for developing antitubercular drugs, Dr. Tyagi's laboratory characterized its gene products. *fadD13*, the last gene of the *mymA* operon, encodes a Fatty Acyl-CoA Synthetase. Dr. Tyagi and colleagues developed several site-directed mutants of FadD13 and analyzed them for the structural-functional integrity of the enzyme. This study revealed that mutation of Lys487 resulted in 95% loss of the activity thus demonstrating its crucial requirement for the enzymatic activity. Comparison of the kinetic parameters by Dr. Tyagi et al. showed the residues Lys172 and Ala302 to be involved in the binding of ATP and Ser404 in the binding of CoenzymeA. The influence of mutations of the residues Val209 and Trp377 emphasized their importance in maintaining the structural integrity of FadD13. Besides, Dr. Tyagi and colleagues showed a synergistic influence of fatty acid and ATP binding on the conformation and rigidity of FadD13. FadD13 represents the first Fatty Acyl-CoA Synthetase to display biphasic kinetics for fatty acids. The studies by Dr. Tyagi and colleagues provided a significant understanding of the FadD13 protein including the identification of residues important for its activity as well for the maintenance of structural integrity.

(ii) Identification of "switch residues" or "interface hot spots" involved in the self assembly and function of bacterioferritin B of *M. tuberculosis*

By employing site-directed mutagenesis Dr. Tyagi and colleagues identified important residues for interactions between subunits of this ferritin that are required for molecular assembly, structural integrity, thermodynamic stability, and ferroxidase activity to provide an improved understanding of the determinants of self-assembly and the structure–function relationship.

To identify the crucial residues involved in the self assembly and function of BfrB, Dr. Tyagi and colleagues constructed various mutants by employing site-directed mutagenesis. The analysis of mutants led to the identification of "interface hot-spot residues" that act as

"switch points" for BfrB oligomerization. These studies demonstrated the importance of 4-fold axis residues in assembly formation. Moreover, it was demonstrated that single-point mutations can enhance the thermal stability of the protein without affecting its assembly. Importantly, a comparative analysis of various mutations by Dr. Tyagi and colleagues revealed that the function of various homologous positions in different ferritins could be at variance; hence, predicting the function of a residue just based on sequence−structure comparisons may not be appropriate. Thus, these studies showed that single-point mutations have a remarkable potential for alteration of multiple properties of ferritins. Besides, "switch residues" or "interface hot spots" identified in this study could also prove to be helpful for the rational design of interfacial inhibitors.

(D) Identification of inhibitors against *M. tuberculosis*

(i). Identification of inhibitors against Fatty Acyl-CoA Synthetase (FadD13, Rv3089) of *M.tuberculosis*

Dr. Tyagi et al. earlier demonstrated that exposure to acidic pH results in the upregulation of the *mymA* operon of *M. tuberculosis* (Rv3083 -Rv3089). The functional loss of the *mymA* operon leads to alterations in the colony morphology, cell wall structure, mycolic acid composition and drug sensitivity and results in markedly reduced intracellular survival of *M.tb* in macrophages. Besides, the *mymA* mutant of *M.tb* shows a drastic reduction (800fold) in its ability to survive in the spleen of guinea pigs as compared to the parental strain and hence, represents an important drug target for *M.tuberculosis*. *fadD13*, the last gene of the *mymA* operon, encodes a Fatty Acyl-CoA Synthetase (FACS), which catalyzes the activation of various fatty acids by converting them into fatty acyl-CoA thioesters.

Dr. Tyagi and colleagues generated the three–dimensional structure of FadD13 by comparative homology modeling. The predicted active site covered parts of both the N- and C-terminal domains along with the cleft region placed between both the domains. Moreover, the active site was similar to that seen in other homologous proteins.

Dr. Tyagi and colleagues employed the NCI Open Database comprising of 2,60,071 compounds for virtual screening against the FadD13 model with the ATP binding site as the target for docking by using AutoDock4. Based on the results, the top 40 compounds were requested from National Cancer Institute - Developmental Therapeutics Program (NCI-DTP). The compounds were experimentally evaluated for their potential to inhibit the activity of FadD13. Among the compounds evaluated, 13 exhibited inhibition of the activity. Seven compounds were selected for further studies based on their ability to inhibit FadD13 activity by more than 20%.

For further assessment, Dr. Tyagi and colleagues first examined the effect of various compounds on the growth of *M.smegmatis* (a fast grower) by using the alamar blue dye method. It was observed that two compounds exhibited a marked inhibition of *M.smegmatis* growth with MIC_{99} value of 6.25 µg/ml. Besides, one more compound also exhibited a significant inhibition of *M.smegmatis* growth with MIC_{99} value of 12.5 µg/ml. The compounds were simultaneously also evaluated for their ability to inhibit the growth of

M.tuberculosis by broth macrodilution as well as microplate alamar blue method. The results revealed that one of the compounds exhibited the highest inhibition with an MIC_{99} value of 6.25 μg/ml. Optimization of lead obtained in this study would provide valuable inputs towards the development of inhibitors against *mymA* operon, an important target for the development of antitubercular drugs.

(ii) Identification of Inhibitors against *Mycobacterium tuberculosis* Thiamin Phosphate Synthase

In spite of the availability of drugs for the treatment of TB, the non-compliance to long chemotherapeutic regimens often results in the emergence of multidrug resistant strains of *Mycobacterium tuberculosis* adding to the precariousness of the situation. This has necessitated the development of more effective drugs. Thiamin biosynthesis, an important metabolic pathway of *M. tuberculosis*, is shown to be essential for the intracellular growth of this pathogen. Dr. Tyagi and colleagues constructed a three–dimensional homology model of *M. tuberculosis* thiamin phosphate synthase by using the X-ray crystal structure of thiamin phosphate synthase from *Pyrococcus furiosus.*

Dr. Tyagi and colleagues employed computational screening approach to identify potential small-molecule inhibitors of MtTPS from the NCI diversity set II comprising of 1541 compounds. Compound A, (4-{[(2-hydroxy-5-nitrophenyl) methylidene]amino}-5-methyl-2-(propan-2-yl)phenol), B, (3-benzylsulfanyl-phenanthro [9,10-e][1,2,4]triazine) and C, (Coumarin, 7-[[4-chloro-6-(diethylamino)-s-triazin-2-yl]amino]-3-phenyl-) were identified as potential inhibitors of *M. tuberculosis* growth. All these compounds exhibited inhibition of MtTPS enzymatic activity as well as the growth of *M. tuberculosis* in broth culture. However, one of the compounds A exhibited the highest efficacy with an MIC99 value of 6 μg/ml. In addition, it did not exhibit any significant toxicity in various cell lines till a concentration of 25 μg/ml and also adhered to the Lipinsky rules for drug-likeness. The binding mode of compound A provided key insights into the likely binding sites. The compound A is docked at the large hydrophobic pocket at the active site of MtTPS. The aromatic ring A is placed in a hydrophobic environment surrounded by Ile173, Val193 and Phe171 while the two oxygen atoms of the nitro group appear to be making hydrogen bonds with the hydrogen atoms of the adjacent Cys136 and Cys11 both present within 2.5A° distance from the oxygen atoms. Moreover, the hydroxyl group of the aromatic ring B can form hydrogen bond with the carboxyl group of Asp98 present at a distance of 1.78A°. Inhibition of MtTPS by compound A in the presence of varying concentrations of the substrate HMPPP showed that an enhancement in the concentration of the substrate causes a decline in the inhibition and vice versa, which clearly indicated that it inhibits MtTPS by competing with HMP-PP for binding at the active site thus substantiating the docking results. In conclusion, Dr. Tyagi and colleagues have identified a promising lead molecule (compound A) for the development of sterilizing agents against *M. tuberculosis* and further efforts are in progress to optimize and enhance the inhibitory potency of this lead compound.

3. The first completed genome of a new bacterial species (*Mycobacterium indicus pranii*) from India

This work on *Mycobacterium indicus pranii* (MIP) was responsible for the publication of the first completed genome of a new bacterial species from India and was covered in Nature as "Science News" item in September 2012.

MIP is a saprophytic mycobacterial species that is known for its immunomodulatory properties. MIP, which shares antigens with both *M. leprae* and *M. tuberculosis*, provides protection against *M. tuberculosis* infection in mice and accelerates sputum conversion in both type I and type II category of tuberculosis (TB) patients when used as an adjunct to chemotherapy. In HIV/TB co-infections, a single dose of MIP converted tuberculin -ve patients into tuberculin +ve in >95% of the cases. This attribute is unique to MIP because similar application of other saprophytic mycobacteria such as *M. vaccae* does not provide commensurate protection. Based on its demonstrated immunomodulatory action in various human diseases, MIP has been the focus of several clinical trials and successful completion of one such trial has led to its use as an immunotherapeutic vaccine 'Immuvac' against leprosy. However, very little information was available about MIP's molecular, biochemical, genetic and phylogenomic features. Thus, in a collaborative effort, Dr. Tyagi and colleagues in a molecular phylogenetic study by using candidate marker genes and FAFLP (fluorescent-amplified fragment length polymorphism techniques) fingerprinting assay showed that MIP belongs to a group of opportunistic mycobacteria and is a predecessor of *M. avium* complex (MAC). A comprehensive analysis of cellular and biochemical features of MIP along with chemotaxonomic markers such as FAME (fatty acid methyl ester) analysis and comparison with other mycobacterial species established that MIP is endowed with specific attributes.

In a collaborative effort with Dr. Tyagi being the Principal Investigator, the complete MIP genome was sequenced to gain an insight into its unique life style and molecular basis of immunomodulation. In addition, they employed comparative genomics to understand the habitat diversification and bases and means of functional genetic correlates responsible for evolution of pathogenicity in ancestral mycobacterial lineages.

Different analyses performed in an earlier study established that MIP represents an organism at a unique phylogenetic point as the immediate predecessor of opportunistic mycobacterial species of MAC. It also became evident that natural selection in MAC has acted in a preferential manner on specific categories of genes leading to reduced habitat diversity of pathogenic bacteria, and thus facilitating host tropism. The genome of MIP was ~5.6Mb in size and was found to be shaped by a large number of lateral gene acquisitions thus revealing, for the first time, mosaic architecture of a mycobacterial genome. Thus, this study by Dr. Tyagi and colleagues offered a paradigm shift in our understanding of evolutionary divergence, habitat diversification and advent of pathogenic attributes in mycobacteria. A scenario for mycobacterial evolution was envisaged wherein the earliest evolving soil derived mycobacterial species like MIP underwent massive gene acquisitions to attain a unique soil–water interface habitat before adapting to an aquatic and parasitic lifestyle. These lateral acquisition events were selective and possibly facilitated by the presence of specific genetic factors (i.e. ComEC) that induce competence to acquire large chunks of DNA to confer immediate survival advantage to the recipient organism.

Subsequently, mycobacterial species tuned their genetic repertoires to respective host adapted forms with a high degree of genomic fluidity aided by selective lateral gene acquisitions and gene loss by deletion or pesudogenization. Importantly, a significant increase in transposon elements in the pathogenic mycobacteria as compared with MIP, for the first time, suggested their possible role toward mycobacterial virulence. In addition, comparative genomic analysis revealed a higher antigenic potential of MIP subscribing to its unique ability for immunomodulation against various types of infections and presented a template to develop reverse genetics based approaches to design better strategies against mycobacterial infections.

Details of Research Contributions

Some important research contributions of Dr. Tyagi are described below in a chronological order.

1973-1977

During these formative years, while pursuing his Ph.D., Dr. Tyagi worked on mycobacteria. This group of microorganisms comprises various pathogenic and non pathogenic organisms including the pathogens that cause tuberculosis and leprosy. The special emphasis was on understanding the mechanism of oxidative phosphorylation in mycobacteria in order to understand the slow growing nature of mycobacteria.

Role of various dehydrogenases in energy production in *M. tuberculosis* BCG

The levels of all the dehydrogenases associated with electron transport chain in *M.tuberculosis* BCG were investigated. NAD-dependent malate dehydrogenase was found to be the most active, and was exclusively present in the soluble fraction. Isocitrate dehydrogenase was fairly active; however, other enzymes like malate vitamin K reductase, succinic dehydrogenase, alpha-ketoglutarate dehydrogenase and pyruvate dehydrogenase were present in low levels. Malic enzyme and beta-hydroxy-butyrate dehydrogenase could not be detected in BCG. Localization and specific activity of some enzyme complexes of the electron transport chain like NADH oxidase, NADH-cytochrome *c* oxidoreductase, succinate cytochrome *c* oxidoreductase, NADH-DCIP oxidoreductase and cytochrome oxidase in BCG was also studied.

Spectal studies using the ETP from *M.tuberculosis* BCG revealed the occurrence of cytochromes *a, b* and *c*. The carbon monoxide difference spectrum, however, demonstrated the presence of only cytochrome a but not cytochrome O.

Studies on the specificity of phosphate acceptor system in BCG revealed that only ATP but not AMP could replace ADP. Judged by the rates of oxidation and phosphorylation it was concluded that the organism could utilize only malate, succinate and isocitrate for its energy requirements. Malate was shown to be oxidized only by NAD dependent (MAL_{NAD} pathway) malate dehydrogenase. All three sites of phosphorylation were found functional in *M.tuberculosis* BCG.

Conclusion

Of the dehydrogenases associated with electron transport chain of *M.tuberculosis* BCG, malate dehydrogenase is the most active one. The organism could generate energy only by oxidizing malate, succinate and isocitrate. Only MAL_{NAD} pathway was operable for malate oxidation. All three sites of ATP production were functional in slow growing *M.tuberculosis* BCG.

Determination of site of action of non heme iron protein in electron transport chain

Studies were also carried out on the site of action of nonheme iron protein in the malate vitamin K reductase pathway of *Mycobacterium phlei*. Irradiation with ultraviolet light destroyed malate oxidase activity of both cell free extracts as well as reconstituted system and the loss of activity could not be significantly restored by vitamin K1 alone, which suggested the participation of another light sensitive component. Using the techniques of irradiation with ultraviolet light (360 nm), o-phenanthroline and electron acceptors like

MTT, it was shown that nonheme iron protein combination with flavin (metalloflavoprotein) acts at a site prior to vitamin K in the MAL_{FAD} pathway of *M.phlei*.

To support the above view, electron paramaganetic resonance studies were carried out. Electron transport particles from *M.phlei* upon reduction with malate exhibited electron paramagnetic resonance signals at g=2.002 and 1.94, characterisitic of napthosemiquinone and nonheme iron protein respectively. Upon irradiating the particles with ultraviolet light (360 nm) these signals were not observed suggesting that ultraviolet irradiation destroyed the environment around the metal in such a way that malate failed to reduce the metal.

Conclusion

Site of action of non heme iron protein in the electron transport chain of *M.phlei* (in the MAL_{NAD} pathway) was established. It was found that non heme iron protein participates before or in combination with flavin in electron transport chain of *M.phlei*.

Purification and characterisation of malate dehydrogenase

Malate dehydrogenase (EC 1.1.1.37) was purified from *M.phlei* to homogeneity. The enzyme was found to be composed of four subunits of equal molecular weight (21, 554). Tyrosine and isoleucine were identified as the N- and C-terminals of the malate dehydrogenase of *M.phlei*. Amino acid composition of the malate dehydrogenase was determined to understand the chemical structure of the protein molecule. Studies on the effect of acid and urea on the structure of malate dehydrogenase demonstrated that treatment of the enzyme with acid and urea results in the dissociation of the enzyme followed by loss of catalytic activity. This dissociated enzyme could however be reconstituted by bringing the pH back to neutrality or by removing the urea from the enzyme solution. Slow removal of urea by dialyzing in cold proved a better extent for reconstitution.

Conclusion

The native enzyme probably has only one active site and the catalytic monomer is the tetrameric form of the protein. Inactivation followed by dissociation of protein by acid and urea treatment therefore reveals that for making up a single active site cooperative interaction and folding of the four polypeptide chains is essential.

1978-1982 (Post-doctoral research at NIH, USA)

Studies on a novel, natural and unique anticancer agent

The scientific literature during these years marked the blossoming of knowledge concerning the treatment of cancer specially the designing, biological effects, mechanism of action and application of cancer drugs. Dr. Tyagi's efforts during this period focussed on studying the pharmacology, metabolism and mechanism of action of an antiviral, antimicrobial and antitumor antibiotic L-alanosine 2-amino-3- [(N-hydroxy-N-nitroso) amino] propionic acid. A natural product L-alanosine is structurally distinctive as this compound was unique among natural compounds to have both N-nitroso functionality and a hydroxy group on a single nitrogen atom and it had already shown very promising anticancer activity.

Interaction of L-alanosine with enzymes metabolizing L-aspartic acid, L-glutamic acid and their amides

First a comprehensive analysis was made of the manner in which L-alanosine interacts with the enzymes responsible for the metabolism of the dicarboxylic amino acids and their amides. It was found that the drug impedes the transport of L-aspartic acid and, to a lesser degree, than of L-glutamic acid, L-asparagine and L-glutamine by lymphoblasts, *in vitro*. In each of these instances, inhibition was apparently competitive in type. Of the enzymes involved in the metabolism of L-aspartic acid, adenylosuccinate synthetase, SAICAR synthetase (5-amino-4-imidazole-N-succino-carboxamide ribonucleotide synthetase) L-aspartyl tRNA synthetase L-aspartate transcarbamylase and L-aspartate aminotransferase were inhibited by L-alanosine; moreover, each of these enzymes except L-aspartyl tRNA synthetase accepted the antibiotic as substrate, although at substantially diminished rates. Of the enzymes involved in the metabolism of L-glutamic acid, L-alanosine inhibited only L-glutamine synthetase and L-glutamate decarboxylase to a prominent degree. Although L-alanosine provoked a rise in the concentration of inosinic and (IMP) *in vitro*, pointing to the conclusion that the drug was capable of inhibiting adenylosuccinate synthetase under these circumstances, no such rise was seen *in vivo* either in tumor or in liver. However, 1 and 5 hr after administration L-alanosine depressed hepatic ATP and NAD pools, an effect which indicated that the drug is, in fact, restricting the intracellular concentration of adenine nucleotides. Of the metabolites of L-alanosine *in vitro*, α-decarboxy alanosine, α-keto alanosine, α-hydroxy alanosine, alanosyl IMP and N-carbamyl L-alanosine did not inhibit adenylosuccinate synthetase to any prominent degree, whereas the metabolite generated by SAICAR synthetase powerfully inhibited this enzyme, with a K_i of 0.3 μM. Parenteral therapeutic doses of L-alanosine produced striking increases in the concentrations of L-aspartic acid in tumor and liver as well as of L-aspartic and L-glutamic acid in urine.

Conclusion

In quantitative terms, transamination of L-alanosine and reduction of the resultant α-ketocarboxylic acid appeared to be the principal metabolic fate of the antibiotic. In qualitative terms, with therapeutic, toxicologic and enzymologic actions as end points, the most important metabolic fate of L-alanosine was its condensation with 5-amino-4-imidazole carboxylic acid ribonucleotide to yield a fradulent anabolite capable of powerfully inhibiting adenylosuccinate synthetase (K_i 0.3 μM).

Studies on the mechanism of action of L-alanosine

L-alanosine, like azaserine, is a derivative of L-alanine and contains a negatively charged nitrogenous α-substituent. However, azaserine arrests the synthesis of all purines, but L-alanosine interrupts the synthesis of adenine alone. Now, the pathway of the synthesis of adenine is common upto the step at which 5-formamidoimidazole-4-carboxamide ribonucleotide undergoes ring closure to form IMP. Thus, L-alanosine's site of action seemed likely to be subsequent to this step, most probably at the level of the reactions involved in the conversion of IMP to AMP. Consequently, the two steps of primary concern became (1) the addition of L-aspartate to IMP and (2) the removal of fumarate from the adenylosuccinate thus formed to yield AMP.

The finding that L-aspartic acid, even at high concentrations, wholly failed to alleviate the effects of L-alanosine on cell replication or AMP formation suggested that the

antibiotic was not behaving as a competitive inhibitor of that amino acid in the adenylosuccinate synthetase reaction, or that the drug might require conversion to the formally competitive species.

Further, support for the latter alternative was provided by an examination of the chronology of inhibition of adenylosuccinate synthetase. When a therapeutic dose of L-alanosine (500 mg/kg) was given to mice bearing nodules of leukemia L5178Y and the inhibition of adenylosuccinate synthetase was followed over time, it was observed that there was a 30-minute lag before inhibition became prominent, but, thereafter, the drug inhibited tumoral adenylosuccinate synthetase for an 8-hour period. Subsequently, a gradual restitution of activity was observed. Virtually all the inhibition seen was reversible by dialysis.

When the inhibition of adenylosuccinate synthetase produced by L-alanosine *in vitro* was compared to that exerted by the drug *in vivo* marked disparities emerged: *in vitro*, the inhibition by L-alanosine of adenylosuccinate synthetase, partially purified from leukemia L5178Y cells, using L-aspartic acid as a variable substrate, was non-competitive and weak, with a K_i of 57 mM; with GTP, and IMP as variable substates, inhibition was also non-competitive and feeble, with K_i of 30 mM and 37 mM, respectively.

Since L-alanosine itself can inhibit adenylosuccinate synthetase, it became important to determine whether the nodules of L5178Y cells used in these studies contained the antitumor agent *in vivo* at a concentration commensurate with the kinetics of inhibition measured *in vitro*. It was found that the concentration of L-alanosine in these tumors fell to 110 μM within 2 hours after the administration of the drug, and to 170 μM within 8 hours, despite the fact that inhibition of adenylosuccinate synthetase had been found to persist at approximately 70% over this time span. Obviously, these concentrations of L-alanosine were incapable of exerting the magnitude of enzyme inhibition observed, a finding that ruled out the possibility that the antibiotic itself was functioning *in vivo* as the proximate inhibitor of adenylosuccinate synthetase.

In spite of the postulation that the active metabolite of L-alanosine was the adduct of the antitumor agent with AICOR. Our early attempts to demonstrate this molecule in the tumors of mice treated with the drug met with failure. However, because its identification was central to any explanation of the mechanism of action of L-alanosine, more comprehensive studies on the *in vivo* formation of L-alanosyl-AICOR were undertaken using L5178Y cells growing as subcutaneous nodules in mice. L-alanosyl-AICOR was prepared from L-alanosine and AICOR by the catalytic action of a preparation of SAICAR synthetase partially purified from avian liver. This compound was a strong inhibitor of adenylosuccinate synthetase, and Bratton-Marshall reaction positive.

Studies on the inhibition by L-alanosyl-AICOR of partially purified adenylosuccinate synthetase from leukemia L5178Y showed that the anabolite was a formally competitive inhibitor versus IMP, with an apparent K_i of 0.228 μM.

In as much as it was possible to condense L-alanosine with AICOR *in vitro*, and in view of the fact that the resulting antimetabolite, L-alanosyl-AICOR, was a very potent inhibitor of adenylosuccinate synthetase, a search was mounted to demonstrate the occurrence of this anabolite in living tumors. To this end, mice bearing L5178Y nodules were given a very large dose (50 μCi) of radioactive L-alanosine along with nonradioactive L-alanosine (500 mg/kg); tumors were excised, flash frozen, and extracted, and the extracts

subjected to high resolution chromatography. A prominent radioactive peak, co-eluting with L-alanosyl-AICOR and unique to the tumors of treated recipients, was detected at concentrations of 70, 53 and 20 μM at 2, 4, and 8 hours respectively.

To confirm that this material was indeed L-alanosyl-AICOR, all chromatographic fractions from these studies were tested for inhibition of partially purified adenylosuccinate synthetase and for Bratton-Marshall positively. Fractions corresponding to the peak coeluting with L-alanosine had no effect on the enzyme; however, those corresponding to the peak coeluting with L-alanosyl-AICOR strongly inhibited the partially purified preparation of adenylosuccinate synthetase. Only the fractions corresponding to this peak were observed to be Bratton-Marshall reaction positive.

Conclusion

These studies led to the understanding of mechanism of L-alanosine. L-alanosine acts as anticancer agent not directly but after being converted to L-alanosyl-AICOR. The later inhibits adenylosuccinate synthetase very potently resulting in depletion of purine nucleotides.

Mechanism of Resistance against L-alanosine

Sublines of P388 and L12010 leukemia were rendered resistant to L-alanosine and designated P388/LAL and L1210.LAL. Assessments were made to certain biochemical and pharmacological determinants of the sensitivity or resistance to L-alanosine of these sensitive and resistant lines. It was observed that the antibiotic strongly inhibited adenylosuccinate synthetase and DNA synthesis only in the parent or sensitive lines; moreover, after a therapeutic dose of the drug, the concentration of L-alanosyl-AICOR, the putative active anabolite of L-alanosine, was dramatically higher in these parent lines as compared with the resistant variants. Enzymologic studies established that, in P388/LAL, the specificity activity of the enzyme SAICAR synthetase was depressed significantly. In both resistant lines, however, the enzymes of purine salvage were present at levels about 200 per cent higher than those measured in the native strains.

Conclusions

The two mechanisms were found to be dominant in the state of resistance to L-alanosine - a significantly diminished ability to accumulate L-alanosyl-AICOR and significantly enhanced ability to re-utilize preformed purines which are responsible for the development of resistance against L-alanosine.

This period also saw the extension of Dr. Tyagi's research activities into the area of polyamines. Polyamines play a crucial role in various cellular processes. Cell growth and differentiation does not occur in the absence of polyamines. For this reason polyamine biosynthesis has gained widespread importance as a target for metabolic and pharmacological intervention. His investigations during this period focussed on regulation and role of polyamines in *Saccharomyces cerevisiae*.

Regulation of ornithine decarboxylase in *S.cerevisiae*

Ornithine decarboxylase (ODC) was purified to homogeneity (1500 folds) from yeast and characterized. It was discovered that the enzyme is synthesized as a precursor of 86 kDa and then is converted to 68 kDa form during purification. This conversion was inhibited

by proleolytic inhibitors. We were also able to isolate this 86 kDa form of the enzyme using an antibody - sepharose column with antibodies against 68 kDa form.

In view of these new findings, it was decided to study the effect of addition of spermine and spermidine to the growth medium on the amount of ornithine decarboxylase protein found in the yeast cells. It was shown that addition of amines to the medium resulted in the complete loss of ornithine decarboxylase activity within 6 hours; this inactivation required protein synthesis. In contrast to the loss of enzymatic activity, there was no significant loss of immunoreactive 68 kDa protein. When this experiment was repeated with our improved immunoprecipitation procedure, complete retention of the 86kDa protein, despite complete loss of enzyme activity was observed. Thus, we found evidence that a post-translational modification of the 86 kDa form occurs following growth in amine-supplemented medium. This modification is unrelated to the proteolytic cleavage of the native enzyme.

Immunoprecipitates from one of the *spe*10 mutants which lack ornithine decarboxylase activity were prepared, to determine if these strains contain residual inactive protein. It was found that these inactive extracts contained an amount of 86 kDa protein equal to that found in the very active extracts obtained from the derepressed *spe*2 strain. This was an evidence for regulation of the enzyme activity by a modification which is not related to the proteolytic changes.

Conclusion

The addition of polyamine causes loss of ODC activity by negative control and this loss which is dependent on protein synthesis results from post translational modification of the enzyme.

Requirement of polyamines for the replication and maintenance of dsRNA plasmids (killer plasmids) of yeast

Double-stranded RNA (ds RNA) genomes are found in all major groups of organisms such as viruses of mammals, insects, plants, fungi and bacteria etc. Of the stably maintained ds RNA systems, the best studied one is the killer system of *S.cerevisiae*. Certain strains of yeast secrete protein toxins, also called killer toxins to which they are resistant but that kill other members of the same species. Atleast two distinct killer specificities have been recognized which are known as K1 and K2 killers. These are encoded by two double-stranded RNAs namely M1 and M2. *S.cerevisiae* is of increasing interest as model eukaryote and the killer systems permit detailed study of genetics of model eukaryote. Thus we had undertaken to study whether polyamines are required for the replication and maintenance of these killer plasmids.

The killer systems involve a group of cytoplasmic or non mendelian genetic elements. Most of them are located on ds RNA molecules, which are encapsulated in virus like particles called VLPs but they are not 'autonomously replicating' elements, as both virus and plasmids are often described. Studies have defined 39 chromosomal genes and six plasmids involved in various ways in the maintenance, replication and expression of various components of killer system.

Various strains of yeast were taken which are mutants and thus are defective in one of the steps of polyamines biosynthesis and either by mating these strain with the strains that carry a specific killer component and selecting the sergeants or by the process of

cytoduction generated strains which are mutants for a specific step of polyamine biosynthesis and at the same time carry a killer component of interest such as M2 dsRNA, EXL, HOK, NEX, L-A HN or combination of any of these.

After testing for both these characteristics these mutants were depleted of polyamines by growing them on a polyamine free medium. On this medium, *spe*2 mutants, which contain putrescine but lack spermidine and spermine grow indefinitely but with a 3-4 times longer doubling time. *spe*10 mutants which lack all - putrescine, spermidine and spermine stop growing after several colony isolations on this medium. At this stage these mutants were again replica plated onto a polyamine containing medium, they were grown and again tested whether they still contain the killer specificity in question or have lost it during polyamine depletion.

The strains containing the KIL-K1 or KIL-K2 plasmid and *spe*2 and *spe*10 mutation are killers in nature when they are grown on a rich YPAD medium which contains polyamines but when they were grown in the absence of polyamines and had exhausted their polyamine contents they became non-killers and sensitive to killer toxin thus showing that polyamines are required for the maintenance and replication of these plasmids. Also, it showed that Putrescine is not enough to maintain these plasmids and spermidine or spermine are specifically required, because *spe*2 mutant continue to make large amounts of putrescine and lack only spermidine and spermine yet they lose both M1 and M2 dsRNAs. When 100 μM spermidine was included in the polyamine free medium during the growth of these strains then neither *spe*2 nor *spe*10 strains showed any loss of killer plasmids but ones the killer plasmid is lost from either *spe*2 or *spe*10 strains it could not be restored back by growing these strains in the presence of polyamines. After polyamine deprivation both M1 and M2 dsRNAs were lost from these strains.

Both these *spe*2 and *spe*10 strains carry EXL plasmid which prevents replication of KIL K2. When either of these was mated with strain 1387 which carries KIL-K2, the diploids generated did not show any killing because KIL-K2 is excluded in the presence of EXL. When the *spe*10 strain was depleted of polyamine contents by extended growth on polyamine free medium and was again mated with strain 1387 the diploids now clearly show killing. This indicates that *spe*10 strain has lost EXL and that polyamines are required for the replication of EXL. Once lost, EXL could not be restored by addition of polyamines.

When *spe*2 strain was depleted of spermidine and spermine by extended growth on polyamine free medium, the EXL is not lost, as the diploids generated did not show any killing.

The *spe*2 strains in contrast to spe10 strains continue to make putrescine in greater than wild type amount when grown on a polyamine free medium. Thus, these results showed that putrescine alone in the absence of spermidine and spermine was sufficient to maintain the EXL plasmid. It was also observed that addition of 100 μM putrescine to polyamine free medium during growth prevented the loss of EXL. The polyamine requirement for another variety of dsRNA that is designated L-A-HN were then studied. It carries two cytoplasmic genes HOK i.e. helper of killer and NEX i.e. neutralizer of EXL. A detailed study of polyamine requirement of this plasmid showed that this plasmid does not require polyamines for its maintenance and replication.

Conclusion

M1, M2 and L-A-E dsRNAs all require polyamines for their replication and maintenance. These requirements are not identical for all these dsRNAs. M1 and M2 require spermidine or spermine but putrescine alone is of no help. However, for L-AE any of the polyamine, putrescine, spermidine or spermine is good enough. While L-A-E requires polyamines another variety of L-dsRNA i.e. L-A-HN does not require any of the polyamines. This is rather striking because these two RNA molecules have 99% sequence homology. This data showed that polyamines are important in the replication of KIL-K1, KIL-K2 and EXL for specific steps and that these steps were not involved in the replication or maintenance of HOK and NEX.

Scientific career in India after returning from USA

1983-1989

After returning from USA, Dr. Tyagi did not continue with his post-doctoral work that he was doing at NIH in relation to cancer research. He instead started investigations on mycobacteria as TB was a more important problem for India. His efforts were focussed to develop strategies which could lead to prevention and control of tuberculosis. It was thought that polyamine biosynthesis would be a very useful target for this purpose and thus he initiated work to understand the biosynthesis of polyamines and its regulation in mycobacteria in order to delineate the key target points for inhibition of polyamine biosynthesis. In addition, the work was also started on the role of polyamines in transcription in mycobacteria to understand whether polyamines might have a special role in gene expression in mycobacteria as the latter has highly GC rich genome and polyamines have been shown to exert their effect by transition of B-DNA to Z-DNA apart from the condensation of DNA. Work was initiated on the promoter regions of slow and fast growing mycobacteria in order to understand their involvement, if any, in slow growth of some mycobacterial species and also to study their structure and function and use strong mycobacterial promoters for generation of more soluble expression vectors to study molecular genetics of mycobacteria and for expression of specific protective antigens for tuberculosis and leprosy.

Regulation of putrescine biosynthesis in mycobacteria

It was found that activities of both arginine decarboxylase and ornithine decarboxylase are closely associated with mycobacterial growth polyamines were required during the period of high metabolic activity. Conversely, polyamines were not required by resting or non-proliferating cells. This work represented the first report on the activities of arginine decarboxylase and ornithine decarboxylase during the growth of *M.smegmatis* and their relationship to polyamine biosynthesis. Both ornithine decarboxylase and arginine decarboxylase exhibit highest activities during the log phase of growth curve, however, the maximal activity of arginine decarboxylase is four time higher than the maximal activity exhibited by ornithine decarboxylase, leading to a situation hitherto unknown in bacteria.

Assay of arginine decarboxylase using both, 1-^{14}C arginine or U-^{14}C arginine exhibited that while decarboxylastion of the 1-carboxy group of arginine would result in the formation of agmatine, (a decarboxylated guanidino compound) the guanidino group of

arginine was further metabolized to labelled CO_2. Hence, for every arginine molecule, two molecules of CO_2 will be formed.

Labelling of ornithine decarboxylase and arginine decarboxylase products showed that the putrescine formed as a result of the above two activities in dialysed crude extracts of *N.smegmatis* corresponded to the activities of the two enzymes measured *in vitro*.

Conclusion

The results indicate that for polyamine biosynthesis the contribution of putrescine from ornithine decarboxylase: arginine decarboxylase is in the ratio, 1:6.

Studies on arginine decarboxylase from *M.smegmatis* TMC 1546

In an attempt to study the enzyme arginine decarboxylase in order to evaluate its role in putrescine biosynthesis in *M.smegmatis*, its purification and study of its properties were undertaken. Arginine decarboxylase was purified by a new, hitherto unpublished procedure resulting in 311 fold purified preparation with a specific activity of 2577 nmoles CO_2/mg protein/hour and a yield of 10.0 per cent. The purified enzyme had a molecular weight of 232,000 and a subunit Mw. of between 58,000 to 59,000. The results indicated the native tetrameric enzyme to be made up of four equivalent subunits. Purified arginine decarboxylase exhibited a pH optimum at pH 8.4, an optimum temperature for decarboxylation at 37^{O} to 40^{O}C and was moderately labile to heat denaturation.

The holo-arginine decarboxylase was completely resolved into its apoenzyme form by dialysis of the former against hydroxylamine. The apoenzyme form showed negligible activity at pH 8.4 in the absence of added pyridoxal-5' -phosphate and regained almost 100 per cent of its activity, in the presence of 0.5 mM pyridoxal-5'-phosphate. However, the activity of the reconstituted preparation at pH 6.2 was observed to be only 30 per cent of that shown at pH 8.4. These results demonstrated a strong correlation with results obtained when the holoenzyme activity was determined as a function of pH and that arginine decarboxylase from *M.smegmatis* was strongly dependent on pyridoxal-5'-phosphate for its activity. Unlike the enzyme from *E.coli* arginine decarboxylase from *M.smegmatis* did not require Mg^{++} for activity at pH 8.4. However, at pH 6.2, Mg^{++} enhance enzyme activity by 23.0 per cent.

The holo-arginine decarboxylase at pH 8.4 showed a characteristic absorption maximum at 415 nm, whereas the apo-arginine decarboxylase showed a characteristic absorption of protein at 280 nm, along with a minor peak at 333 nm, absorption of holo-arginine decarboxylase from *M.smegmatis* with a peak at 415 nm was consistent with the formation of a Schiff-base through an azomethine linkage. Addition of 0.5 mM pyridoxal-5'-phosphate to the apoarginine decarboxylase at pH 8.4 resulted in the appearance of a peak indicating the formation of an azomethine bond vis-a-vis Schiff base. The formation of such an absorption species is concomitant with a 99.0 percent regain of enzyme activity. Further, the reconstitution studies with apo-arginine decarboxylase indicated that at pH 6.2 pyridoxal-5'-phosphate is involved in a different type of Schiff base formation with an absorption at 333 nm. The addition of Mg^{++} apparently creates a more favourable conformation. We then carried out differential spectrometry at pH 8.4 on apo-arginine decarboxylase at pH 8.4. These studies indicated that pyridoxal-5'-phosphate induced

positive co-operativity at optimal pH leading to a conformational change resulting in an increased catalytic activity.

Conclusion

It was evident from our studies that at pH 8.4 tautomeric form I is the preferred Schiff base resulting in maximum catalytic activity. At pH 6.2 the preferred tautomer is form III with absorption maxima at 333 nm which does not promote positive cooperativity induced by pyridoxal 5'-phosphate. The addition of Mg^{2+} at pH 6.2 apparently creates a more favourable conformation. This pH-induced change in the preferred tautomeric form is most likely mediated through a pyridoxal-5'-phosphate-dependent conformational change in the enzyme. Spectrophotometric analyses indicate that the pH-labile active-site polarity may have a role to play in the regulation of enzyme activity.

Role of polyamines in transcription and its implication in gene regulation

RNA polymerase was purified from *M.phlei* to a 467 fold purified preparation. All three polymines i.e. putrescine, spermidine and spermine stimulated the RNA synthesis in a dose-dependent manner. Spermidine and spermine showed a biphasic effect on RNA synthesis. Both inhibition as well as stimulation of transcription could be observed depending upon the concentration of polyamines employed. Thus, in a growing cell where the concentration of polyamines is changing with the growth status, these molecules can have a regulatory effect on transcription of various genes.

To study whether these effects were the result of polyamine interactions with DNA template or with the enzyme, experiments were performed in which the enzyme concentration was kept constant with varying concentration of template at two different (i.e. a suboptimal and an optimal) concentrations of polyamines. The result of this study suggested that this modulation results from a change in the conformation of the DNA as a result of interaction with polyamines.

Stimulation of RNA synthesis by dilution of reaction mixture after attainment of plateau suggested that the product of the reaction might inhibit the RNA synthesis. More pronounced stimulation was obtained by addition of polyamines to the reaction mixture at plateau point. These results were further confirmed by the fact that addition of RNA isolated from *M.phlei* or yeast inhibited RNA synthesis and this inhibitory effect was significantly reversed by polyamines. This suggests that the hybrid formed between nascent RNA and DNA may act as a barrier for movement of the enzyme along the template. Polyamines can destabilize the RNA-DNA hybrid, thus, effecting the smooth movement of the enzyme along the template.

RNA synthesis by polyamines could be influenced either by affecting initiation or elongation of RNA chains. The initiation was studied by following incorporation of [gamma-^{32}P] labelled ATP and elongation was studied in the presence of rifampicin/sarkosyl to block further initiation of RNA chains. This study showed that influence on RNA synthesis by polyamines resulted from their effect on both initiation as well as elongation of RNA chains.

Conclusion

Polyamines influence transcription by facilitating binding of enzyme to template as well as movement of enzyme along the template. These effects result from conformational changes in the template. More significantly, however, it appears that different

concentrations of polyamines can have a variable effect on the transcriptional activity, as also a given concentration of polyamines can exert diverse effect on the transcription of various genes. Thus in the milieu of the cell wherein the concentration of polyamines is changing with the growth status, these molecules can impose a remarkable regulatory effect on the transcriptional activity of the cell.

Discovery of an RNA inhibitor to ornithine decarboxylase

A study was undertaken to demonstrate the presence of an inhibitor to ornithine decarboxylase in *M.smegmatis* as our preliminary studies indicated the presence of such a non-dialysable inhibitor in the crude extracts. The results of these studies carried out to isolate and characterize the inhibitor of ornithine decarboxylase demonstrated that (i) It was a ribonucleic acid, 0.194 kb in size (ii) It was specific for ornithine decarboxylase from *M.smegmatis* and did not inhibit ornithine decarboxylase from *E.coli* and *S.cerevisiae* (iii) The concentration of this inhibitor increases four fold when cells of *M.smegmatis* were grown in medium supplemented with 0.5 mM putrescine and 1.0 mM spermidine (iv) Studies carried out on the mode of interaction of the inhibitor with ornithine decarboxylase showed that inhibition was linear upto 40 per cent, however, a maximum of 70 per cent may be achieved. The inhibition was independent of temperature and time.

Conclusion

Based on these results a unique mode of regulation of ornithine decarboxylase in mycobacteria was apparent wherein its activity is modulated by a specific RNA inhibitor. It seems that transcription of a particular gene in mycobacteria is controlled by the level of polyamines in the cell, the RNA product of which in turn regulates the activity of ornithine decarboxylase. This novel mode of control of ornithine decarboxylase wherein an RNA specifically inhibits mycobacterial ornithine decarboxylase opened an exciting new vista in the regulation of polyamine biosynthesis.

1990-1998

Studies on the pathogenesis of *M.tuberculosis* - identification and characterization of virulence associated genes.

The establishment of infection by a pathogen depends upon its ability to enter, survive and multiply within the host cell. Pathogens usually employ several mechanisms which may act individually or in concert to produce infection and disease. We still seem to be far from knowing anything definite about the nature of genes that are responsible for the pathogenesis of *M. tuberculosis*. Several attractive approaches are being pursued to identify such genes in *M. tuberculosis*.

virS* and *mymA* genes of *M. tuberculosis

Dr. Tyagi's laboratory identified a new gene (*virS*) from *M. tuberculosis* H_{37}Rv, the 38 kDa protein product of which shows homology with virF protein of *Shigella*, virFY protein of *Yersinia* and Cfad, Rns and FapR proteins from various enterotoxigenic *E.coli* (ETEC) strains. All of these proteins act as positive modulator of transcription. VirF and VirFy proteins of Shigella and Yersinia, respectively, regulate the transcription of structural genes required for host invasion and intracellular survival. VirF in addition, also controls the infection of adjacent cells. Likewise Cfad, Rns and FapR, which constitute a family of analogous

regulatory proteins from different enterotoxigenic strains of *E.coli*, regulate transcription of structural genes required for adhesion and colonization of epithelial cells. The protein product of the gene from mycobacteria, like in the cases of its homologs, contains a helix-turn-helix motif in the C-terminal region. This gene was found to be present only in the species belonging to the *Mycobacterium tuberculosis* complex. The sequence and structural homology of VirS with virulence regulating proteins along with its presence exclusively in the organisms of MTB complex strongly suggest its involvement in the establishment of disease.

Another gene designated as *mymA* (for mycobacterial monooxygenase) was divergently arranged to *virS* and codes for a 55 kDa protein that exhibits homology with cyclohexanone monooxygenase from *Acinetobacter* sp. and N,N-dimethylaniline monooxygenase from mammals. PCR and Southern blot analysis of genomic DNAs from several mycobacterial species show that this gene is present exclusively in the members of the *M .tuberculosis* complex. Expression of *mymA* in *M .tuberculosis* was detected by immunoblotting with antibodies against the *mymA* protein. Deletion analysis of the upstream region of *mymA* showed that its expression is subjected to regulation through the possible involvement of trans-acting factor(s) specific to *M .tuberculosis* that are absent in *M. smegmatis. mymA* and the *virS* gene are located divergent to each other.

mymA could be detected both in the avirulent and virulent strains of *M .tuberculosis* by using specific polyclonal antiserum, its expression being dependent on the growth status of cells, and showed a maximum at an A_{600nm} of 3.0 representing the log phase in the growth curve. However, the overall expression was very weak suggesting that *mymA* is not expressed well under the *in vitro* culture conditions. The observed expression could possibly represent the basal level of *mymA* expression which could be induced to optimal level under specific environmental and physiological conditions.

The analysis of the upstream region of *mymA* revealed that *mymA* is under the transcriptional control of both down- and up- regulating elements in *M .tuberculosis* possibly with the involvement of trans- acting factors. *M.smegmatis* which lacks *mymA* coding sequence appears to lack one or more of these trans-acting regulators. None of the constructs with the upstream DNA sequences of *mymA* showed any transcriptional activity in *M.smegmatis*.

Studies on the transcriptional signals of Mycobacteria

E.coli and *Streptomyces lividans* have been used to study expression of mycobacterial genes. The efficiency of these heterologous systems is, however, variable and does not permit the expression of majority of mycobacterial genes. In addition, to understand the genetic responses elicited by mycobacteria during host pathogen interactions it is important to study the regulation of mycobacterial gene expression in homologous systems that would respond faithfully to various physiological constraints imposed by the host environment. Although various excellent vectors have been developed for this purpose, the repertoire of such systems is limited. A major obstacle in the development of such vectors has been the lack of information on mycobacterial transcriptional signals. Moreover, the rate of transcription in mycobacteria has been found to be relatively very low and the initiation of transcription has been found to be specially poor although studies have shown that these differences can not be attributed to inherent low activity of RNA polymerase. The answer presumably lies in the promoter regions of

mycobacteria. Hence, it was proposed that a detailed study of mycobacterial promoters may not only shed light on the divergence of mycobacterial transcriptional machinery from those of other bacteria, it may also provide a basis for the observed differences in the growth rate of various mycobacteria. More significantly, it promised the availability of tools to generate versatile expression systems for mycobacteria.

A promoter selection vector was constructed for mycobacteria to analyze the sequences involved in mycobacterial transcriptional regulation. The vector pSD7 contains extrachromosomal origins of replication from *Escherichia coli* as well as from *Mycobacterium fortuitum* and a kanamycin resistance gene for positive selection in mycobacteria. The promoterless chloramphenicol acetyltransferase (CAT) reporter gene has been used to detect mycobacterial promoter elements in a homologous environment and to quantify their relative strengths. Using pSD7, Dr. Tyagi and colleagues isolated 125 promoter clones from the slow growing pathogen *Mycobacterium tuberculosis* H37Rv and 350 clones from the fast-growing saprophyte *Mycobacterium smegmatis*. The promoters exhibited a wide range of strengths, as indicated by their corresponding CAT reporter activities (5 to 2,500 nmol/min/mg of protein). However, while most of the *M.smegmatis* promoters supported relatively higher CAT activities ranging from 100 to 2,500 nmol/min/mg of protein, a majority of those from *M. tuberculosis* supported CAT activities ranging from 5 to only about 100 nmol/min/mg of protein. These results indicate that stronger promoters occur less frequently in the case of *M.tuberculosis* compared with *M. smegmatis*.

The extent of divergence of mycobacterial promoters has been studied *vis a vis* those of *E.coli*. Of the 100 promoter clones tested from *M.smegmatis* only 12 transformed *E.coli* for chloramphenicol resistance and out of 100 promoter clones tested from *M.tuberculosis* none of the clones transformed *E.coli* for chloramphenicol resistance. The CAT activities of mycobacterial promoters was found to be very low in *E.coli* exhibiting differences of several hundred fold in their activities in mycobacteria and *E.coli*. In order to dissect the specific sequence requirements for transcription initiation in mycobacteria, we have carried out the DNA sequencing and promoter-mapping and *in vitro* studies. Dr. Tyagi's group has shown that the recognition of mycobacterial promoters is similar in the fast growing saprophyte *M.smegmatis* and the slow growing *M.tuberculosis* and *M.bovis* BCG. Analysis of sequences of these promoters shows that promoters of *M.tuberculosis* are more GC rich (56%) than the promoters of *M.smegmatis* (41%). Higher GC content of *M.tuberculosis* promoters may contribute to a relatively lower transcription observed in this species. Alignment of promoter sequences based on the transcriptional start points shows that the -10 regions of mycobacteiral and *E.coli* promoters are highly similar. However, the absence of TTGACA like sequences in the -35 region of most of the mycobacterial promoters seems to be their distinct feature. The degeneracy of sequences in the -35 region of mycobacterial promoters places them close to *Streptomyces* promoters. Comparison of sequences in the -10 and -35 binding regions of MysA, HrdB and RpoD (the principal sigma factors of *M.smegmatis*, *Streptomyces* and *E.coli*, respectively) shows that (i) all three sigma factors have identical -10 binding domain, (ii) the -35 binding domain of MysA is identical to HrdB but is very different compared to the corresponding region of RpoD. Thus mycobacterial transcriptional machinery may be highly similar to Streptomyces but different from that of *E.coli* and the major cause for this difference lies in the -35 region of the promoters and the corresponding binding domain of sigma factor.

Further, a detailed analysis was carried out to identify what other sequences/ features apart from –10 region contribute to the activity of mycobacterial promoters. Since majority of the known housekeeping promoters of mycobacteria are weak and are unlikely to carry consensus / nearly consensus recognition sequences, it required to generate strong promoters, which bind efficiently with the RNAP of mycobacteria, which was obtained by following a strategy, similar to the saturation mutagenesis. However, due to lack of sufficient knowledge about the mycobacterial promoter elements (except the Pribnow Box), Dr. Tyagi and colleagues started with background information about the promoters from other prokaryotic systems. A DNA sequence library harboring ~100 bp long DNA fragments containing random sequences in a stretch of 29 bases was generated, which represented the number of bases acquired between –35 and –10 positions in a typical prokaryotic promoter (number of bases in 2 hexamers separated by a distance of 17 bp = [2x6]+17. Despite using the incomplete library of DNA sequences, it was possible to select a few strong promoter sequences. A_{37} from this library based on its extremely high activity and near-perfect score was chosen for further characterization.

Thorough analysis of A_{37} revealed that its extremely high activity could be subscribed to cumulative effect of several features such as a purine at +1, a conserved –10 sequence along with an extended –10 motif. It was observed that replacing the base at +1 by any of the purine residues resulted in ~2-fold increase in the promoter's activity in mycobacteria. In the DNase I footprinting experiments, hyperactivities of DNase I at –24/–25 positions of A_{37} indicated overexposure of the bases to DNAse I due to the presence of RNAP. This suggested that interaction of RNAP with A_{37} may result in the generation of a favorable conformation of the promoter possibly due to bending at –24/–25 positions for a better binding of holoenzyme to both the –35 and the –10 sequences.

It was further shown that for the optimal activity and recognition of RNAP, a sequence at –35 region, 5'-TTGCGA-3' was preferred by mycobacterial transcriptional machinery. Significant changes in the activities of the promoters, A_{37TG-}, *sigA*, *mmsA* and *gcvH* on the substitution of their respective –35 regions substantiated the importance of –35 region in the activity of a mycobacterial promoter. Further evidence for the role of –35 sequence in promoter function was provided by enhanced binding of the mycobacterial RNAP with A_{37TG-}*con* and *sigAprocon* promoter derivatives containing 5'-TTGCGA-3' sequence at –35 region. However, substitutions of various individual bases at –35 site still resulted in substantial promoter activities, indicating that mycobacterial transcriptional machinery can tolerate variety of sequences at –35 position, as was reported by Dr. Tyagi and colleagues in their previous studies.

Alterations in the distance between –35 and –10 sequences revealed that unlike *E. coli* RNAP (where the optimum distance between –35 and –10 sequences is 17 bp), mycobacterial enzyme requires an 18 bp long spacer sequence for optimal promoter activity. Around 40% of the putative promoter sequences in 5'UTRs, obtained by pattern search analysis, exhibited a distance of 18 bp between putative –35 and –10 sequences, suggesting that a distance of 18 bp between –35 and –10 sequences represents an optimal spacer length for mycobacterial promoters. Further support for this comes from analysis of several known mycobacterial promoters, which revealed the presence of a spacer of 18 bp in most of the strong promoters.

Despite the similarities with *E. coli* promoters, the mycobacterial promoters do not function efficiently in *E. coli*. Recently, in a study, it was shown that the presence of GC rich sequences in the spacer region drastically influences the strength of promoters in *E. coli*. This observation was further substantiated by the fact that majority of strong *E. coli* promoters have an AT content of >75-80%. Analysis of A_{37TG}-*con E. coli* promoter derivative indicated the presence of high GC content (~60%) in the spacer region. Hence, the GC-rich spacer sequence of this promoter (from position –13 to –20) was replaced by a sequence resulting in 75% AT richness in the spacer region. This enhanced AT richness resulted in 15-fold higher activity of this promoter in *E. coli*. It has been observed that the inter-domain distance between regions 2.4 and 4.2 of *E. coli* σ^{70} is much shorter than the distance between –10 and –35 promoter elements. Hence, the AT rich spacer sequence may be better suitable for appropriate binding of this region required by RNAP to establish optimal contacts with –10 and –35 hexameric sequences. Although, σ^{A} from mycobacteria has not been crystallized as yet, possibly, it may have a more appropriate distance between 2.4 and 4.2 regions, thus, making it less dependent on the maneuvering of promoter region affected by bending of the spacer sequence. This may provide an explanation as to why mycobacterial promoters may function with highly GC rich spacer regions but exhibit significantly reduced activity in *E. coli*.

Dr. Tyagi has also analyzed the role of the TGN motif present immediately upstream of the -10 region of mycobacterial promoters. Sequence analysis and site-specific mutagenesis of a *Mycobacterium tuberculosis* promoter and a *Mycobacterium smegmatis* promoter revealed that the TGN motif is an important determinant of transcriptional strength in mycobacteria. It was shown that mutation in the TGN motif can drastically reduce the transcriptional strength of a mycobacterial promoter. The influence of the TGN motif on transcriptional strength is also modulated by the sequences in the -35 region. Comparative assessment of these extended -10 promoters in mycobacteria and *E.coli* suggested that functioning of the TGN motif in promoter of these two species is similar.

Designing and construction of vectors for study of mycobacterial molecular genetics and for expression of genes in mycobacteria

The nodal expression vector

During the past decade considerable progress has been made to develop systems for studying molecular genetics of mycobacteria, yet many limitations in the study of mycobacterial genetics still remain to be overcome. The existing vectors mostly depend on mycobacterial hsp60 and hsp70 gene promoters for expression and this has obstructed the development of versatile expression systems that would permit modulation of gene expression in mycobacteria. Using the mycobacterial promoters of different strength isolated in Dr. Tyagi's laboratory a system has been developed that will permit the expression of genes in mycobacteria at a desired level. A shuttle vector pSD5 has been constructed which can propagate in both mycobacteria and *E.coli*. It carries a modular expression cassette which provides site for cloning of promoters, ribosome binding site with an appropriately placed initiation codon and multiple cloning site for cloning of genes. The expression level of any gene can be altered as desired by the use of mycobacterial promoters of different strength.

Blue-white selection based promoter trap vector

Another derivative of pSD5 contains promoterless ß-galactosidase gene for isolation of transcriptional signal from mycobacteria. The vector provides a rapid selection for mycobacterial promoters in a homologous environment by simple blue white selection. Secondly, the chronological order of appearance and colour intensity of the blue colonies provides an index of the strength of cloned promoter. Furthermore, this selection strategy permits cloning of a wide range of promoters without incorporating any bias towards the promoters of a certain range as can occur in the vectors using drug resistance genes as basis for promoter selection.

Vector for construction of expression libraries in mycobacteria

Another derivative of pSD5 namely pSD5C has been designed to construct mycobacterial genomic libraries and express the cloned inserts as fusion proteins with maltose binding protein in mycobacteria. The expression of fusion proteins is controlled by the *Ptac* promoter thereby allowing regulation of expression with the inducer IPTG in *E.coli* XL1-Blue strain, whereas in mycobacteria the gene is expressed in a constitutive manner. This vector works as an excellent vector system for generating expression libraries of mycobacteria, which can be screened in *E.coli* by a nucleic acid or antibody probe using induction of *Ptac* promoter by IPTG. The clone so selected can be directly subjected to expression studies in mycobacteria wherein its expression can be achieved without any further subcloning step. Such libraries in addition can be useful for genetic complementation of nonpathogenic mycobacterial species with genomic libraries of pathogenic species such as *M. tuberculosis* H_{37}Rv for identifying the genetic determinants responsible for the disease causing ability of the latter. The vector can also be used for expression of heterologous DNA fragments from other pathogenic organisms in mycobacteria.

Integration proficient vector

In one of the pSD5 derivatives the origin of replication of mycobacteria and the gene for kanamycin resistance have been excised out and the integration specific sequences of L5 bacteiophage have been cloned. This vector can stably express a gene under a mycobacterial promoter by integrating site specifically into mycobacterial genome. Such a vector should serve as an excellent tool for stable expression of a mycobacterial or foreign gene in *Mycobacterium bovis* BCG for the purpose of producing recombinant DNA based improved BCG vaccines.

1999 onwards

Use of Recombinant BCG based approach for the development of vaccine against infectious diseases

BCG represents the most extensively used vaccine with a record 3 billion doses administered during the last several decades. While the efficacy of BCG as a vaccine against TB can be a matter of debate, what has been proven beyond doubt is that BCG is an extremely immunogenic, safe and stable vaccine, which is given at the time of birth to elicit long term immunity with a single administration. These factors have made large number of investigators focus their efforts on approaches based on recombinant DNA technology to

modify BCG not only into a recombinant BCG vaccine against tuberculosis but also to employ it as a multipurpose vaccine vehicle against several other microbial infections.

Dr. Tyagi has carried out important ground work and has taken lead by developing an expression system, which with its capacity to modulate gene expression, holds very good promise as a tool for development of BCG into a multipurpose vaccine delivery vehicle.

Six different promising immunodominant antigens of *M. tuberculosis* namely 85A, 85B, 85C, 19 kDa antigen, 38 kDa antigen and ESAT-6 were cloned under different mycobacterial promoters and over expressed in BCG. The evaluation of immune responses elicited by different recombinant BCG strains separately expressing the antigens 85A, 85B, 85C, 19 kDa antigen, 38 kDa antigen and ESAT-6 was carried out. Humoral immune responses and cell-mediated immune responses were measured by ELISA and splenocyte proliferation assays, respectively. The Th1/Th2 bias of the immune responses was measured by isotyping the antibody responses as well as by analyzing the cytokine profiles. The protective efficacy of the recombinant BCG strains expressing the above antigens was evaluated in the guinea pig model of tuberculosis. Immunizations were carried out by intradermal injections with $1x10^6$ cfu of BCG or rBCG. The protective efficacy of the rBCG strains was evaluated at various doses of subcutaneous challenge with *M. tuberculosis* viz. $3.5x10^2$ cfu, $5x10^4$ cfu and $7.5x10^5$ cfu. The animals were euthanized 3 and 8 weeks post-challenge and post-mortem virulence scores were assigned. Bacterial load in spleen was determined and histopathological analysis of liver and lung tissue was performed to determine the percentage of granuloma in the organs and cellular composition of the granuloma.

Immunization with wild type BCG (WtBCG) elicited a Th1-Th2 or Th2 type of T cell response against purified mycobacterial antigens (antigens of the 85 complex, 19 kDa antigen and 38 kDa antigen) as well as against BCG sonicate. In general, the recombinant BCG constructs elicited immune responses of higher magnitude as compared to the wild type BCG and the response was markedly shifted towards either Th1 or Th2 phenotype. Overexpression of the antigens 85A, 85B and 85C and the 38 kDa antigen resulted in a predominantly Th1 response characterized by increased titres of antibodies of IgG2a isotype and preferentially increased secretion of IFN-γ against individual purified proteins as well as BCG sonicate. Overexpression of ESAT-6 in BCG resulted in a mixed Th1-Th2 or Th2 type of T cell response against the purified antigen as well as BCG sonicate as observed in the case of immunization with WtBCG although the magnitude of these responses was significantly higher. In contrast, overexpression of the 19 kDa antigen in BCG induced a very predominant, Th2 type immune responses against BCG sonicate although the response against the purified 19 kDa antigen was predominantly Th1 type. It was observed that modulation of the immune responses was dependent on the level of expression of the antigen with highest level of expression usually inducing maximal immuno-modulation.

In case of each antigen, the recombinant BCG strain expressing the antigen at the highest level was evaluated for its protective efficacy in guinea pigs. The BCG vaccination was quite effective in reducing the bacillary load in the spleen of the animals. Some recombinant BCG strains reduced the bacillary load more efficiently than BCG, others did not show any significant improvement over BCG. The immunization with rBCG-19 overexpressing the 19kDa antigen did not provide any protection. In fact, it abrogated even the protective efficacy of BCG completely. In spite of statistical variations within a particular

group, it was observed that recombinant BCG strains overexpressing either ESAT-6 or antigen 85C conferred better protection to animals as compared to the protection imparted by BCG. The immunization with rBCG strains overexpressing either antigen 85A or 85B did not show very clear results although overexpression of 85B seemed to provide slightly better protection than BCG.

Development of candidate DNA vaccines against tuberculosis and their evaluation in mice and guinea pigs

DNA inoculation represents a novel approach to vaccine and immune therapeutic development. The direct introduction of gene expression cassettes into a living host transforms a number of cells into factories for production of the introduced gene products. Expression of these delivered genes has important immunological consequences and results in a specific immune activation of the host against the novel expressed antigens. The recent demonstration by several laboratories that these immune responses are protective in infectious disease experimental models as well as cancers is viewed with optimism. Further, the relatively short development times, ease of large-scale production, low development, manufacturing and distribution costs all combine with immunological effectiveness to suggest that this technology will dramatically influence the production of a new generation of experimental vaccines and immune therapies.

Development and evaluation of candidate DNA vaccines for protection against tuberculosis

Expression of the antigens:

The genes encoding the three selected mycobacterial antigens namely ESAT-6, α-crystallin and Superoxide dismutase were cloned in the eukaryotic expression vectors indigenously developed in Dr. Tyagi's laboratory and expression was analysed in the COS-1 cell line. All three antigens were expressed in the mammalian cells.

Evaluation of protective efficacy of candidate DNA vaccines in guinea pigs:

Immune responses elicited by these candidate DNA vaccines were evaluated by immunization of mice with plasmid DNA and measuring humoral immune responses as well as cellular immune responses.

For the evaluation of the protective efficacy of the candidate DNA vaccines, guinea pigs were immunized with the vaccine constructs and later challenged with *M. tuberculosis*. The protective efficacy was evaluated by measuring the bacillary load in lung and spleen homogenates and histopathological analysis of liver and lung tissues.

The DNA vaccine expressing the gene for ESAT-6 was effective in decreasing the bacterial CFU in spleen and lung by about 1.0 log and 0.5 log, respectively as compared to sham immunized animals. The results of histopathology also revealed a reduction in the percentage of granuloma in liver and lung.

Immunization of mice with alpha-crystallin DNA vaccines resulted in a reduction in the spleen CFU by about 1.0 log. However, this plasmid DNA immunization was not effective at reducing the lung CFU. The histopathological analysis suggested a decrease in granuloma in liver as well as lung.

The plasmid DNA encoding the Superoxide dismutase was found to be the most effective one in decreasing the CFU in lung as well as in spleen. The mice immunized with

this plasmid DNA exhibited a 1.6 log reduction in the spleen CFU and a 1.0 log reduction in the lung CFU. The histopathological analysis also revealed that immunization with this vaccine resulted in maximum reduction in the lung granuloma when compared to the other two vaccine constructs.

Heterologous prime boost approach with aerosol challenge model

It may be stated here that the aerosol route of infection, which is usually employed for infection of guinea pigs, leads to extensive colonization of the bacilli in the lung and further spread of this infection in lung as well as to the other organs such as spleen. The subcutaneous route of infection employed in these studies (it is supposedly the second best route for infection after the aerosol route) on the other hand, leads to a different pattern of initial bacillary distribution among different organs (~90% to liver, ~10% to spleen and only 1-2% to lungs) within 24 hours. Secondly, BCG is known to protect animals more efficiently against hematogenous spread of the tubercle bacilli from the lungs of an infected animal (Dissemination TB). The infection by subcutaneous route does not draw much analogy with dissemination TB for which BCG supposedly acts as a relatively more potent vaccine. Thus, subcutaneous route of challenge does not result in a significant load of bacilli in lungs unless very high dose of *M. tuberculosis* is used for infection as seen in these studies. This is in sharp contrast to the proceedings in the case of aerosol challenge, wherein just a few bacilli can result in extensive colonization of bacilli in lungs. Thus, in spite of use of guinea pigs as a challenge model, the subcutaneous route of infection does not mimic the infection and its progression in a manner similar to humans.

Based on these observations, it was proposed that the protective efficacy of a candidate vaccine in guinea pigs should be evaluated i) by using aerosol route of challenge and ii) by employing an appropriate dose of *M. tuberculosis* for infection in order to determine the exact merit of the candidate vaccine in question.

Recombinant BCG overexpressing antigen 85C

This regimen demonstrated a significant enhancement in the protective efficacy of BCG by over expression of Ag85C- an immuno-dominant antigen of *M. tuberculosis.* The parameters used for the evaluation of protective efficacy following an aerosol challenge with *M. tuberculosis* were, (i) bacillary load in lung and spleen and (ii) pathological changes in lung, liver and spleen. At 10 weeks post-infection, vaccination with rBCG85C resulted in a significantly reduced bacillary load in the lungs (~87 folds) along with a marked reduction in hematogenous spread to the spleen (~360 folds) in comparison to vaccination with the parental BCG strain. This reduced bacillary load was also accompanied by a marked reduction in the pulmonary, splenic and hepatic pathology. On extending the interval between vaccination and challenge (to 12 weeks) and between challenge and euthanasia (to 16 weeks), rBCG85C continued to impart a relatively superior protection with a remarkably greater control on bacillary multiplication in the lungs (~9 folds) and a successful restriction of the hematogenous spread of tubercle bacilli to spleen (~100 folds) in comparison to immunization with the parent BCG strain.

In the absence of vaccination, the clinical manifestation of progressive end-stage TB in guinea pigs is known to be associated with a strong inflammatory response to the persistent antigens or bacilli leading to extensive necrosis and progressive fibrosis. However, an efficient vaccine is expected to prime the immune system to generate an efficiently regulated and targeted response for an effective microbial and antigenic clearance,

minimizing the collateral damage to the host. Immuno-localization of Ag85 complex proteins – some of the most abundant proteins of *M. tuberculosis*, as a marker of the mycobacterial antigen load, showed elevated levels of these antigens in the granulomas as observed in case of saline treated animals. This increased antigen load was found to be associated with the production of superfluous amount of TNF-α, unwarranted inflammation, tissue destruction and excessive collagen deposition. However, in addition to the bacillary clearance, rBCG85C mediated immune responses resulted in reduced antigen load indicating an effective removal of mycobacterial antigens and/or the bacillary remnants. A corresponding reduction in the extent of granulomatous inflammation and fibrosis in this group further substantiated the fact that an effective removal of the residual antigenic depots from the sites of infection is essential for the resolution of granulomatous lesions. More over, reduction in the levels of IFN-γ and TNF-α, towards the later stage of disease in case of the rBCG85C-immunized animals further signifies the fact that, although, induction of these cytokines following *M. tuberculosis* infection is known to be essential for the initial containment of the bacilli, a subsequent reduction in the levels of these cytokines is crucial for the resolution of granulomatous lesions, as observed in this study.

BCG as priming agent followed by boosting with a DNA vaccine expressing α–crystallin

In view of the enormous number of individuals vaccinated with BCG, it becomes imperative to develop efficient booster vaccines in order to enhance the BCG induced immunity and sustain protection even in the old age. Besides, due to lack of adequate immune response to latency-associated antigens, BCG is often unable to provide sterilizing immunity against primary *M. tb* infection leading to occurrence of latent TB. Thus, in this study an attempt was made to enhance the protective immunity of BCG by heterologous boosting with a DNA vaccine-expressing α-crystallin – one of the most prominent antigens recognized during latency. The demonstration of a significantly reduced bacillary load in lung (~ 37 fold) and spleen (~ 96 fold) at 10 weeks post-infection by the 'BCG prime DNAacr boost' regimen, provides substantial evidence for its superiority over BCG. More over, a rigid control on bacillary multiplication (~100 fold and ~47 fold reduced bacillary load in lung and spleen, respectively) along with a significant reduction in pathological damage up to an extended period of 16 weeks post-infection suggests a robust and sustained enhancement in the protective efficacy of B/D regimen in comparison to classical BCG vaccination.

On histological analysis, unvaccinated animals showed extensive multi-focal coalescing granulomas with prominent central coagulative necrosis occupying more than 60% of the lung sections at 10 weeks post-infection. BCG immunization significantly reduced granulomatous infiltration in the lungs characterized by the presence of well-organized granulomas covering ~35% of the lung sections. However, animals vaccinated with B/D regimen showed well-preserved alveolar spaces with only a few scattered areas of diffused infiltration in peribronchial and perivascular areas (~5%). Corresponding to the aggravated pulmonary pathology, unvaccinated animals showed widespread infiltration with scattered areas of necrosis occupying more than 40% of the liver sections. However, all the BCG based regimens irrespective of the boosting agent, remarkably reduced the hepatic inflammation with a very few or no influx of inflammatory cells. At 16 weeks post-infection, both BCG vaccinated as well as unvaccinated animals showed a considerable increase in the pulmonary pathology. However, a booster dose of DNA vaccine significantly reduced the granulomatous inflammation in lung, when compared to a solitary immunization with BCG as well as B/V regimen. Moreover, B/D regimen conferred complete protection in liver with

no evident sign of infiltration in comparison to the animals belonging to both BCG and B/V regimens, which showed scattered areas of granulomatous inflammation in liver.

Commensurate with the negligible granulomatous inflammation, B/D group showed no evident signs of collagen staining in the lungs other than the usual occurrence of collagen in the peri-bronchial and peri-vascular areas at both the time points. In contrast, widespread fibrosis was observed in and around the pulmonary granulomas in the unvaccinated animals causing loss of alveolar and micro-vasculature structure. BCG immunized animals, in comparison to significantly reduced collagen deposition at 10 weeks showed a relatively increased collagen staining at 16 weeks. Examination of relationship between the extent of collagen deposition, bacillary load and granulomatous inflammation revealed a strong positive correlation among these parameters.

Although, the importance of heterologous prime boost immunization in the context of TB has been reported by several investigators, in this study, for the first time a latency-associated antigen (α-crystallin) was successfully employed as a booster DNA vaccine subsequent to BCG. The superior protection imparted by 'BCG prime and DNAacr boost' heterologous prime boost regimen provides several advantages, when viewed in clinical context. BCG, according to WHO guide lines, is given only once after the birth. However, the immunomodulatory effect of boosting the BCG induced immunity by employing an effective booster vaccine remains unaltered irrespective of the time span between the primary BCG vaccination and boosting. Thus, a booster dose of DNAacr to BCG immunized and unexposed individuals at any time can be expected to enhance immunity against perceived *M. tuberculosis* infection. Moreover, since, BCG protects against childhood TB, replacing it with a vaccine regimen that does not include BCG would be neither ethical nor practical, thus, employing DNAacr as a booster vaccine would simplify the matters related to the clinical testing of this regimen without hampering the child hood immunization program. In addition, the α-crystallin based memory immunity elicited by this regimen would help circumvent the occurrence of latent and reactivation TB due to enhanced recognition and clearance of the latent bacilli. However, a separate study to evaluate the effect 'DNAacr boost' on the reactivation of latent TB in a suitable animal model would be necessary to further strengthen this particular hypothesis.

Recombinant BCG overexpressing α-crystallin as the priming agent followed by boosting with a DNA vaccine expressing the same antigen

Over expression of α-crystallin in BCG imparted a significantly improved protection against *M. tuberculosis* infection, when compared to the parental BCG vaccination. However, a booster dose of this latency antigen in the form of a DNA vaccine subsequent to rBCG priming (R/D), resulted in a far superior protection. Even up to an extended period of 16 weeks post-infection, the R/D regimen was able to exhibit a rigid control on bacillary multiplication as was evident from 750 fold and 65 fold fewer bacilli in the lungs and spleen of animals immunized with R/D regimen, when compared to BCG vaccinated animals. Histopathological analysis of animals vaccinated with R/D regimen also exhibited a commensurate lesser granulomatous inflammation and associated pathological damage.

Vaccination induced alterations in the cytokine milieu dictate the variations in the disease trajectories. Measurement of immune responses at the later stages of disease in this study and their correlation with disease progression, provided an understanding about how the dynamic changes in the cytokine milieu of the lungs influence the fate of an infection.

The increased levels of inflammatory cytokines such as IFN-γ and TNF-α along with reduced levels of immuno-suppressive cytokines like TGF-β and IL-10 corresponded well with the increased disease severity as observed in the case of unvaccinated animals. Both the heterologous prime boost regimens (R/D and D/R) elicited apparently similar immune responses marked by enhanced but comparable levels of inflammatory as well as immunosuppressive cytokines, however, the protection imparted by these regimens varied – while the R/D regimen provided sustained protection till 16 weeks post-infection, protection afforded by D/R regimen declined considerably after 10 weeks. This suggested that merely the measurement of levels of cytokines may not provide appropriate correlations with disease severity and/ or level of protection, which led us to analyze the cytokine milieu based on the relative proportions of various cytokines in addition to their individual levels. As can be seen from Fig. 4B, the analysis based on the relative proportions of cytokines, guided us to draw better correlations between the distribution of cytokines and their consequential influence on protection. While, the R/D regimen with a superior protection showed a considerably increased relative proportion of IL-12 along with proportionate decline in IL-10 with time, the D/R regimen, in contrast, showed an exactly opposite trend resulting in a decline in protection after 10 weeks post-infection. Moreover, rBCGacr-immunized animals, which showed enhanced protection in the lungs at 16 weeks, also exhibited increased proportion of IL-12 along with a concomitantly reduced proportion of IL-10 as observed in case of R/D regimen. These observations from various vaccinated groups suggest that the increase in the proportion of IL-12 and decrease in the proportion of IL-10 at 16 weeks in comparison to their relative proportions observed at 10 weeks time point may be critical for the observed protection against the disease and a concomitantly reduced pathology.

Also, this study further demonstrated a close association of *M. tuberculosis* antigen load and extent of collagen deposition with the bacillary load and granulomatous inflammation observed in lung, suggesting that an efficient vaccine regimen in addition to providing protection against the initial infection should also prevent development of pathological lesions allowing the restoration of normal lung architecture.

An important corollary of these results pertains to their clinical relevance. The superior protection imparted by α-crystallin based 'BCG prime - DNA boost' and 'rBCG prime - DNA boost' regimens provides multiple advantages and possibilities in terms of their clinical relevance as stated below:

BCG, according to WHO guide lines, is given only once after the birth. However, it has been recently reported that the immunomodulatory effect of an efficient booster vaccine remains unaltered irrespective of the time span between the primary BCG vaccination and boosting. It has been observed that there was no significant difference in the magnitude of immune responses generated, when the booster is administered shortly after, or many years after BCG vaccination. In light of this, a booster dose of DNAacr to the BCG immunized individuals as described in this dissertation under "Boosting BCG" strategy, at any time, can be expected to enhance protective immunity against a perceived M. tb infection. Hence, this regimen could provide an effective strategy to boost the immunity of BCG immunized individuals.

The 'rBCG prime - DNA boost' regimen, on the other hand, can be effectively useful for the child hood immunization program. Firstly, in this regimen, the use of rBCG in place of BCG in the newborn children will not only preserve the valuable attributes of BCG, but will also result in an efficient immune response and superior protection against pulmonary TB. Secondly, a booster dose of DNA vaccine would further enhance and sustain the rBCG-induced immunity.

Since, production of α-crystallin is up regulated by M. tb during its transition from actively dividing to latent phase, prevalence of α-crystallin specific memory immunity in case of both BCG/DNAacr and rBCGacr/DNAacr regimens will aid in the enhanced recognition and clearance of latent bacilli. Hence, vaccination with these regimens is likely to reduce the incidence of latent and reactivation TB.

Conclusions

In all TB vaccine related studies, BCG has been used as the gold standard to pronounce the worthiness of a new vaccine candidate, because it is the failure of BCG in the adult human population that has necessitated the development of a new TB vaccine in the first place. However, this convention suffers from a caveat – a new vaccine is required for protection in humans, wherein, BCG does not work well; on the other hand, a new vaccine cannot progress to human trials without proving its superiority to BCG in animal models in which BCG works rather efficiently. Hence, it has been difficult to develop vaccines, which would ensure a superior protection over BCG in animal models. It is thus not surprising that in spite of a large number of vaccine related studies, merely 9 vaccine regimens have progressed to various stages of human clinical trials. These vaccines have shown a better or equal performance in comparison to BCG in their ability (i) to reduce the bacillary load in lung and spleen and/or (ii) to reduce pathological damage and/or (iii) to perform better in time to death assay. The 16 weeks assay carried out in this study to evaluate protective efficacy in a highly relevant guinea pig model of TB showed that on the basis of their comparison with all the vaccines that have already progressed to clinical trials, these three regimens imparted a remarkable protection. These vaccine regimens have been approved for human clinical trials by the Tuberculosis Vaccine Clinical Trial Expert Group (TVCTEG) of the Department of Biotechnology, Government of India. Currently, some upstream pre-clinical work on these candidate vaccines is in progress so that the human clinical trials can be initiated.

Study of *M. tuberculosis* genes involved in the establishment and progression of tuberculosis - identification of new targets for the development of anti-tubercular drugs

Dr. Tyagi's group has been working on genes involved in the establishment and progression of tuberculosis to understand the mechanism of pathogenesis and identification of new targets for the development of novel anti-tubercular drugs.

MymA operon

Dr. Tyagi and colleagues have identified and characterized the *mymA* operon (*Rv3083-Rv3089*) of *M. tuberculosis*, which is arranged in a divergent manner to *virS* (*Rv3082c*) which was identified by Dr. Tyagi's labroatory earlier. The investigations by his group showed that the transcription of the *mymA* operon is dependent on the presence of VirS protein. To identify the environmental cues that might trigger an up-regulation of the *mymA* operon, its expression under various *in vitro* conditions that simulate those faced by

M. tuberculosis in the host environment was studied. It was observed that VirS is essential for transcription from the *mymA* operon promoter. However, a 4-5 fold induction of the promoter of the *mymA* operon by VirS occurs specifically at acidic pH. This may be due to increased synthesis of VirS at acidic pH. Alternatively, the acidic pH might change the phosphorylation state of VirS, which could improve its affinity for the promoter region of the *mymA* operon. The primary sequence analysis of VirS shows the presence of 9 putative protein kinase C phosphorylation motifs, [ST]-x-[RK]. However, induction of *mymA* operon at acidic pH and on infection of macrophages with *M. tuberculosis* underscores the importance of the encoded gene products, in processes that are important during the mycobacterial residence in the host environment.

An extensive analysis of the conserved domains and the core motifs present in the gene products encoded by *mymA* operon suggested that mycobacteria might use it for modification, activation and transfer of fatty acids to the appropriate acceptor(s) in their cell wall. *mymA*, a monooxygenase encoded by *Rv3083* could potentially oxygenate mycobacterial fatty acids. The oxygenated fatty acids could be further modified by the acetyl hydrolase/esterase (*Rv3084*), short chain alcohol dehydrogenase (*Rv3085*) and zinc containing alcohol dehydrogenase (*Rv3086*). Finally, the acyl CoA synthase homologue (*Rv3089*) could then activate the fatty acids (modified by the products of genes *Rv3083-Rv3086*), which could subsequently be transferred to an acceptor in the cell wall of mycobacteria by acyl transferases (*Rv3087* and *Rv3088*).

It is known that under acidic conditions there is a two-fold reduction in the expression of genes present in the FAS II operon. FAS II operon that are responsible for the biosynthesis of meromycolic acids in *M. tuberculosis* by elongating long chain fatty acid precursors like C24 and C26 generated by the FAS I system. Down-regulation of the FAS II system at low pH would be expected to decrease fatty acid elongation, leading to an accumulation of C24 and C26 fatty acids. However, since the *mymA* operon is up-regulated at acidic pH, it can utilize the C24 or C26 fatty acids and as suggested above, modify and transfer them to appropriate biological acceptor(s) on the mycobacterial cell wall. Thus, induction of the *mymA* operon can play an important role in remodeling the envelope of intracellular *M. tuberculosis* under acidic conditions in the macrophages.

Dr. Tyagi and colleagues showed that Mtb∆*virS* and Mtb*mym:hyg* have an altered cell wall structure. Both strains exhibited a much denser and darker staining of cell surface, indicating an alteration in the electron transparent zone (ETZ), which is thought to be composed primarily of mycolic acids arranged perpendicular to the plane of cell surface. Such dense staining of the cell wall has also been observed after treatment of *M. avium* with isoniazid resulting from the inhibition of mycolic acids synthesis by the drug. The alterations in the cell surface of Mtb∆*virS* and Mtb*mym:hyg* strains were further substantiated by the HPLC profiles of mycolic acids from the mutants and the parental strains. Furthermore, both mutants produced less mycolic acids in comparison to the parental strain as analyzed by TLC. These findings suggest that the observed alterations in the cell wall ultrastructure result from the altered mycolic acid composition although the effect of latter on the arrangement of other cell surface lipids and proteins and their consequent contribution on the observed phenotype cannot be completely ruled out. On exposure to acidic pH, the reduction in mycolic acids synthesis was markedly more prominent in the Mtb∆*virS* and Mtb*mym:hyg* strains in comparison to the parental strain. The accumulation of fatty acids (C24:0/C26:0) at acidic pH was also observed to be higher in the mutants as compared to the parental strain.

Although, a general reduction in the synthesis of mycolic acids at acidic pH can be expected to stem from the repression of Fas II operon, a much sharper decline in mycolic acid synthesis in case of both the mutant strains implicates *mymA* operon in the synthesis of mycolic acids on exposure of the pathogen to acidic pH. The emergence of new mass peaks corresponding to C88-C92 chain length of mycolic acids (1328, 1356 and 1384) in the parental strain, but not in the mutants clearly suggested the role of *mymA* operon in the synthesis of these mycolic acids at acidic pH. Further, the enhanced accumulation of C24:0/C26:0 fatty acids in the mutant strains substantiates their role in the synthesis of mycolic acids by *mymA* operon. Conventionally mycolic acids are believed to be synthesized by elongating long chain fatty acids (C16-C26) to meromycolic acids by Fas II operon of *M. tuberculosis* and the final Claisen type condensation of C24:0/C26:0 fatty acid with meromycolates results in the production of full length mycolic acids. However, an alternate approach of mycolic acid synthesis by "head–to- tail" condensation of long chain fatty acids has also been suggested. The synthesis of mycolic acids by this approach involves the condensation of three common fatty acids. First, two of these are subjected to the omega-oxidation followed by condensation to produce meromycolic acids which in turn condenses with C24:0/C26:0 fatty acids to produce mycolic acids. This approach of mycolic acid synthesis requires enzymes that can carry out omega oxidation of fatty acids and their subsequent condensation. Interestingly, analysis of gene products of *mymA* operon revealed that Rv3083 (*mymA*) is a homologue of flavin containing monooxygenases, which can carry out omega-hydroxylation of fatty acids - the first step in omega oxidation of fatty acids, while Rv3085 and Rv3086 show homologies with dehydrogenases and could possibly carry out subsequent steps to convert terminal methyl groups of fatty acids to carboxylic groups for condensation as described. Release of acly carrier protein (ACP) esterified to the fatty acids by thioesetrase, LipR (Rv3084) leads to generation of diaacids for the condensation. Rv3087 and Rv3088 contain HHxxxDG motif required for the thioesterification or Claisen type condensation of fatty acids, the last gene Rv3089 is an acyl-CoA synthase and can activate the fatty acids. Thus, Rv3087 and Rv3088 can carry out "head to tail" condensation of fatty acids which were previously omega oxidized by Rv3083-Rv3086 gene products and further activation of the condensed fatty acids by Rv3089 can yield long chain fatty acids (keto acids). These keto acids can then be subjected to functional group modification like methylation, decarboxylation, cyclopropanation to generate meromycolic acids. The condensation process described above can produce long chain fatty acids that are indistinguishable from mycolic acids. Thus, the genes present in *mymA* operon can assemble meromycolic acids beginning from the omega oxidation of fatty acids followed by their condensation with fatty acids (C24:0/C26:0) to produce mycolic acids.

Both the mutants showed increased sensitivity to major antitubercular drugs along with enhanced susceptibility to SDS and acidic pH. Enhanced susceptibility of *M. tuberculosis* to antibiotics, detergents and environmental stresses has been shown to be associated with the alterations in the mycolic acid contents and composition.

The induction of *mymA* operon at acidic pH and a significantly reduced ability of Mtb□*virS* and Mtb*mym:hyg* to survive in the activated macrophages as compared to the parental strain supports the hypothesis that *mymA* operon may play an important role in the survival of *M. tuberculosis* upon exposure to severely acidic conditions in activated macrophages or caseating granuloma in the later stages of infection. This was substantiated by a drastic reduction (~2.8log) observed in the ability of the mutant strains to specifically

survive in spleen as compared to the parental strain at 20 weeks post infection. The genes present in the *mymA* operon apparently are involved in remodeling the cell wall integrity required for the persistence of *M. tuberculosis* in the host.

Conclusion

The involvement of *mymA* operon in the persistence of *M. tuberculosis* together with its role in maintaining appropriate mycolic acid composition to resist antitubercular drugs at acidic pH indicate that precise targeting of *mymA* operon gene products may increase effectiveness of combination chemotherapy and impede the mechanisms involved in the persistence of *M. tuberculosis*.

Characterization of Fad13 and identification of important residues

Mycobacterium tuberculosis (*M.tb*), an intracellular pathogen, is exquisitely adapted for human parasitization. It has evolved a number of distinct strategies to survive in the hostile environment of macrophages. The drugs for the treatment of tuberculosis (TB) are available but the long and demanding regimens lead to erratic and incomplete treatment often resulting in the development of drug resistance. Hence, the importance of identification and characterization of new drug targets cannot be overemphasized.

It has been earlier demonstrated that exposure to acidic pH results in upregulation of the *mymA* operon of *M.tb* (Rv3083 - Rv3089). The functional loss of the *mymA* operon leads to alterations in the colony morphology, cell wall structure, mycolic acid composition and drug sensitivity and results in markedly reduced intracellular survival of *M.tb* in macrophages. Besides, the *mymA* mutant of *M.tb* shows a drastic reduction (800 fold) in its ability to survive in the spleen of guinea pigs as compared to the parental strain. To gain further insight into the functioning of *mymA* operon, a potential target for developing antitubercular drugs, it was necessary to characterize its gene products. *fadD13*, the last gene of the *mymA* operon, encodes a Fatty Acyl-CoA Synthetase.

Eight site-directed mutants of FadD13 were designed and constructed by Dr. Tyagi and colleagues and analyzed for the structural-functional integrity of the enzyme. The study revealed that mutation of Lys487 resulted in 95% loss of the activity thus demonstrating its crucial requirement for the enzymatic activity. Comparison of the kinetic parameters showed the residues Lys172 and Ala302 to be involved in the binding of ATP and Ser404 in the binding of CoenzymeA. The influence of mutations of the residues Val209 and Trp377 emphasized their importance in maintaining the structural integrity of FadD13. Besides, these studies showed a synergistic influence of fatty acid and ATP binding on the conformation and rigidity of FadD13. FadD13 represents the first Fatty Acyl-CoA Synthetase to display biphasic kinetics for fatty acids. FadD13 exhibits a distinct preference for C26/C24 fatty acids, which in the light of earlier reported observations further substantiates the role of the *mymA* operon in remodeling the cell envelope of intracellular *M.tb* under acidic conditions.

Conclusions

Thus, these studies by Dr. Tyagi and colleagues provided a significant understanding of the FadD13 protein including the identification of residues important for its activity as

well as in the maintenance of structural integrity. The findings of this study will provide valuable inputs in the development of inhibitors against the *mymA* operon, an important target for the development of antitubercular drugs.

Tyrosine phosphatases of *M. tuberculosis* and their role in the survival of *M. tuberculosis* in the host tissue

Protein phosphorylation and dephosphorylation play a significant role in transducing signals involved in cellular processes such as adhesion, internalization and killing of pathogens. The analysis of the genome of *M. tuberculosis* revealed the presence of two genes for tyrosine phosphatases designated as MptpA and MptpB.

To investigate the role of MptpB in the pathogenesis of *M. tuberculosis,* Dr. Tyagi's group constructed a mutant strain of *M. tuberculosis* lacking the activity of MptpB. The gene encoding MptpB was inactivated in *M. tuberculosis* genome by homologous recombination using a non-replicative suicidal vector, pBKΔB. Southern blot and immunoblot analysis confirmed the verity of the mutant strain. Disruption of *mptpB* had no significant effect on the morphology and growth of *M. tuberculosis* in defined liquid culture medium suggesting that MptpB is not required for the growth of *M. tuberculosis* under *in vitro* conditions. Similar results were also observed when macrophage cell line was infected with the mutant and wild type strains. Both the strains were comparable in their ability to infect and survive in the mouse macrophage cell line J774A.1. To evaluate the role of MptpB in pathogenesis of *M. tuberculosis,* the survival of mutant strain in the guinea pig model of tuberculosis was studied. In this model of infection, a significant reduction was observed in the ability of the mutant strain to survive in the host organs. An approximately 70-fold (1.7 log) reduction in bacillary load was observed in the spleen of the animals infected with mutant strain as compared to the bacillary load from the animals infected with wild type strain at 6 weeks post-infection. This difference in the splenic bacillary load in both the groups of animals was not observed at the earlier time point of sacrifice (3 week post-infection). These observations suggest that initially both the strains (mutant and wild type) of *M. tuberculosis* are capable of establishing the infection to a similar extent. However, the ability of the strains to withstand the assault by the host was significantly different. The host was able to clear the mutant strain more efficiently than the parental strain. The influence of disruption of *mptpB* gene on survival of *M. tuberculosis* specifically in guinea pigs but not in macrophages suggests that although experiments involving infection of a macrophage cell line by *M. tuberculosis* have yielded useful information about several aspects related to the survival of pathogen in the host, a macrophage cell line may not represent the exact context encountered by mycobacteria in the host.

In order to demonstrate that the loss of virulence of *M. tuberculosis* was a direct consequence of disruption of *mptpB,* the gene was reintroduced in the mutant strain and the complemented strain was evaluated for its ability to survive in the guinea pigs. The complemented strain could establish an infection and survive in the host tissues even at the 6-week time point at levels comparable to those observed in the case of wild type *M. tuberculosis*. These observations clearly suggest that MptpB plays an essential role in the survival of *M. tuberculosis* in host.

Dr. Tyagi's group also investigated the role of *mptpA* operon in the virulence of *M. tuberculosis* by constructing a mutant strain of *M. tuberculosis* inactivated in *mptpA* locus.

Disruption of *mptpA* in the *M. tuberculosis* genome was confirmed by Southern blot and immunoblot analysis. Similar growth characteristics in MB 7H9 media and colony morphology on MB 7H10 plates suggested that MptpA is not required for *in vitro* growth of *M. tuberculosis*. Next, the ability of *mptpA* mutant and parental strain to survive in IFN-□ activated macrophages was compared. At 2 days post-infection, an approximately 2-fold reduction in the survival of intracellular *mptpA* mutant (30% survival) was observed in comparison to the intracellular parental strain (55% survival). However, this difference in survival increased to approximately 10-folds and 14-folds at 4 and 6 days post-infection, respectively. At six days post-infection, the intracellular *mptpA* mutant showed 2% survival in comparison to the internalized parental strain that showed 28.4% survival suggesting that the *mptpA* mutant strain was impaired in its ability to survive in the activated macrophages.

Disruption of *mptpA* also impaired the ability of *M. tuberculosis* to survive in lungs and spleens of infected guinea pigs. An approximately 8-fold difference was observed in the bacillary load in spleens and lungs of guinea pigs infected with the *mptpA* mutant strain in comparison to the bacillary load in the spleens and lungs of guinea pigs infected with the parental strain of *M. tuberculosis* at 3 weeks post-infection. At 6 weeks post-infection, this difference in the bacillary load increased from 8-fold to 80 folds in case of spleen and 90 folds in case of lungs in comparison to bacillary load in spleens and lungs of animals infected with the parental strain.

Upon histopathological analysis of lung at 3 weeks post-infection, it was observed that tissue damage was comparable among the animals infected with the parental or *mptpA* mutant or *mptpA* complemented strain of *M. tuberculosis*, with similar extent of granulomatous tissue present in all cases. However, at six weeks post-infection, a significantly reduced pathological damage was observed in the lungs of animals infected with the *mptpA* mutant strain in comparison to the parental strain. This reduction in the extent of tissue damage in animals infected with the *mptpA* mutant strain suggested a healing response of the host, which was commensurate with impaired survival and reduced number of *mptpA* mutant strain in the lungs.

An 80 and 90 folds reduced bacillary load in spleens and lungs, respectively, along with markedly reduced pathological damage in lungs of animals infected with the *mptpA* mutant strain as compared to infection with the parental strain clearly implies an essential role of *mptpA* operon in the virulence of *M. tuberculosis.*

Conclusion

Thus, both MptpA and MptpB are important genes that are required for the survival of pathogen in the host tissue. Hence, both these phosphatases represent attractive targets for the development of new anti-tubercular drugs.

Iron storage proteins and their importance in the survival and pathogenesis of *Mycobacterium tuberculosis*

Iron is an essential nutrient for almost all microbes, including pathogens such as *Mycobacterium tuberculosis*. It is an indispensable cofactor for proteins involved in critical cellular processes, such as electron transfer, oxygen transport, DNA synthesis, etc. Although iron is essential, excess free iron is potentially toxic for the cells because it catalyzes the production of reactive oxygen radicals by a Fenton reaction, leading to oxidative damage. Thus, all living organisms tightly regulate the cellular levels of iron by employing efficient

iron acquisition and storage mechanisms. Microorganisms have evolved two types of proteins for storing iron, ferritins (Ftn) and bacterioferritins (Bfr); these are distinguishable by the presence of heme in the latter. The primary function of bacterioferritins and ferritins is to store iron during iron adequacy and supply it to the cell for various functions. It has been observed that prokaryotes possess a homolog of either an Ftn or Bfr; however, some microorganisms, such as *Escherichia coli*, *Vibrio cholerae*, *Clostridium acetobutylicum*, and *M. tuberculosis*, have evolved with the presence of both Ftn and Bfr. The sequencing of the *M. tuberculosis* H37Rv genome revealed the presence of two putative iron storage proteins, namely, BfrA (Rv1876), a bacterioferritin, and BfrB (Rv3841), a ferritin-like protein. The expression of both *bfrA* and *bfrB* is regulated by the binding of iron-activated IdeR (iron-dependent regulator) to the tandem operator sites present upstream of these iron storage genes. The regulation of the expression of *bfrA* in response to iron levels perhaps serves as a crucial mechanism for the adaptation and survival of *M. tuberculosis* in the host. In view of the well-established importance of iron for *M. tuberculosis*, the role of BfrA and BfrB in iron storage and supply as well as in protection against iron-mediated oxidative stress and their overexpression during hypoxic conditions, which is often associated with the latent phase, these proteins represent attractive targets for the development of new therapeutic molecules against tuberculosis.

However, the biological significance of these iron-storing proteins for *M. tuberculosis* has not been genetically proven. Hence, Dr. Tyagi and colleagues generated mutants of *M. tuberculosis* lacking *bfrA* (Rv1876) and *bfrB* (Rv3841) encoding the iron storage proteins. They showed that the mutant of *M. tuberculosis*, H_{37}Rv *_bfrA_bfrB*, which lacks the function of both *bfrA* and *bfrB*, has significantly reduced growth under iron-deprived conditions, is markedly vulnerable to oxidative stress, and exhibits the attenuation of growth in human macrophages. Moreover, reduced bacillary load in the lung and spleen of H_{37}Rv *_bfrA _bfrB*-infected guinea pigs, resulting in a significant reduction in pathology, clearly implied that these proteins play a crucial role in the pathogenesis of *M. tuberculosis.* Mycobacteria are continuously exposed to oxidative stress generated by the activated macrophages that they inhabit. When they evaluated the ability of *M. tuberculosis* mutants lacking the function of *bfrA* and *bfrB* to resist oxidative stress, it was observed that simultaneous mutations in *bfrA* and *bfrB* in *M. tuberculosis* (H_{37}Rv *_bfrA _bfrB*) tremendously reduced its ability to withstand oxidative stress, implying the role of these iron storage proteins in restricting oxidative damage. BfrA and BfrB are iron storage proteins that reduce the freely available ferrous form, thereby limiting the production of oxygen radicals by Fenton reaction and protecting the bacteria from the harmful oxidative damage. When the *M. tuberculosis* mutants lacking the function of a single Bfr protein (BfrA or BfrB) were evaluated for their ability to withstand oxidative stress, it was observed that these mutants also exhibited a moderate ability to withstand the oxidative damage; however, the magnitude of influence was less than that of the double mutant. Thus, the studies by Dr. Tyagi and colleagues clearly demonstrated the importance of these iron storage proteins in the mycobacterial response to oxidative stress.

The most substantial evidence for the role of bacterioferritins in *M. tuberculosis* pathogenesis emerged from their guinea pig studies, wherein at 10 weeks postinfection a marked reduction was observed in the CFU of H_{37}Rv*_bfrA_bfrB* in the spleen of guinea pigs compared to that of the parental strain (25-fold reduction). The bacillary load of H_{37}Rv

_bfrA _bfrB compared to that of the parental strain was further reduced when the disease was allowed to progress up to 16 weeks of infection. At this time point, a 52-fold lower bacillary load was observed in the spleen along with a 5-fold reduction in the lung of guinea pigs infected with the H_{37}Rv *_bfrA _bfrB* strain compared to that of infection with the parental strain. Thus, they showed that BfrA and BfrB together are required for the survival and pathogenesis of *M. tuberculosis* in the guinea pig model, as measured by bacillary load in lung and spleen and the pathological insult to the organs.

Conclusions

BfrA and BfrB proteins play a crucial role in protecting the pathogen against oxidative stress encountered during infection. In addition, BfrA and BfrB proteins are important for the survival and hematogenous spread of the pathogen. Our studies clearly establish these proteins as attractive drug targets for the development of new therapeutic molecules against mycobacterial infections.

Crystallization of *M. tuberculosis* proteins and structural determination

Iron is required for the growth of *Tubercle bacilli* in broth culture as well as in macrophages and thus represents a crucial requirement for infection by this pathogen. Due to its two readily interchangeable oxidation states (II) and (III), iron is an extremely useful redox mediator in biology. It is an indispensable cofactor for proteins participating in critical cellular processes such as electron transfer, oxygen transport, DNA synthesis, nitrogen fixation and for production of haemoproteins. Though iron is essential, the excess of free iron is potentially toxic as it catalyzes the production of reactive oxygen by Haber-Weiss/Fenton reactions, which cause oxidative damage to the cell. Thus, the cellular levels of iron have to be tightly regulated, for which efficient iron acquisition and storage mechanisms have been developed by all living organisms. Safe iron storage, detoxification and appropriate delivery of iron for biosynthetic functions in a cell are carried out by a superfamily of proteins known as ferritins that are widely found in all domains of life.

The Mtb genome revealed the presence of two putative ironstorage proteins, namely, BfrA (Rv1876)–a bacterioferritin and BfrB (Rv3841)–a ferritin like protein. It was expected that the expression of these genes would be upregulated in high-iron conditions and reduced in low-iron conditions as has been shown in other bacteria. As anticipated, the transcription of *bfrB* has been found to be repressed *in vitro* under iron-limited conditions. Interestingly, *bfrA* in *Mtb* is controlled by three promoters, of which two are repressed by iron, whereas, the third is activated by high levels of iron. Therefore, intriguingly, mRNA of *bfrA* gene in *Mtb* is produced under both low- and highiron conditions, thus suggesting that BfrA may have an additional role than storage of iron *in vivo*. It is quite possible that the mRNA pool of this gene has to be always available so that under iron overload conditions the gene for the storage of toxic iron can be translated quickly. The firmly regulated expression of BfrA appears to be crucial for the adaptation and survival of tubercle bacilli in the host. Hence, it represents a promising target for structure determination.

To further enhance the understanding about the proteins, Dr. Tyagi and colleagues determined the crystal structure of the selenomethionyl analog of bacterioferritin A (SeMet-

BfrA) from *Mycobacterium tuberculosis* (*Mtb*). Unexpectedly, electron density observed in the crystals of SeMet-BfrA analogous to haem location in bacterioferritins, showed a demetallated and degraded product of haem. This unanticipated observation was a consequence of the altered spatial electronic environment around the axial ligands of haem (in lieu of Met52 modification to SeMet52). Furthermore, the structure of *Mtb* SeMet-BfrA displayed a possible lost protein interaction with haem propionates due to formation of a salt bridge between Arg53-Glu57, which appeared to be unique to *Mtb* BfrA, resulting in slight modulation of haem binding pocket in this organism. The crystal structure of *Mtb* SeMet-BfrA provided novel leads to physiological function of haem in Bfrs. It may also serve as a scaffold for designing specific inhibitors. In addition, this study provided evidence against the general belief that a selenium derivative of a protein represents its true physiological native structure.

Dr. Tyagi and colleagues also determined a 3.0 A° crystal structure of BfrB from *Mycobacterium tuberculosis* (Mtb). Similar to the other members of ferritin family, the Mtb BfrB subunit exhibited the characteristic fold of a four-helical bundle that possesses the ferroxidase catalytic centre. Dr. Tyagi and colleagues compared the structure of Mtb BfrB with representatives of the ferritin family belonging to the archaea, eubacteria and eukarya. Unlike most other ferritins, Mtb BfrB has an extended C-terminus. To dissect the role of this extended C-terminus, truncated Mtb BfrB was purified and biochemical studies implicate this region in ferroxidase activity and iron release in addition to providing stability to the protein. Based on the comparative studies, they identified the slowly evolving conserved sites as well as the rapidly evolving variable sites and analyze their role in relation to structure and function of Mtb BfrB. Further, electrostatic computations demonstrated that although the electrostatic environment of catalytic residues is preserved within the family, extensive variability was exhibited by residues defining the channels and pores, in all likelihood keeping up with the diverse functions executed by these ferritins in varied environments.

The first committed step in lipid biosynthesis is the biotinylation of Acetyl Coenzyme A Carboxylase (ACC) mediated by biotin acetyl-CoA carboxylase ligase/biotin protein ligase (BirA). A recent biochemical study on *Mtb*-BirA has revealed significant differences in the ligand-binding properties of this enzyme compared to BirAs from various other organisms. Therefore, on one hand, BirA appears to be an attractive target for the development of broad spectrum therapeutic agents against multiple infections, while on the other, it also appears to be ideal for the development of species-specific novel anti-infective agent. All the apo BirA crystal structures have revealed the presence of disordered flexible loops, which undergo a conformational transition upon biotin and biotinyl-59-AMP binding. These loops are known to participate in either dimer interface or ligandbinding or both. The apo *Escherichia coli* (*Ec*) BirA has four disordered loops - biotin binding loop:BBL, adenylate binding loop:ABL, dimer loop I:DLI and dimer loop II:DLII. Binding of ligands induces dimerization of *Ec*BirA and structural ordering of these loops. However, *Pyrococcus horikoshii* (*Ph*) BirA exists as a dimer in both the liganded and unliganded forms and the crystal structure of its apo form shows only one disordered loop (BBL).

Dr. Tyagi and colleagues have shown that dehydration of *Mtb*-BirA crystals traps both the apo and active conformations in its asymmetric unit, and for the first time provides

structural evidence of such transformation. Recombinant *Mtb*-BirA was crystallized at room temperature, and diffraction data was collected at 295 K as well as at 120 K. Transfer of crystals to paraffin and paratone-N oil (cryoprotectants) prior to flash-freezing induced lattice shrinkage and enhancement in the resolution of the X-ray diffraction data. Intriguingly, the crystal lattice rearrangement due to shrinkage in the dehydrated *Mtb*-BirA crystals ensued structural order of otherwise flexible ligand-binding loops L4 and L8 in apo BirA. In addition, crystal dehydration resulted in a shift of 3.5 A° in the flexible loop L6, a proline-rich loop unique to *Mtb* complex as well as around the L11 region. The shift in loop L11 in the C-terminal domain on dehydration emulates the action responsible for the complex formation with its protein ligand biotin carboxyl carrier protein (BCCP) domain of ACCA3. This is contrary to the involvement of loop L14 observed in Pyrococcus horikoshii BirA-BCCP complex. Another interesting feature that emerged from this dehydrated structure was that the two subunits A and B, though related by a noncrystallographic twofold symmetry, assembled into an asymmetric dimer representing the ligand-bound and ligand-free states of the protein, respectively. In-depth analyses of the sequence and the structure also provided answers to the reported lower affinities of *Mtb*-BirA toward ATP and biotin substrates. This dehydrated crystal structure not only provided key leads to the understanding of the structure/function relationships in the protein in the absence of any ligand-bound structure, but also demonstrated the merit of dehydration of crystals as an inimitable technique to have a glance at proteins in action.

Conclusions

Thus, Dr. Tyagi and colleagues crystallized and determined the structures of three important proteins of *M. tuberculosis* namely BfrA, BfrB and BirA. These studies brought out important information regarding their structure-function relationship more importantly, the unrevealed the unique features of these *M. tuberculosis* proteins which would be crucial in targeting them for the development of new therapeutic molecules against mycobacterial infections.

Importance of mycobactin biosynthesis in the physiology, growth and pathogenesis of *M. tuberculosis*

Iron deficiency can prevent growth and excess of iron can lead to the generation of reactive oxygen radicals. Hence, successful pathogens carefully control the levels of intracellular iron. *M.tuberculosis* has developed an efficient mechanism to sequester iron from the host by secreting siderophores known as mycobactins. Mycobactins bind to iron more strongly than the iron storage proteins of the host and play a crucial role of scavenging iron from the iron limiting host environment. Although *M.tuberculosis* can uptake exogenous heme and utilize it as iron source, mycobactin mediated iron uptake remains its major iron acquisition mechanism. *M.tuberculosis*, *mbt* cluster is induced under low iron conditions as well as in IFNγ-stimulated macrophages thus indicating that *M.tuberculosis* can adapt its transcriptional machinery to environment by producing and secreting mycobactins required for increased uptake of iron by the pathogen. However, no studies have been carried out to evaluate the importance of mycobactin biosynthesis during the survival of *M.tuberculosis* in the host.

Dr. Tyagi and colleauges disrupted the *mbtE* gene (Rv2380c) of *M.tuberculosis* that encodes a non ribosomal peptide synthetase in the *mbt* cluster. Disruption of this gene rendered *M.tuberculosis* incapable of synthesizing mycobactins. The Mtb∆*mbtE* mutant displayed an altered colony morphology and was drastically affected in its ability to grow on agar medium and in broth culture as compared to the parental strain. Supplementation of agar and broth medium with Fe3+CMBT or Fe3+MBT restored the growth of Mtb∆*mbtE* to levels similar to that of the parental strain. Moreover, increasing the concentration of iron in the medium did not enhance the growth of the mutant, unless the medium was supplemented with mycobactins. Genetic complementation of Mtb∆*mbtE* with *mbtE* gene restored the in vitro growth phenotype of the mutant similar to that of the parental strain. From these observations, it was evident that mycobactin mediated iron acquisition is important for the normal growth of the pathogen. Transmission electron microscopy studies demonstrated that Mtb∆*mbtE* displayed a much denser and darker staining of the cells along with the cytoplasm emphasizing an altered cell wall permeability. Earlier, it was reported that mycobactins represent upto 10% of the cell mass and 1% of these are present in the cell membrane itself. Supplementation of growth medium with Fe3+CMBT restored the staining of Mtb∆*mbtE* similar to that of the parental strain. The altered colony morphology, cell wall permeability and growth characteristics of Mtb∆*mbtE* suggested that in the absence of mycobactins, several iron requiring systems of Mtb∆*mbtE* might have been affected (emanating as a consequence of inability of the mutant to synthesize mycobactins). The restoration of normal growth, cell wall permeability as well as colony morphology resulting from the addition of mycobactins in the media suggested that due to its essential role in procuring iron, mycobactin biosynthesis plays an important role in the biology of the pathogen.

Dr. Tyagi and colleagues demonstrated that Mtb∆*mbtE* mutant displayed a significantly reduced ability to infect and grow inside the human THP-1 macrophages in comparison to the parental strain, emphasizing that mycobactins are vital for mycobacterial growth. Their studies in guinea pigs provided further evidence that Mtb∆*mbtE* is highly attenuated for its growth and ability to cause pathology. The animals infected with the parental strain exhibited normal pathology, which increased from 4 weeks to 10 weeks post infection, as expected. However, in comparison, the animals infected with Mtb∆*mbtE* although did show pathology at 4 weeks post infection, the pathological damage was less at 10 weeks post infection. In the case of infection with the parental strain, a substantial number of CFU was recovered from the lungs and spleen of animals, at 4 as well as 10 weeks post infection, while no CFU was obtained from the animals infected with Mtb∆*mbtE* at both the time points. These observations 16 demonstrated that the mutant strain could survive in the host only for a limited period of time. In addition, a crucial proof of this came from the observation that while in the case of infection with the parental strain, the ZN staining could identify the acid fast bacilli in the lungs of animals at 4 as well as 10 weeks post infection, no such identifiable bacilli were present in the lungs of animals infected with the Mtb∆*mbtE*. These observations demonstrate a severe attenuation in the ability of the mutant to grow in the host and cause disease.

Conclusion

Disruption of mycobactin biosynthesis results in altered colony morphology, increased cell wall permeability and a severe defect in the ability of *M.tuberculosis* to grow in broth culture as well as in macrophages and renders the pathogen significantly attenuated for growth in the host thus severely limiting its ability to cause disease. Thus, this study highlights the importance of mycobactins for the normal physiology of *M.tuberculosis*, in vitro as well as in the host and establishes the enzymes of mycobactin biosynthesis as novel targets for the development of therapeutic interventions against tuberculosis.

Development of first oligonucleotidew microarray for global gene expression profiling in guinea pigs: defining the transcription signature of infectious diseases

The Guinea pig (Cavia porcellus) is one of the most extensively used animal models to study infectious diseases. However, despite its tremendous contribution towards understanding the establishment, progression and control of a number of diseases in general and tuberculosis in particular, the lack of fully annotated guinea pig genome sequence as well as appropriate molecular reagents has severely hampered detailed genetic and immunological analysis in this animal model. Dr. Tyagi and colleagues reported the development of first comprehensive microarray for studying the global gene expression profile in guinea pigs and validation of its usefulness with tuberculosis as a case study. An important gap in the area of infectious diseases was addressed by Dr. Tyagi and colleagues and a valuable molecular tool was provided to optimally harness the potential of guinea pig model to develop better vaccines and therapies against human diseases.

Since, fully annotated guinea pig genome sequence was not available, Dr. Tyagi and colleagues employed cross-species hybridization technology to develop a 44 K microarray platform to study gene expression profile in guinea pigs. Initially a 244 K microarray was designed to contain 60 mer oligonucleotide probes from multiple mammalian species (human, mouse, rat, guinea pig, rhesus monkey, dog, horse, cat, sheep, pig, chimpanzee, chinchilla, gray-tailed opossum and cattle) based on all the probe sequences available from Agilent Catalogue arrays and NCBI mRNA sequences. Especially, the array included 1132 probes based on annotated gene sequences of guinea pig and 92,815 probes corresponding to guinea pig ESTs. The 244 K array was then hybridized with Cy3 labeled cRNA produced from pooled RNA obtained from various guinea pig tissues (lung, liver, spleen, brain, muscle, kidney and bone marrow) and Cy5 labeled genomic DNA isolated from guinea pig spleen tissue. Following hybridization, the array was scanned and features were extracted. The filtration criteria during the probe selection, while developing microarray by cross-species hybridization technology on Agilent platform, were based on comparison of specific signal intensity viz. the background signal intensity. Probes exhibiting significantly higher signal intensity ($p < 0.05$), at least 2 fold higher as compared to the background are selected for array development. Based on this criterion, a total of 20,023 out of 62,560 probes representing different mammalian genes were selected from the 244 K array. Similarly, a total of 9,823 out of 92,815 probes were selected for ESTs. However, irrespective of the intensities, all the 1,132 probes for guinea pig were included. Further, an additional of 12,825 best probes out of 19,975 newly added guinea pig EST's from NCBI database were

added to the 44 K array. Thus, the final design of the guinea pig 44 K microarray comprised of a total number of 45,220 features including 29,846 valid features from different mammalian species, 1,132 probes for guinea pig transcripts and 12,825 probes for guinea pig ESTs, 1,264 Agilent positive controls and 153 Agilent negative controls.

In their study, the pulmonary transcriptional profiling of M. tuberculosis infected guinea pigs revealed a significant regulation of 3200 unique targets. While, 1344 unique genes exhibited a marked up regulation, 1856 genes were significantly down regulated. Differentially regulated genes were further classified into different categories based on their direct or indirect involvement in various biological processes or pathways. A massive re-alignment of metabolic pathways, mostly associated with catabolism, emerged as one of the interesting themes from this analysis. Extensive necrosis observed in the pulmonary granulomas in our study as well as a marked up regulation of several of these lipid homeostasis related genes, such as, ABHD2, ABHD8, ACSL1, ACSL5, CYP27A1, CYP2B18A, CYP26B1, CYP2F1, CYP2A13, CYP1A2, CYP11A1, CYP2D40, CYP2F1, FDPS, HADHA and LPL corresponded well with the observations associated with human caseous granulomas. On comparing the entire list of up and down regulated genes from our guinea pig study with that obtained from human TB granuloma study [GEO Accession no. GSE20050], Dr. Tyagi and colleagues observed that 38% of the up regulated genes of guinea pig [512 out of 1344 genes] exhibited an overlap with the genes up regulated in humans. Further, on comparing the microarray data available in the public database for TB infection in case of humans [GEO Accession no. GSE20050], mouse [GEO Accession no. GSE15335] and non-human primates [GEO Accession no. GPL10183], while, the nonhuman primates and humans exhibited a 19% overlap between up regulated genes, the overlap between mouse and humans was 18%. The guinea pig model is known for its close similarity to humans in terms of pathological response to *M. tuberculosis* infection. The observations of Dr. Tyagi and colleagues indicated that guinea pigs also exhibit higher resemblance to humans in terms of transcriptional response to M. tuberculosis infection, which further validates it as an excellent animal model to study TB. Hence, findings of this study would have a direct implication towards the development of novel therapeutic interventions. Besides, it would also permit the development and validation of biomarkers for effective vaccines and drugs in guinea pig model. A concurrent up regulation in the expression of oxidative phosphorylation related genes (expected to result in increased ATP levels), purinergic receptors and IL-1β in this study provided the first in vivo evidence for the involvement of these pathways in TB. Further, the lungs of the infected guinea pigs also exhibited a marked perturbation in the expression of several key genes associated with chemokine signaling (CCL27, CCL5, CXCL9, CXCR3, CCL21 and CCL11), cell adhesion molecules (CAMs) (HLA, ALCAM, MPZL1, CADM3, CADM1, CD34, CD8A, CD99, CDH3, CLDN4, CLDN6, NCAM1, ITGB2, ITGB8 and ITGA9) and cytokine and cytokine receptors (IL1β, IL1RAP, IL2RG, IL8, IL9, IL23A, IL23R, TGFB1, TGFB3, IFNGR2, TNFα, TNFSF10, CSF1R, BMP4, BMP8A, BMPR1A, BMPR2, LTA and ACVR2A), which are known to contribute to leukocyte trans-endothelial migration, inflammation and granulomatous pathology. Perturbation in the cellular signaling pathways is another typical theme that emerged from the study of Dr. Tyagi and colleagues. The most prominent observation related to the repression of numerous genes related to MAPK, Wnt and calcium signaling pathways.

Conclusion

This study by Dr. Tyagi and colleagues for the first time reported the development of a 44 K oligonucleotide microarray for guinea pigs and provided an important tool to capture the genome wide transcriptional changes in this model. The transcriptional profiling of M. tuberculosis infected guinea pig lungs not only revealed modulation of key immunologically relevant genes but also demonstrated involvement of novel metabolic and signaling pathways in TB pathogenesis. Moreover, in silico analysis revealed a higher resemblance of guinea pigs to humans in terms of transcriptional response to M. tuberculosis infection when compared to mouse and non-human primates. Development of the 44 K GPOM is thus, a critical step towards characterization of the guinea pig model, which will greatly aid in improving our understanding of host responses to a number of infectious diseases.

Identification of "switch residues" or "interface hot spots" involved in the self assembly and function of bacterioferritin B of *M. tuberculosis*

Dr. Tyagi and colleagues previously reported the crystal structure of bacterioferritin B (BfrB) of *M. tuberculosis*, and its comparative analysis with the representatives of the ferritin families belonging to the archaea, eubacteria, and eukarya identified the slowly evolving conserved sites as well as the rapidly evolving variable sites and analyzed the role of a unique and extended C-terminus in relation to the structure and function of the protein. Further in this study, they employed site-directed mutagenesis to identify residues important for interactions between subunits of this ferritin that are required for molecular assembly, structural integrity, thermodynamic stability, and ferroxidase activity to provide an improved understanding of the determinants of self-assembly and the structure–function relationship.

To identify the crucial residues involved in the self assembly and function of BfrB, Dr. Tyagi and colleagues constructed various mutants by employing site-directed mutagenesis. The analysis of mutants led to the identification of "interface hot-spot residues" (R69, L129, and F159) that act as "switch points" for BfrB oligomerization, and our observations show the importance of 4-fold axis residues in assembly formation. Moreover, they demonstrated that single-point mutations Q51A, Q126A, and E135A can enhance the thermal stability of the protein without affecting its assembly. Importantly, a comparative analysis of various mutations revealed that the function of various homologous positions in different ferritins could be at variance; hence, predicting the function of a residue just based on sequence−structure comparisons may not be appropriate. Thus, Dr. Tyagi and colleagues reported the identification of novel residues in the assembly formation and function of BfrB and show that single-point mutations have a remarkable potential for alteration of multiple properties of ferritins. Besides, "switch residues" or "interface hot spots" identified in their study could also prove to be helpful for the rational design of interfacial inhibitors.

Genome Sequence of *Mycobacterium indicus pranii* provides a perspective on mycobacterial evolution

The work described below on *Mycobacterium indicus pranii* (MIP) has led to the publication of the first completed genome of a new bacterial species from India and was covered in Nature as "Science News" item in September 2012.

MIP is a saprophytic mycobacterial species that is known for its immunomodulatory properties. In late 70s, this bacterium, initially coded as *Mycobacterium 'w'*, was selected

from a panel of atypical mycobacteria for its ability to evoke cell mediated immune responses against *M. leprae* in leprosy patients. MIP, which shares antigens with both *M. leprae* and *M. tuberculosis*, provides protection against *M. tuberculosis* infection in mice and accelerates sputum conversion in both type I and type II category of tuberculosis (TB) patients when used as an adjunct to chemotherapy. In HIV/TB co-infections, a single dose of MIP converted tuberculin _ve patients into tuberculin +ve in >95% of the cases. This attribute is unique to MIP because similar application of other saprophytic mycobacteria such as *M. vaccae* does not provide commensurate protection. Based on its demonstrated immunomodulatory action in various human diseases, MIP is the focus of several clinical trials and successful completion of one such trial has led to its use as an immunotherapeutic vaccine 'Immuvac' against leprosy. However, very little information is available about MIP's molecular, biochemical, genetic and phylogenomic features. In a molecular phylogenetic study by using candidate marker genes and FAFLP (fluorescent-amplified fragment length polymorphism techniques) fingerprinting assay, Dr. Tyagi and colleauges showed that MIP belongs to a group of opportunistic mycobacteria and is a predecessor of *M. avium* complex (MAC). A comprehensive analysis of cellular and biochemical features of MIP along with chemotaxonomic markers such as FAME (fatty acid methyl ester) analysis and comparison with other mycobacterial species established that MIP is endowed with specific attributes.

Dr. Tyagi and colleagues sequenced complete MIP genome to gain an insight into its unique life style and molecular basis of immunomodulation. In addition, they employed comparative genomics to understand the habitat diversification and bases and means of functional genetic correlates responsible for evolution of pathogenicity in ancestral mycobacterial lineages.

Different analyses performed in this study established that MIP represents an organism at a unique phylogenetic point as the immediate predecessor of opportunistic mycobacterial species of MAC. It was also evident that natural selection in MAC has acted in a preferential manner on specific categories of genes leading to reduced habitat diversity of pathogenic bacteria, and thus facilitating host tropism. The genome of MIP was ~5.6Mb in size and was shaped by a large number of lateral gene acquisitions thus revealing, for the first time, mosaic architecture of a mycobacterial genome. Thus, this study offers a paradigm shift in our understanding of evolutionary divergence, habitat diversification and advent of pathogenic attributes in mycobacteria. A scenario for mycobacterial evolution was envisaged wherein the earliest evolving soil derived mycobacterial species like MIP underwent massive gene acquisitions to attain a unique soil–water interface habitat before adapting to an aquatic and parasitic lifestyle. These lateral acquisition events were selective and possibly facilitated by the presence of specific genetic factors (i.e. ComEC) that induce competence to acquire large chunks of DNA to confer immediate survival advantage to the recipient organism. The genes, such as members of 'Hr' family, acquired to assist mycobacteria survive in fluctuating oxygen levels, would have been instrumental in the initial advent of pathogenicity in the aquatic opportunistic mycobacterial species. Subsequently, mycobacterial species tuned their genetic repertoires to respective host adapted forms with a high degree of genomic fluidity aided by selective lateral gene acquisitions and gene loss by deletion or pesudogenization. Importantly, a significant increase in transposon elements in the pathogenic mycobacteria as compared with MIP, for the first time, suggests their possible role toward mycobacterial virulence and would be interesting to explore. In addition, comparative genomic analysis revealed a higher

antigenic potential of MIP subscribing to its unique ability for immunomodulation against various types of infections and presents a template to develop reverse genetics based approaches to design better strategies against mycobacterial infections.

Conclusions

In this work, which represents the first completed genome of a new bacterial species from India, we proposed an original idea, accepted as current model of mycobacterial evolution that the progenitors of *M. avium* complex, an opportunist group of pathogens, and *M. tuberculosis* complex shared a common aquatic phase in their early life history that shaped up their virulence. In this paradigm shifting work, we showed, for the first time, the mosaic nature of a mycobacterial genome shaped up by extensive gene transfer events. This work led to the identification of 3 new families and 2 new sub-families of CYP450 in prokaryotes and was adjudicated in top 5% article for scientific excellence and originality by the editorial board of *Nucleic Acids Research*.

Identification of Inhibitors against *Mycobacterium tuberculosis* Thiamin Phosphate Synthase

In spite of the availability of drugs for the treatment of TB, the non-compliance to long chemotherapeutic regimens often results in the emergence of multidrug resistant strains of *Mycobacterium tuberculosis* adding to the precariousness of the situation. This has necessitated the development of more effective drugs. Thiamin biosynthesis, an important metabolic pathway of *M. tuberculosis*, is shown to be essential for the intracellular growth of this pathogen and hence, it is believed that inhibition of this pathway would severely affect the growth of *M. tuberculosis*.

A three–dimensional homology model of *M. tuberculosis* thiamin phosphate synthase was constructed by Dr. Tyagi and colleagues by using the X-ray crystal structure of thiamin phosphate synthase from *Pyrococcus furiosus.*

Computational screening approach was employed to identify potential small-molecule inhibitors of MtTPS from the NCI diversity set II comprising of 1541 compounds. Out of the 39 selected compounds evaluated for their inhibitory activity, compound 9 (4-{[(2-hydroxy-5-nitrophenyl) methylidene]amino}-5-methyl-2-(propan-2-yl)phenol), 33 (3-benzylsulfanyl-phenanthro [9,10-e][1,2,4]triazine) and 35 (Coumarin, 7-[4-chloro-6-(diethylamino)-s-triazin-2-yl]amino]-3-phenyl-) were identified as potential inhibitors of *M. tuberculosis* growth. All these compounds exhibited inhibition of MtTPS enzymatic activity as well as the growth of *M. tuberculosis* in broth culture. However, compound 9 exhibited the highest efficacy with an MIC_{99} value of 6 mg/ml. In addition, it did not exhibit any significant toxicity in various cell lines till a concentration of 25 mg/ml and also adhered to the Lipinsky rules for drug-likeness. The binding mode of compound 9 provided key insights into the likely binding sites. The compound 9 or NSC 33472 is docked at the large hydrophobic pocket at the active site of MtTPS. The aromatic ring A is placed in a hydrophobic environment surrounded by Ile173, Val193 and Phe171 while the two oxygen atoms of the nitro group appear to be making hydrogen bonds with the hydrogen atoms of the adjacent Cys136 and Cys11 both present within 2.5A° distance from the oxygen atoms. Moreover, the hydroxyl group of the aromatic ring B can form hydrogen bond with the carboxyl group of Asp98 present at a distance of 1.78A°. Inhibition of MtTPS by compound

9 in the presence of varying concentrations of the substrate HMPPP showed that an enhancement in the concentration of the substrate causes a decline in the inhibition and vice versa, which clearly indicates that compound 9 inhibits MtTPS by competing with HMP-PP for binding at the active site thus substantiating the docking results.

Conclusions

In conclusion, Dr. Tyagi and colleagues identified a promising lead molecule (compound 9) for the development of sterilizing agents against *M. tuberculosis* and further efforts are being made to optimize and enhance the inhibitory potency of this lead compound.

Studies on the importance of SapM in the physiology, growth and pathogenesis of *M. tuberculosis*

Macrophages are equipped with a plethora of antimicrobial mechanisms to kill pathogens. However, the success of *M. tuberculosis* as a highly adapted human pathogen has largely been attributed to its ability to survive successfully in the infected macrophages. *M. tuberculosis* blocks the biogenesis of phagolysosome, the very organelle responsible for the routine elimination of microorganisms by phagocytic cells. This strategy employed to arrest phagosomal maturation helps *M. tuberculosis* prevent its killing inside the host macrophage. In fact, by employing a genetic screen it has been demonstrated that *M. tuberculosis* mutants that are defective in the arrest of phagosome maturation show a reduced intracellular survival. Hence, *M. tuberculosis* proteins and lipids involved in the phagosome maturation blockage hold great promise as a target for the design of anti-tubercular molecules.

Previous studies had reported divergent observations with respect to the role of SapM in phagosomal maturation arrest in mycobacteria. Hence, Dr. Tyagi and colleagues first attempted to reascertain the involvement of SapM in phagosomal maturation arrest in *M. tuberculosis* and also evaluated the influence of sapM mutation on the growth of the pathogen in macrophages. Further, for the first time, they also evaluated the importance of SapM in the pathogenesis of *M. tuberculosis* by conducting animal studies with a *sapM* mutant.

The results of the studies by Dr. Tyagi and colleagues demonstrated that sapM is dispensable for the in vitro growth of *M. tuberculosis* in the broth culture. However, the growth kinetics of *Mtb⊿sapM* in human THP-1 macrophages up to 6 days post-infection revealed an attenuated growth phenotype when compared with the parental strain. Further, to reascertain the involvement of SapM in arresting the phagosomal maturation in *M. tuberculosis*, Dr. Tyagi and colleagues carried out colocalization studies. On examining the colocalization of FITC labeled *M. tuberculosis* containing phagosomes with Lysotracker, it was observed that while *M. tuberculosis* primarily resided in non-acidified compartments of THP-1 cell line, a mutation in *sapM* significantly increased the number of *M. tuberculosis* in the acidified compartments. Thus, by clearly demonstrating the inability of *Mtb⊿sapM* to arrest phagosomal maturation and its reversal by *Mtb⊿sapMComp*, their studies demonstrated the involvement of SapM in arresting the maturation of phagosomes in *M. tuberculosis.*

The most substantial evidence for the role of SapM in *M. tuberculosis* pathogenesis emerged from the studies by Dr. Tyagi and colleagues in the guinea pig model of infection which demonstrated that while *M. tuberculosis* exhibited normal growth in the organs of guinea pigs, the growth of *Mtb⊿sapM* was highly attenuated. In fact, at the end of 16 weeks, no mycobacteria were recovered from the lungs or spleens of *Mtb⊿sapM* infected animals. *M. tuberculosis* and *Mtb⊿sapMComp* exhibited normal growth in the guinea pig organs although the growth of *Mtb⊿sapMComp* was a bit less than *M. tuberculosis* at the end of 16 weeks post-infection. Thus, for the first time their observations demonstrated that SapM is indispensable for the growth of *M. tuberculosis* in the host, which was further substantiated by the observations that guinea pigs infected with *Mtb⊿sapM* exhibited a significantly reduced pathological damage as compared to the animals infected with *M. tuberculosis*.

As survival of infected animals is one of the best parameters to evaluate the involvement of a gene in the pathogenesis of an organism. Dr. Tyagi and colleagues also evaluated the effect of the disruption of *sapM* on the survival of the infected animals. The animals infected with *M. tuberculosis* gradually succumbed to death within 120 days post-infection with an MST of 98.5 days. *Mtb⊿sapMComp* infected guinea pigs also exhibited comparable survival time with an MST of 129 days. However, the influence of the deletion of *sapM* gene on the survival of the animals was unambiguous as during the total duration of the experiment (210 days) not even a single *Mtb⊿sapM* infected animal succumbed to death. This was the most substantial evidence for the role of *SapM* in the pathogenesis of *M. tuberculosis*. Thus, Dr. Tyagi and colleagues demonstrated the importance of *SapM* in arresting the phagosomal maturation as well as in the pathogenesis of *M. tuberculosis*, establishing it as an important target for the development of new anti-tubercular molecules.

Conclusions

To summarize, Dr. Tyagi and colleagues demonstrated that SapM mediates an important role in the protection of *M. tuberculosis* against the host defense by subverting the phagosomal maturation pathway. Disruption of *sapM* in *M. tuberculosis* resulted in a highly attenuated strain with an impaired ability to grow in the THP-1 macrophages as well as in the guinea pig tissues. Thus, these studies established SapM as a potential drug target. The fact that there are no known human analogues of SapM makes it even more important target for the development of new therapeutic molecules against TB. In addition, the secretory nature of SapM presents a unique opportunity in order to avoid the drug permeability issue due to thick hydrophobic cell envelope of *M. tuberculosis*.

Characterization and role of *M. tuberculosis* AP endonucleases in DNA repair and pathogenesis

During the establishment of an infection, bacterial pathogens encounter oxidative stress resulting in the production of DNA lesions. Majority of these lesions are repaired by base excision repair (BER) pathway. Amongst these, abasic sties are the most frequent lesions in DNA. Class II apurinic/apyrimidinic (AP) endonucleases play a major role in BER of damaged DNA comprising of basic sites. *Mycobacterium tuberculosis* resides in the human macrophages and is continually subjected to oxidative assault.

To maintain its genome integrity, the bacterium must possess robust DNA repair machinery. Further, the GC rich (~66%) genome of this pathogen renders it much more susceptible to cytosine deamination (generating uracil) and guanine oxidation [predominantly generating 8-oxoguanine (8-oxoG)] than other intracellular bacteria. This has led to special interest in the BER pathways that repair uracil and 8-oxoG in mycobacteria and it is thought that BER may play a central role in maintaining the integrity of DNA in this bacterium in the absence of any recognized homologs of mismatch repair. However, no studies have focused on the role and characterization of AP endonucleases in *M. tuberculosis*.

The sequencing of *M. tuberculosis* genome revealed the presence of Ec-EndoIV and Ec-ExoIII homologs namely Endonuclease IV (End) and Exonuclease III (XthA), that are encoded by the genes *end* (Rv0670) and *xthA* (Rv0427c), respectively. Dr. Tyagi and colleagues, for the first time, carried out the biochemical and functional characterization of these proteins in *M. tuberculosis*.

The experimental evidence gathered by Dr. Tyagi and colleagues has made interesting revelations in support of End being the more important AP endonuclease of *M. tuberculosis*. They evaluated the enzymatic activities of the annotated AP endonucleases of *M. tuberculosis*, and showed that both Endonuclease IV (End) and Exonuclease III (XthA) are multifunctional enzymes. These enzymes exhibit AP endonuclease and 3′→5′ exonuclease activities with differences in their substrate specificities. More importantly, they showed that Endonuclease IV is the major AP endonuclease of *M. tuberculosis* that also plays an important role in protecting the pathogen against oxidative DNA damage.

Evaluation of *M. tuberculosis* AP endonucleases revealed that End is a highly efficient AP endonuclease while XthA displays weaker AP endonuclease activity. This difference in the efficiency of End was attributed to a ~4 fold higher K_{cat} value of End in comparison to XthA when acting on a double-stranded DNA containing an AP site. Mycobacterial End cleaved both the double and single-stranded DNA containing an AP site. The AP endonuclease activity of the End enzyme exhibited almost 2-fold higher AP endonuclease activity on double-stranded DNA in comparison to single-stranded DNA.

The observations by Dr. Tyagi and colleagues demonstrated that the activity of AP endonucleases of *M. tuberculosis* is stimulated in the presence of Mg^{2+} or Ca^{2+} and these metals may play an important role in the catalysis of these enzymes. Inhibitory effect of EDTA on the AP endonuclease activity of both the proteins further substantiated that like other members of Endonuclease IV and Exonuclease III family, both the mycobacterial AP endonucleases appeared to require transition metals for their activity. Both End and XthA were five times as active on a THF residue opposite C as compared to T. End and XthA incised AP·A and AP·G mismatches with a similar efficiency. The preferential recognition of AP site opposite the cytosine residue is an important characteristic of mycobacterial AP endonucleases, probably indicating the importance of AP endonucleases in recognizing the modifications in the guanine base. 7,8-dihydro-8-oxoguanine (8-oxoG) is one of the most common damage resulting from the oxidation of DNA, and failure to replace it with the correct base results in mutations . Moreover, it has been demonstrated that DNA

polymerase(s) from mycobacteria display a preference for the incorporation of G opposite the 8-oxoG as opposed to an A in *E. coli*, which further enhances the risk of accumulating guanine modifications like 8-oxoG, in their GC rich DNA. Further, several class II AP endonucleases such as yeast Apn1 and human Ape1 have been demonstrated to remove 3′ incorporated 8-oxoG damaged nucleotide. In light of the above, it is not unlikely that the AP endonucleases of *M. tuberculosis* play a role in the removal of 8-oxoG damaged nucleotide.

Dr. Tyagi and colleagues observed that XthA is a less efficient enzyme than End for its AP endonuclease activity. Also, incubation of XthA with metal ions enhanced the AP endonuclease activity of XthA, but to lesser extent than that observed in the case of End. Overall, they observed a lower AP endonuclease activity of XthA when compared to End.
The measurement of AP endonuclease activity in the mycobacterial cell-free extracts of the parental and mutant strains of *end* or/and *xthA,* demonstrated End as the major apurinic endonuclease under normal growth conditions. However, the activity of XthA in these cultures was only conspicuous by its absence. In spite of exposure to the DNA damaging agents, Dr. Tyagi and colleagues did not find any measurable activity of XthA even at the highest concentration of these DNA damaging agents used under their experimental conditions. Based on these results, it appears that End and represents the major AP endonuclease in *M. tuberculosis*.

To investigate the role of these AP endonucleases in repairing the DNA damage and thereby protecting *M. tuberculosis*, Dr. Tyagi and colleagues employed disc diffusion assay. The response of *M. tuberculosis* to DNA damage resulting from the exposure of reactive oxygen intermediates in mutant strains lacking one or both the AP endonucleases was measured by growth inhibition zone around a paper disc impregnated with CHP. The results of these studies suggested that in the *M.tuberculosis* BER pathway, the removal of damaged DNA resulting from oxidative stress is primarily carried out by End. XthA that possesses only a weak AP endonuclease activity with a predominant 3′→5′ exonuclease activity plays a less significant role in the repair of such damage.

Dr. Tyagi and colleagues demonstrated that End and XthA were able to compensate the absence of each other to repair the DNA damaged in response to alkylation stress. However, a simultaneous disruption of both the AP endonucleases in *M. tuberculosis* (Mtb∆*end*∆*xthA*) significantly reduced the ability of the pathogen to withstand alkylation stress when compared with *M. tuberculosis*, thereby indicating the importance of AP endonucleases in protecting the pathogen against alkylation damage. The experimental evidence gathered by Dr. Tyagi and colleagues showed that disruption of both the AP endonucleases in *M.tuberculosis* (Mtb∆*end*∆*xthA*) significantly reduced the growth of the pathogen in THP-1 cell line. However, in the guinea pig infection model, they observed that disruption of either one or both the AP endonucleases did not affect the growth of *M. tuberculosis* apparently indicating that these AP endonucleases may not be indispensable for the growth and pathogenesis of *M. tuberculosis*. Their observations were substantiated by gross pathological and histopathological damage.

Conclusion

In summary, the study by Dr. Tyagi and colleagues provided the first evidence for the presence of active AP endonucleases in *M. tuberculosis* and for distinct substrate preferences of these AP enzymes. They demonstrated that End is not only a more efficient AP endonuclease enzyme than XthA but it also represents the major AP endonuclease activity in *M. tuberculosis* and plays a crucial role in defense against oxidative stress in comparison to XthA. In addition, while End possesses a metal ion independent exonuclease activity; XthA is a metal ion dependent enzyme which predominantly acts as a 3′→5′ exonuclease possessing weak AP endonuclease activity. The AP endonuclease activity of both the *M. tuberculosis* AP endonucleases is stimulated by $Mg^{2+}$and Ca^{2+} and displays a preferential recognition for abasic site paired opposite to a cytosine residue in DNA. Based on the animal studies, it appears that *M. tuberculosis* may possess repair pathways or proteins that overlap with AP endonucleases in order for them to protect the DNA from damage during its survival in the host. This is consistent with the robustness, which this pathogen is known for.