PSEUDOMONAS SYRINGAE PATHOVARS AND RELATED PATHOGENS

Developments in Plant Pathology

VOLUME 9

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Pseudomonas Syringae Pathovars and Related Pathogens

Edited by

K. RUDOLPH

*Institut für Pflanzenpathologie und
Pflanzenschutz der Universität,
Göttingen, Germany*

T. J. BURR

*Department of Plant Pathology,
NYSAES-Cornell University,
Geneva, NY, U.S.A.*

J. W. MANSFIELD

*Department Biological Sciences,
Wye College, University of London,
Wye, Ashford, Kent, U.K.*

D. STEAD

*Central Science Laboratory
Sand Hutton,
York, U.K.*

A. VIVIAN

*Department of Biological Science,
UWE-Bristol, Frenchay,
Bristol, U.K.*

J. VON KIETZELL

*Biologische Bundesanstalt für
Land- und Forstwirtschaft,
Braunschweig, Germany*

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Contributors

Attila L. Adam, Plant Protection Institute, Hungarian Academy of Sciences, 1525 Budapest, P.O. Box 102, Hungary

Tika Adhikari, Dept. of Plant Pathology, Inst. Agricult. and Animal Science, Tribhuvan University, P.O.Box: 984, Kathmandu, Nepal

Maia Alexandrova, Ist. di Patologia Vegetale, Università di Bologna, via Filippo Re 8, I-40026 Bologna, Italy

James R. Alfano, Cornell University, Department Plant Pathology, 334 Plant Science Building, Ithaca, NY 14853-4203, U.S.A.

Caitilyn Allen, Dept. of Plant Pathology, University of Wisconsin, 1630 Linden Drive, Madison, WI 53706, U.S.A.

Fuad Al-Sallami, University of Agriculture, Mendeleev Str. 12, 4000 Plovdiv, Bulgaria

Tahsin Al-Shinawi, Institut für Pflanzenpathologie und Pflanzenschutz, Grisebachstr. 6, 37077 Göttingen, Germany

Todi Amlai, University of Fort Hare, Dept. of Agronomy, Private Bag X 1314, Alice 5700, South Africa

Maryke Appel, Dept. of Biochemistry, University of Stellenbosch, Stellenbosch 7602, South Africa

Simon A. Archer, Biology Dept. East Wing, Imperial College of Science, Technology and Medicine, Prince Consort Rd., London SW7 2BB, U.K.

Dawn Arnold, Faculty of Applied Sciences, Univ. of the West of England, Frenchay Campus, Coldharbour Lane, Bristol, BS16 1QY, U.K.

Momcilo Arsenijevic, Faculty of Agriculture, Trg D. Obradovica 8, 21000 Novi Sad, Yugoslavia

Georg Auling, Institut für Mikrobiologie der Universität, D-30167 Hannover, Schneiderberg 50, Germany

Andre S. Bachmann, Biotechnology Program, Pacific Biomedical Research Center, University of Hawaii, Honolulu, Hawaii 96822, U.S.A.

R.C. Bachmann, Dept. of Biology, Utah State University, Logan, U.S.A.

Baharuddin, Dept. Plant Pathology, Faculty of Agriculture, Hasaruddin University, Ujung Pandang, 90245, Indonesia

Ana M. Bailey, CINVESTAV-IPN, Ap. Postal 629, Irapuato Gto, Mexico 36500, Mexico

Giorgio M. Balestra, Dipartimento di Protezione delle Piante, Università dagli Studi, Viterbo, Italy

A. Ballio, Dipt. di Scienze Biochimiche „A. Rossi Fanelli“ e Centro di Biologia Molecolare del CNR, Università „La Sapienza“, Roma, Italy

Balász Barna, Plant Protection Institute, Hungarian Academy of Sciences, 1525 Budapest, P.O. Box 102, Hungary

D. Barra, Dipt. di Scienze Biochimiche „A. Rossi Fanelli“ e Centro di Biologia Molecolare del CNR, Università „La Sapienza“, Roma, Italy

Yoav Bashan, Dept. of Microbiology, The Center for Biological Research of the Northwest (CIB), A.P. 128, La Paz, 23000, B.C.S., Mexico

Ramchandra Basnyat, Dept. of Plant Pathology, Inst. Agricult. and Animal Science, Tribhuvan University, P.O.Box: 984, Kathmandu, Nepal

Lisa M. Bateham, Dept. of Botany and Plant Pathology, Oregon State University, Cordley Hall 2082, Corvallis, Oregon 97331-2902, U.S.A.

Henri Batoko, Lab. de Cytogénétique, Univ. Catholique de Louvain, Place Croix du Sud 5 bte 13, 1348 Louvain le Neuve, Belgium

David W. Bauer, Cornell University, Department Plant Pathology, 334 Plant Science Building, Ithaca, NY 14853-4203, U.S.A.

Carlo Bazzi, Ist. di Patologia Vegetale, Università di Bologna, via Filippo Re 8, I-40026 Bologna, Italy

Dirk U. Bellstedt, Dept. of Biochemistry, University of Stellenbosch, Stellenbosch 7602, South Africa

Carol L. Bender, Dept. Plant Pathology, Oklahoma State University, 110, Noble Research Center, Stillwater, OK 74078, U.S.A.

Mark A. Bennett, Dept. Biological, Sciences, Wye College, University of London, Wye, Ashford, Kent. TN25 5AH, U.K.

Stefan Bereswill, Max-Planck-Institut für med. Forschung, Jahnstr. 29, D-69120 Heidelberg, Germany

Katarina Björklöf, Department of Biosciences, Division of General Microbiology, University of Helsinki, P.O. Box 56, SF-00014 Helsinki, Finland

Nevena Bogatzewska, Plant Protection Institute, 2230 Kostinbrod, Bulgaria

F. Bossa, Dipt. di Scienze Biochimiche „A. Rossi Fanelli“ e Centro di Biologia Molecolare del CNR, Università „La Sapienza“, Roma, Italy

Jules Bouharmont, Lab. de Cytogénétique, Univ. Catholique de Louvain, Place Croix du Sud 5 bte 13, 1348 Louvain le Neuve, Belgium

Lamine Boukris, University of Algiers, Institute of Agronomy, Department of Plant Protection, El Harrach, Algiers, Algeria

Marc Boutry, Unité de Biochimie Physiologique, Place Croix du Sud 2 bte 20, Université catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium

Zoltán Bozsó, Plant Protection Institute, Hungarian Academy of Sciences, 1525 Budapest, P.O. Box 102, Hungary

Helmut Bröther, Landesamt für Ernährung, Landwirtschaft und Flurneueordnung, -Pflanzenschutzdienst-, Hermannswerder 20 A, 14473 Potsdam, Germany

Thomas J. Burr, Dept. Plant Pathology, NYSAES - Cornell University, Geneva, NY 14456, U.S.A.

Ippolito Camele, Dipt. di Biologia, Difesa e Biotecnologie Agro Forestale, Università della Basilicata, Via N. Sauro 85, 85100 Potenza, Italy

L. Camoni, Dipt. di Scienze Biochimiche „A. Rossi Fanelli“ e Centro di Biologia Molecolare del CNR, Università „La Sapienza“, Roma, Italy

Marilyn L. Canfield, Dept. of Botany and Plant Pathology, Oregon State University, Cordley Hall 2082, Corvallis, Oregon 97331-2902, U.S.A.

Francisco, M. Cánovas, Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Málaga, E-29071 Málaga, Spain

Francisco R. Cantón, Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Málaga, E-29071 Málaga, Spain

Renato Capasso, Dipt. Sci. Chimico-Agrarie, Università di Napoli "Federico II", 80055 Portici, Italy

Arturo Caponero, Dipartimento di Patologia vegetale, Università degli Studi di Bari, Via G. Amendola 165/A, 70126 Bari, Italy

Francisco M. Cazorla, Dpt. Microbiologia, Fac. Ciencias, Univ. Málaga, 29071 Málaga, Spain

Zoya Cheusova, Kiev University, Faculty of Biology, Vladimirskaia 64, Kiev 252017, Ukraine

Nataliya Chitchevitch, Institute of Microbiology and Virology, National Academy of Ukraine, Zabolotny Str. 154, 252143 Kiev, Ukraine

Svitlana Chodos, Inst. of Microbiology and Virology, Zabolotny Str. 154, 252143 Kiev, Ukraine

Giuseppe Ciuffreda, Dipt. di Biologia, Difesa e Biotecnologie Agro Forestale, Università della Basilicata, Via N. Sauro 85, 85100 Potenza, Italy

Juan A. Codina, Dpt. Microbiologia, Fac. Ciencias, Univ. Málaga, 29071 Málaga, Spain

Alan Collmer, Cornell University, Department Plant Pathology, 334 Plant Science Building, Ithaca, NY 14853-4203, U.S.A.

Benoit Cournoyer, Faculty of Applied Sciences, Univ. of the West of England, Coldharbour Lane, Frenchay, Bristol, BS16 1QY, U.K.

Gennaro Cristinzio, Istituto di Patologia Vegetale, Università di Napoli "Federico II", 80055 Portici, Italy

Raffaella Curci, Dipt. di Scienze Biochimie, Università di Roma "La Sapienza", P.le Aldo Moro 5, 00185 Roma, Italy

José Da Cruz Machado, ESAL Depto de Fitossanidade, Cx. Postal, 37 - 37200-000 Lavras, MG, Brazil

Fábio K. Dal Soglio, Universidade Federal de Santa Catarina/CCA/Depto. de Fitotecnia, Caixa Postal, 476, 88049-900 Florianopolis/SC, Brazil

Holger Deising, Universität Konstanz, Fakultät für Biologie, Lehrstuhl Phytopathologie, D-78464 Konstanz, Germany

Ioannis Deligiosas, Dipt. di Scienze Biochimie, Università di Roma "La Sapienza", P.le Aldo Moro 5, 00185 Roma, Italy

Senka Deneva, Plant Protection Institute, 2230 Kostinbrod, Bulgaria

Yves Dessaux, ISV-CNRS, BAT. 23, Av. de la Terrasse, 91198 Gif sur Yvette Cedex, France

Antonio De Vicente, Dpto. Microbiologia, Fac. Ciencias, Campus Teatinos, Univ. Málaga, 29071 Málaga, Spain

D. Di Giorgio, Dipt. di Scienze Biochimiche „A. Rossi Fanelli“ e Centro di Biologia Molecolare del CNR, Università „La Sapienza“, Roma, Italy

A. Di Nola, Dipartimento di Chimica, Università „La Sapienza“, Roma, Italy

Talmir Duarte da Silva, Universidade Federal de Santa Catarina/CCA/Depto. de Fitotecnia, Caixa Postal, 476, 88049-900 Florianopolis/SC, Brazil

Maria Heloisa Duarte de Moraes, USP/ESALQ Depto de Fitopatologia, Cx. Postal, 9 - 13418-900 Piracicaba, SP, Brazil

Claire B. Durant, Biology Dept. East Wing, Imperial College of Science, Technology and Medicine, Prince Consort Rd., London SW7 2BB, U.K.

Miena Elasri, ISV-CNRS, BAT. 23, Av. de la Terrasse, 91198 Gif sur Yvette Cedex, France

Farouk El-Banoby, Plant Pathology Department, Faculty of Agriculture, EL-Azhar University, Cairo, Egypt

John G. Elphinstone, Central Science Laboratory, Hatching Green, Harpenden, Hertfordshire, AL5 2BD, U.K.

Mostafa A. El-Sayed, Dept. of Botany, Faculty of Science, Tanta University, Tanta, Egypt

Abd El-Raheem El-Shanshoury, Dept. of Botany, Faculty of Science, Tanta University, Tanta, Egypt

Wagih El-Shouny, Dept. of Botany, Faculty of Science, Tanta University, Tanta, Egypt

Rangasamy P. Elumalei, C.A.S. in Botany, University of Madras, Madras-600025, India

Antonio Evidente, Dipt. di Sci. Chimico-Agrarie, Università di Napoli, Via Università 100, 80055 Portici, Italy

Eric Ewbank, Lab. de Phytopathologie, Univ. Catholique de Louvain, Place Croix du Sud 2 bte 3, 1348 Louvain-le-Neuve, Belgium

P. Ferranti, Servizio Spettrometria di Massa del CNR e Dipartimento di Chimica Organica e Biologica, Università „Federico II“, Napoli, Italy

Giovanni Figliuolo, Dipt. di Biologia, Difesa e Biotecnologie Agro Forestale, Università della Basilicata, Via N. Sauro 85, 85100 Potenza, Italy

Marie-Christine Flamand, Lab. de Phytopathologie, Univ. Catholique de Louvain, Place Croix du Sud 2 bte 3, 1348 Louvain-le-Neuve, Belgium

Wolfgang Fritsche, Friedrich-Schiller Universität, Inst. f. Mikrobiologie, Philosophenweg 12, 07743 Jena, Germany

Leopold Fucikovsky, Centro de Fitopatologia, Colegio de Postgraduados, Montecillo, C.P. 56230, Edo de Mexico, Mexico

Louis Gardan, I.N.R.A., Station de Pathologie vegetale et Phytobacteriologie, 42 Rue George Morel, 49071 Beaucouze Cedex, France

Klaus Geider, Max-Planck-Institut für Medizinische Forschung, Jahnstr. 29, D-69120 Heidelberg, Germany

Marjorie Gibbon, Faculty of Applied Sciences, Univ. of the West of England, Frenchay Campus, Coldharbour Lane, Bristol, BS16 1QY, U.K.

Robert L. Gilbertson, Plant Pathology Dept., University of California, Davis CA 95616, U.S.A.

Eric Glickmann, ISV-CNRS, BAT. 23, Av. de la Terrasse, 91198 Gif sur Yvette Cedex, France

Bernard Goret, Lab. de Phytopathologie, Univ. Catholique de Louvain, Place Croix du Sud 2 bte 3, 1348 Louvain-le-Neuve, Belgium

Ingeborg Grgurina, Dipt. di Scienze Biochimie, Università di Roma "La Sapienza", P.le Aldo Moro 5, 00185 Roma, Italy

Charlotte Grimm, Institute of Botany, University of Vienna, Rennweg 14, A-1030 Vienna, Austria

Patrick A.D. Grimont, Unité des Entérobactéries, Institut Pasteur, 75724 Paris, Cedex 15, France

Ute Grolms, Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Grisebachstr. 6, 37077 Göttingen, Germany

Catherine Grondeau, INRA Station de Pathologie Végétale, 42 Rue Georges Morel, BP 57, 49071 Beaucouze Cedex, France

Dennis C. Gross, Washington State University, Dept. of Plant Pathology, Pullman WA 99164-6430, U.S.A.

Michael Groß, Tierärztliches Institut der Universität Göttingen, Groner Landstr. 2, 37073 Göttingen, Germany

Gábor Gullner, Plant Protection Institute, Hungarian Academy of Sciences, 1525 Budapest, P.O. Box 102, Hungary

Rostislav Gvozdyak, Institute of Microbiology and Virology, Ukrainian Academy of Sciences, Zabolotny str. 154, Kiev, 252143, Ukraine

Trimurti Habazar, Fak. Pertanian, Univ. Andalas, Kampus Limau Manih, Padang, 25166, Indonesia

Joe Hanus, Dept. of Botany and Plant Pathology, Oregon State University, Cordley Hall 2082, Corvallis, Oregon 97331-2902, U.S.A.

Efstathios Hatziloukas, USDA/ARS/NAA/FD-WSRU, Fort Detrick, Bldg. 1301, Frederick, MD 21702, U.S.A.

Judy Hennessy, Central Science Laboratory, Hatching Green, Harpenden, Hertfordshire, AL5 2BD, U.K.

Ursula Hettwer, Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Grisebachstr. 6, 37077 Göttingen, Germany

Maria Hevesi, University of Horticulture and Food Industry, Department of Plant Pathology, 1118 Budapest XI, Menesi út, 44, Hungary

Tze-Chung Huang, Dept. Plant Pathology, NYSAES - Cornell University, Geneva, NY 14456, U.S.A.

Qi Huang, Dept. of Plant Pathology, University of Wisconsin, 1630 Linden Drive, Madison, WI 53706, U.S.A.

Steven Hutcheson, Univ. of Maryland at College Park, Dept. of Botany, H.J. Patterson Hall, College Park, Maryland 20742-5815, U.S.A.

Michael L. Hutchison, Washington State University, Dept. of Plant Pathology, Pullman WA 99164-6430, U.S.A.

Nicola S. Iacobellis, Dipt. di Biologia, Difesa e Biotecnologie Agro Forestale, Università della Basilicata, Via N. Sauro 85, 85100 Potenza, Italy

Caterina Iannini, Istituto di Pathologia Vegetale, Università di Napoli "Federico II", 80055 Portici, Italy

Robert Jackson, Faculty of Applied Sciences, Univ. of the West of England, Coldharbour Lane, Frenchay, Bristol, BS16 IQY, U.K.

Jaap D. Janse, Plant Protection Service, Dept. Bacteriology, P.O. Box 9102, 6700 HC Wageningen, Netherlands

Harry Jansing, BBA für Land- und Forstwirtschaft, Inst. für PS in Ackerbau und Grünland, Messeweg 11/12, 38104 Braunschweig, Germany

Karin Jarchow-Redecker, Tauroggener Str. 47, 10589 Berlin, Germany

Carol Jenner, Dept. Biological, Sciences, Wye College, University of London, Wye, Ashford, Kent. TN25 5AH, U.K.

Songmu Jin, Univ. of Maryland at College Park, Dept. of Botany, H.J. Patterson Hall, College Park, Maryland 20742-5815, U.S.A.

Krishan K. Jindal, Dept. of Plant Pathology, Univ. of Horticulture and Forestry, Nauni-173230, Solan, H.P., India

Gilbert M. Kairu, Coffee Research Foundation, P.O. Box 4, Ruiru, Kenia

Grit Kampmann, Friedrich-Schiller Universität, Inst. f. Mikrobiologie, Philosophenweg 12, 07743 Jena, Germany

Yaowei Kang, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Yuan Ming Yuan West Rd., Beijing 10094, P.R. China

Stoycho Karov, Agricultural University of Plovdiv, Mendeleev Str. 12, 4000 Plovdiv, Bulgaria

Noel Keen, Dept. of Plant Pathology, University of California, Riverside, CA 92521, U.S.A.

Islam D. Khan, Science and Technology Infrastructure Development Division, University Grants Commission, Bahadur Shah Zafar Marg, New Delhi 110002, India

Jean-Marie Kinet, Lab. de Cytogénétique, Univ. Catholique de Louvain, Place Croix du Sud 5 bte 13, 1348 Louvain la Neuve, Belgium

Zoltan Király, Plant Protection Institute, Hungarian Academy of Sciences, 1525 Budapest, P.O. Box 102, Hungary

Zoltán Klement, Plant Protection Institute, Hungarian Academy of Sciences, P.O. Box 102, 1525 Budapest, Hungary

Yuriy A. Knirel, N.D. Zelinsky Institute of Org. Chemistry, Leninsky pr. 47, Moscow 117913, Russia

Nina Kocharova, N.D. Zelinsky Institute of Org. Chemistry, Leninsky pr. 47, Moscow 117913, Russia

Birger Koopmann, Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Grisebachstr. 6, 37077 Göttingen, Germany

Alexander Korobko, Inst. of Microbiology and Virology, Zabolotny Str. 154, Kiev 252143, Ukraine

Vladimir Kotlyarov, Kuban State Agrarian University, Kalinin str., 13, 350044 Krasnodar, Russia

Ilona Krämer, BAZ, Institut für Pathogendiagnostik, Postfach 1505, 06435 Aschersleben, Germany

Kai Krohn, Institute of Medical Technology, University of Tampere, Tampere, Finland

Nataliya Kucheryava, Inst. of Microbiology and Virology, Zabolotny Str. 154, Kiev 252143, Ukraine

Paola Lavermicocca, Ist. tossine e micotossine da parassiti vegetali, C.N.R., V.le L. Einaudi, 51, 70125 Bari, Italy

Hajnalka D. Ledó, Plant Breeding Center, Vetomag Trading House Co. LTD, Szentes, Hungary

Seon W. Lee, Dept. of Plant Pathology, University of California, Riverside, CA 92521, U.S.A.

Heinrich Lehmann-Danzinger, Institut für Pflanzenpathologie und Pflanzenschutz, Grisebachstr. 6, 37077 Göttingen, Germany

Michael Lidell, Univ. of Maryland at College Park, Dept. of Botany, H.J. Patterson Hall, College Park, Maryland 20742-5815, U.S.A.

Elisabeth Little, Plant Pathology Dept., University of California, Davis CA 95616, U.S.A.

Amy O. Loneiello, Cornell University, Department Plant Pathology, 334 Plant Science Building, Ithaca, NY 14853-4203, U.S.A.

N. Lyons, Horticulture Research International, Wellesbourne, Warwick, CV35 9EP, U.K.

Ayyamperumal Mahadevan, C.A.S. in Botany, University of Madras, Madras-600025, India

Laurence Malandrin, INRA Station de Pathologie Végétale, 42 Rue Georges Morel, BP 57, 49071 Beaucouze Cedex, France

C. Manetti, Dipartimento di Chimica, Università „La Sapienza“, Roma, Italy

John Mansfield, Dept. Biological Sciences, Wye College, University of London, Wye, Ashford, Kent. TN25 5AH, U.K.

Lucienne E.C. Mansvelt, Infruitec, P.B. X5013, Stellenbosch 599, South Africa

Mohamed Marahiel, Phillips University, Hans Meerwein Str., 35032 Marburg, Germany

Henri Maraite, Unité de Phytopathologie, Université Catholique de Louvain, Place Croix du Sud 2 bte 3, 1348 Louvain-la-Neuve, Belgium

C. Marchiafava, Dipt. di Scienze Biochimiche „A. Rossi Fanelli“ e Centro di Biologia Molecolare del CNR, Università „La Sapienza“, Roma, Italy

G. Marino, Servizio Spettrometria di Massa del CNR e Dipartimento di Chimica Organica e Biologica, Università „Federico II“, Napoli, Italy

Feliciano Mariotti, Dipt. di Scienze Biochimiche, Università di Roma "La Sapienza", P.le Aldo Moro 5, 00185 Roma, Italy

Mary C. Matteson, Dept. Plant Pathology, NYSAES - Cornell University, Geneva, NY 14456, U.S.A.

Rudolf May, FSU Jena, Techn. Mikrobiologie, Philosophenweg 12, 07743 Jena, Germany

Gilda L. Mena, CINVESTAV-IPN, Ap. Postal 629, Irapuato Gto, Mexico 36500, Mexico

Kurt Mendgen, Universität Konstanz, Fakultät für Biologie, Lehrstuhl Phytopathologie, D-78464 Konstanz, Germany

José Otávio M. Menten, USP/ESALQ Depto de Fitopatologia, Cx. Postal, 9 - 13418-900 Piracicaba, SP, Brazil

Manfred Meyer, Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Grisebachstr. 6, 37077 Göttingen, Germany

Timothy M. Milos, Cornell University, Department Plant Pathology, 334 Plant Science Building, Ithaca, NY 14853-4203, U.S.A.

Paola Minardi, Ist. di Patologia vegetale, Università degli Studi, Via Filippo Re 8, 40126 Bologna, Italy

Horst Mittelstädt, Universität Potsdam, Institut für Biochemie und Molekulare Physiologie, Fahrländer Str., 14476 Marquardt, Germany

Larry W. Moore, Dept. of Botany and Plant Pathology, Oregon State University, Cordley Hall 2082, Corvallis, Oregon 97331-2902, U.S.A.

Maria Morea, Ist. tossine e micotossine da parassiti vegetali, C.N.R., V. 1e L. Einaudi, 51, 70125 Bari, Italy

Nataliya Moskalenko, Institute of Microbiology and Virology, National Academy of Ukraine, Zabolotny Str. 154, 252143 Kiev, Ukraine

K.A. Mott, Dept. of Biology, Utah State University, Logan, U.S.A.

Jens Müller, Friedrich-Schiller Universität, Inst. f. Mikrobiologie, Philosophenweg 12, 07743 Jena, Germany

Petra Müller, Stahnsdorfer Damm 81, 14532 Kleinmachnow, Germany

Valentina Muras, Institute of Microbiology and Virology, National Academy of Ukraine, Zabolotny Str. 154, 252143 Kiev, Ukraine

Feridon Nabizadeh-Ardekani, Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Grisebachstr. 6, 37077 Göttingen, Germany

Nachat Nassan Nagha, University of Algiers, Institute of Agronomy, Department of Plant Protection, El Harrach, Algiers, Algeria

Klaus Naumann, Institut für Phytopathologie, Theodor-Roemer-Weg, 06449 Aschersleben, Germany

Mark Newsome, Dept. of Computer Science, Oregon State University, Corvallis, OR 97331 U.S.A.

Frank Niepold, Biologische Bundesanstalt, Messeweg 11/12, 38104 Braunschweig, Germany

Matthias Nöllenburg, Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Grisebachstr. 6, 37077 Göttingen, Germany

Jörg Nüske, FSU Jena, Techn. Mikrobiologie, Philosophenweg 12, 07743 Jena, Germany

Aleksa Obradovic, Center for Vegetables, Smed. Palanka 11420, Karadjordjeva 71, Jugoslavia

Laura Olalla, Dpt. Microbiologia, Fac. Ciencias, Univ. Málaga, 29071 Málaga, Spain

Araceli Oropeza, CINVESTAV-IPN, Ap. Postal 629, Irapuato Gto, Mexico 36500, Mexico

Silvia Ortega, Centro de Fitopatología, Colegio de Postgraduados, Montecillo, C.P. 56230, Edo de Mexico, Mexico

Péter G. Ott, Plant Protection Institute, Hungarian Academy of Sciences, 1525 Budapest, P.O. Box 102, Hungary

Volodymyr Ovod, Institute of Medical Technology, University of Tampere, Tampere, Finland

M. Paci, Dipartimento di Scienze e Tecnologie Chimiche, Università „Tor Vergata“, Roma, Italy

Gioacchino Palumbo, Istituto di Genetica, Università degli Studi di Bari, Via G. Amendola 165A, 70125 Bari, Italy

Cherri Pancake, Dept. of Computer Science, Oregon State University, Corvallis, OR 97331 U.S.A.

Nickolas Panopoulos, Department of Biology, University of Crete, P.O. 2208, Heraklion 71409, Crete, Greece

Lidia Pasichnik, Inst. of Microbiology and Virology, Zabolotny Str. 154, 252143 Kiev, Ukraine

Nandita Pathania, Dept. of Plant Pathology, Univ. of Horticulture and Forestry, Nauni-173230, Solan, H.P., India

Suresh S. Patil, University of Hawai'i at Manoa, Pacific Biomedical Research Center, 1993 East-West Road, Honolulu, Hawai'i 96822, U.S.A.

Alejandro Penaloza-Vazquez, CINVESTAV-IPN, Ap. Postal 629, Irapuato Gto, Mexico 36500, Mexico

Alejandro Pérez-García, Dpt. Microbiologia, Fac. Ciencias, Univ. Málaga, 29071 Málaga, Spain

Minna Pirhonen, Univ. of Maryland at College Park, Dept. of Botany, H.J. Patterson Hall, College Park, Maryland 20742-5815, U.S.A.

Rumyana Popova, Local Service for Plant Protection, 7500 Silistra, Bulgaria

Natalia Porembskaya, Inst. of Microbiology and Virology, Zabolotny Str. 154, Kiev 252143, Ukraine

Mat Poswal, University of Fort Hare, Dept. of Agronomy, Private Bag X 1314, Alice 5700, South Africa

Sutruedee Prathuangwong, Dept. of Plant Pathology, Kasetsart University, Chatuchack, 50 Phaholyothin Rd., Bangkok 10900, Thailand

Gail Preston, Cornell University, Department Plant Pathology, 334 Plant Science Building, Ithaca, NY 14853-4203, U.S.A.

Jay W. Pscheidt, Dept. of Botany and Plant Pathology, Oregon State University, Cordley Hall 2082, Corvallis, Oregon 97331-2902, U.S.A.

P. Pucci, Servizio Spettrometria di Massa del CNR e Dipartimento di Chimica Organica e Biologica, Università „Federico II“, Napoli, Italy

Nakul Puri, Dept. Biological, Sciences, Wye College, University of London, Wye, Ashford, Kent. TN25 5AH, U.K.

Mariá E. Rivera, Dpt. Microbiologia, Fac. Ciencias, Univ. Malaga, 29071 Malaga, Spain

Steven J. Roberts, Horticulture Research Intern., Plant Pathology Section, Wellesbourne, Warwick CV35 9EF, U.K.

Andrea Robinson-Smith, Dept. of Plant Pathology, AFRC-IACR, Rothamsted, Experimental Station, Harpenden, Herts, ALQ 2JQ, U.K.

Roland Rohringer, Institut für Pflanzenpathologie und Pflanzenschutz, Grisebachstr. 6, 37077 Göttingen, Germany

Elena Roine, Department of Biosciences, Division of General Microbiology, University of Helsinki, P.O. Box 56, SF-00014 Helsinki, Finland

Martin Romantschuk, Department of Biosciences, Division of General Microbiology, University of Helsinki, P.O. Box 56, SF-00014 Helsinki, Finland

Daniel Rowley, Univ. of Maryland at College Park, Dept. of Botany, H.J. Patterson Hall, College Park, Maryland 20742-5815, U.S.A.

Klaus Rudolph, Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Grisebachstr. 6, 37077 Göttingen, Germany

Regine Samson, INRA Station de Pathologie Végétale, 42 Rue Georges Morel, BP 57, 49071 Beaucouze Cedex, France

Miguel Santos, Centro de Fitopatologia, Colegio de Postgraduados, Montecillo, C.P. 56230, Edo de Mexico, Mexico

Andrea Scaloni, Servizio Spettrometria di Massa del C.N.R., Via Pansini 5, 80131 Naples, Italy

Norman W. Schaad, Foreign Disease, Weed Science Research, Fort Detrick, Building 1301, Frederick, Maryland 21702, U.S.A.

Heather J. Scheck, Dept. of Botany and Plant Pathology, Oregon State University, Cordley Hall 2082, Corvallis, Oregon 97331-2902, U.S.A.

Brenda K. Scholz, Washington State University, Dept. of Plant Pathology, Pullman WA 99164-6430, U.S.A.

Marco Scottichini, Istituto Sperimentale per la Patologia Vegetale, Via Bertero 22, 00156 Roma, Italy

A. Segre, Istituto di Strutturistica Chimica del CNR „G. Giacomello“, Monterotondo Scalo, Italy

Hussain Shafif, I.N.R.A., Station de Pathologie végétale et Phytobacteriologie, 42 Rue George Morel, 49071 Beaucouze Cedex, France

- M. Simmaco, Istituto di Scienze Biochimiche, Università „G. D'Annunzio“, Chieti, Italy
- Angelo Sisto, Ist. tossine e micotossine da parassiti vegetali, C.N.R., V. 1e L. Einaudi, 51, 70125 Bari, Italy
- Charles A. Smith, Dept. Plant Pathology, NYSAES - Cornell University, Geneva, NY 14456, U.S.A.
- Lyudmila Solyanik, Inst. of Microbiology and Virology, Ukrainian Academy of Sciences, Zabolotny Str. 154, Kiev-143 252143, Ukraine
- Bernd Sonnenberg, BASF AG, 67117 Limburgerhof, Germany
- David Stead, Central Science Laboratory, Sand Hutton, York, YO4 1LZ, U.K.
- Emilio Stefani, Ist. di Patologia Vegetale, Università di Bologna, via Filippo Re 8, I-40026 Bologna, Italy
- Conrad Stevens, Dept. Biological, Sciences, Wye College, University of London, Wye, Ashford, Kent. TN25 5AH, U.K.
- Gary A. Strobel, Dept. of Plant Pathology, Montana State University, Bozeman, U.S.A.
- Giuseppe Surico, Ist. Patol. Zoologia forestale e agraria, Univers. degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy
- Natassa Tabakaki, Department of Biology, University of Crete, P.O. 2208, Heraklion 71409, Crete, Greece
- John Y. Takemoto, Dept. of Biology, Utah State University, Logan, U.S.A.
- Yuichi Takikawa, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka, 422 Japan
- John Taylor, Horticulture Research International, Wellesbourne, Warwick, CV35 9EP, U.K.
- Dawn Teverson, Natural Resources Institute, Chatham Maritime, Kent, ME4 4TB, U.K.
- Hanna Toben, Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Grisebachstr. 6, 37077 Göttingen, Germany
- Juan A. Torés, Estación Experimental „La Mayora“, CSIC, Algarrobo-Costa, E-29750 Málaga, Spain
- George Tsiamis, Dept. Biological, Sciences, Wye College, University of London, Wye, Ashford, Kent. TN25 5AH, U.K.
- Kuersad Turgay, Phillips University, Hans Meerwein Str., 35032 Marburg, Germany
- Matthias Ullrich, Dept. Plant Pathology, Oklahoma State University, 110, Noble Research Center, Stillwater, OK 74078, U.S.A.
- Lyudmilla Varbanets, Institute of Microbiology and Virology, National Academy of Ukraine, Zabolotny Str. 154, 252143 Kiev, Ukraine
- Leonardo Varvaro, Dipartimento di Protezione delle Piante, Università degli Studi, Viterbo, Italy
- Milda Vasinauskienė, Institute of Botany, Zaliuju ezeru 47, 2021 Vilnius, Lithuania
- Vassil Vassilev, Inst. of Introduction and Plant Genetic Resources, 4122 Sadovo, Plovdiv, Bulgaria
- Petya Vassileva, University of Agriculture, Mendeleev Str. 12, 4000 Plovdiv, Bulgaria
- André Vigouroux, INRA, Lab. du Path. Végétale, 2 Place Viala, 34060 Montpellier Cedex 1, France

Carla Visca, Dipt. Sci. Chimico-Agrarie, Università di Napoli "Federico II", 80055 Portici, Italy

Alan Vivian, Dept. Biological Sciences, UWE-Bristol, Frenchay, Coldharbour Lane, Bristol BS16 1QY, U.K.

Beate Völksch, FSU Jena, Techn. Mikrobiologie, Philosophenweg 12, 07743 Jena, Germany

Jan von Kietzell, Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Grisebachstr. 6, 37077 Göttingen, Germany

Svitlana Votseko, Inst. of Microbiology and Virology, Zabolotny Str. 154, Kiev 252143, Ukraine

Helge Weingart, Friedrich-Schiller Universität, Inst. f. Mikrobiologie, Philosophenweg 12, 07743 Jena, Germany

Jeannine Weyns, Unité de Phytopathologie, Université Catholique de Louvain, Place Croix du Sud 2 bte 3, 1348 Louvain-la-Neuve, Belgium

Judith K. Wilson, Central Science Laboratory, Hatching Green, Harpenden, Hertfordshire, AL5 2BD, U.K.

Satish Wimalajeewa, 8 Adele Court, Ringwood, VIC 3134, Australia

Werner Witbooi, University of Fort Hare, Dept. of Agronomy, Private Bag X 1314, Alice 5700, South Africa

Eshetu Wondimagegne, Scientific Phytopathological Laboratory, Ambo, Ethiopia

Kerstin Wydra, Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Grisebachstr. 6, 37077 Göttingen, Germany

Yunlu Xie, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, 100094 Beijing, P.R. China

Lyudmila Yakovleva, Inst. of Microbiology and Virology, Zabolotny Str. 154, Kiev 252143, Ukraine

Petcharatana Yowabutra, Dept. of Plant Pathology, Kasetsart University, Chatuchack, 50 Phaholyothin Rd., Bangkok 10900, Thailand

Filomena Zaccaro, Ist. tossine e micotossine da parassiti vegetali, C.N.R., V. 1e L. Einaudi, 51, 70125 Bari, Italy

Irina Zakharova, Inst. of Microbiology and Virology, Ukrainian Academy of Sciences, Zabolotny Str. 154, Kiev-143 252143, Ukraine

Galina Zdrovenko, Inst. of Microbiology and Virology, Ukrainian Academy of Sciences, Zabolotny Str. 154, Kiev-143 252143, Ukraine

Wolfgang Zeller, Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Biologischen Pflanzenschutz, Heinrichstr. 243, 64287 Darmstadt, Germany

Jian-Hua Zhang, Washington State University, Dept. of Plant Pathology, Pullman WA 99164-6430, U.S.A.

Olga Zhrebilo, Inst. of Microbiology and Virology, Zabolotny Str. 154, Kiev 252143, Ukraine

Preface

During the last decade, research on *Pseudomonas syringae* pathovars and related pathogens has progressed rapidly, opening up many new avenues. The application of molecular genetics has provided new insights into determinants of pathogenicity and virulence. Progress has also been made in elucidating the chemical structures and modes of action of phytotoxins from *Pseudomonas syringae*; by establishing novel strategies for disease control; in biotechnological applications; by studying the resistant reaction of the plant with a combined biochemical and genetic approach; and in the development of new detection and identification methodologies as tools in epidemiological studies. With such rapid advances it becomes more and more difficult to keep abreast of the developments and concepts within disciplines, all involving research on pathovars of *P. syringae*.

In an attempt to provide a balanced overview, recent developments in these rapidly expanding fields have been critically reviewed at the beginning of each chapter by internationally renowned experts. Our comprehensive coverage has been made possible because all the contributors to this volume presented their latest findings at the "5th International Conference on *Pseudomonas Syringae* Pathovars and Related Pathogens" in Berlin, September 3-8, 1995. In this way, it was possible to bring together contributions from a wide range of fields including phytopathology, genetics, bacteriology, plant breeding, plant protection, and taxonomy. This book is not intended simply as a record of the proceedings of the Berlin Conference, but as an extension of recent findings and hypotheses put forward at the meeting. All papers published in this volume have been reviewed by the editors.

The term "Related Pathogens" includes predominantly other fluorescent phytopathogenic pseudomonads, but some contributions on *Pseudomonas* (now *Burkholderia* or *Ralstonia*) *solanacearum* have been included. The areas are divided in seven sections covering all aspects of recent studies: ecology and epidemiology, the resistant reaction of the plant, pathogenesis, determinants of pathogenicity,

genetic analysis of the host/plant-interaction, identification and taxonomy, and finally the important topic of disease control.

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Klaus Rudolph

Thomas J. Burr

John W. Mansfield

David Stead

Alan Vivian

Jan von Kietzell

Foreword

NAUMANN, KLAUS

Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Institut für Pathogendiagnostik,
Theodor-Roemer-Weg 4, D-06449 Aschersleben, Germany

ON THE TRACK OF ROBERT KOCH IN BERLIN

Berlin has played a significant role in the history of bacteriology. Thus, in the thirties of the last century CHRISTIAN GOTTFRIED EHRENBURG has worked in Berlin as secretary of the Prussian Academy of Sciences and as professor at the university. EHRENBURG was an excellent naturalist, who was very much engaged in several fields. During his microscopical studies he was the first scientist to detect sexuality in fungi and to observe flagella on bacterial cells. He described these and other fundamental aspects of microbiology in 1838 in his classical book "Die Infusionstierchen als vollkommene Organismen". As a result of his studies he classified the organisms we now call "bacteria" in three families: Monadina, Vibrionia and Cryptomonadina.

In the following decades further substantial progress towards the development of bacteriology in Germany was made by FERDINAND COHN, a professor at the Breslau (now Wroclaw) University. He later also became the principle scientific mentor of ROBERT KOCH, whose life and scientific activities - especially in connection with Berlin - will be shortly reviewed here.

Childhood and Youth

KOCH's course of life can be arranged in 5 creative stages:

1. childhood and education	23 years
2. physician (assistant and general practitioner) in the Prussian province	15 years
3. officer in the "Kaiserliches Gesundheitsamt" (Imperial Public Health Service) in Berlin	7 years
4. professor of hygiene at the Berlin University	7 years
5. founder and head resp. member of "Königl. Inst. f. Infektionskrankheiten" in Berlin	20 years

Altogether he spent more than 30 years of his life in Berlin.

ROBERT KOCH was born on November 11th, 1843 in Clausthal-Zellerfeld, a provincial town with a mining tradition in the Harz Mountains not far from Göttingen. His father was a respected miner, who later worked his way up to the position of a "Geheimer Bergrat".

ROBERT had 10 brothers and sisters so that his family had to live very economically.

As a pupil ROBERT liked to roam through meadows, fields and forests and to collect herbs, animals and minerals. He also preserved several animals in his room. Consequently, his parents held him responsible for the chicken and rabbits they kept.

Already in those days ROBERT dreamed of travelling to far continents.

School and University Time

Since 1850 ROBERT attended the High School at his home-town. In 1862 he took his school-leaving examination and started his study at the Göttingen University. Among his professors were the famous chemist FRIEDRICH WÖHLER and the anatomist JACOB HENLE who instructed him in microscopical histology.

Medical Practitioner in the Province

In 1866 after his promotion to medical doctor and passing the government examination he left the university and started a 15 year long troublesome career as general practitioner. After short stays in different places he went to Rakwitz, a small town in the province Posen, where he worked as country doctor for 4 years.

As a medical practitioner he was frustrated by his inability to help and to protect children and adults against severe epidemics such as diphtheria or tuberculosis.

In 1872 after passing an additional government examination he was able to improve his economic situation by taking over a position as "Kreisphysikus" at Wollstein, a provincial town near Rakwitz, and opened a new practice.

The Beginning of Scientific Activities

In spite of his many duties in his new position and his extensive practice, in the spring of 1873 he started lively experimental activities. Already during his study at Göttingen he had used each opportunity to carry out scientific investigations. Thus, he successfully took part in a competition at the university and in experiments about the production of succinic acid in the human body. Both treatises were published and proved his scientific qualification impressively. His paper concerning the origin of this acid was the base of his promotion as a medical doctor.

At Wollstein he purchased a microscope from his savings and established a simple laboratory that was separated from his consulting room only by a curtain.

At first he used his microscope without a distinct aim, but soon, inspired by a severe outbreak of anthrax in the province Posen, he devoted himself to the cause of that disease. He studied the blood and the organs of the deceased sheep and cattle microscopically and soon he observed many suspect rod-shaped objects between the blood corpuscles that had never been found in the blood of healthy animals. Thereafter he tried to transmit the disease by injecting blood from dead sheep to experimental animals. Afterwards he attempted to isolate and to cultivate the pathogen. After many futile trials he found that the eyewater of oxen was suitable for cultivation of bacteria. To obtain pure cultures he developed the "hanging drop" technique. By injecting the cells cultivated in this way KOCH was able to induce the characteristic symptoms in white mice. He also detected the ability of the causal agent to form dry resistant spores, which could survive for a long time under pasture conditions (Fig. 1).

Fig. 1: KOCH's original drawings of *Bacillus anthracis* cells producing spores

When he had established the etiology of anthrax, in the spring of 1876 KOCH wanted to discuss his results with an expert in lower organisms including bacteria. Therefore, he requested the already mentioned Prof. FERDINAND COHN at the Breslau University for an opportunity to present his findings. COHN quickly accepted and after a short time KOCH was able to demonstrate his microscopical preparations and transmission experiments in COHN's laboratory. COHN and the famous pathologist JULIUS COHNHEIM who was also in attendance, were very impressed with KOCH's results. Both scholars became his influential promoters in the following years.

Stimulated by the discussions at Breslau KOCH wrote his famous paper "Die Ätiologie der Milzbrandkrankheit, begründet auf der Entwicklungsgeschichte des *Bacillus anthracis*". It was published in COHN's journal "Beiträge zur Biologie der Pflanzen" and established KOCH's scientific reputation. In this publication he prophetically expressed the suggestion, that cholera and typhus, in the same manner as anthrax, could also be caused by schizophytic organisms.

Thereafter he turned to wound infections. Shortly after greatly improved microscopes were available, he was able to demonstrate that several wound infections were caused by bacteria. In this time he also succeeded in isolating and cultivating the causal agent of the relapsing fever, *Borrelia recurrentis*.

As a Health Service Officer in Berlin

KOCH found it difficult to find employment which would allow him to work exclusively in research, despite help of COHN. However in 1880 with the vigorous support of its director, Dr. STRUCK, KOCH was appointed an ordinary member of the newly founded "Kaiserliches Gesundheitsamt", the German National Health Service, in Berlin with the task to head the laboratory.

At first this new institution worked under simple conditions in the Luisenstraße in the centre of the city. By now for the first time KOCH had the opportunity to conduct experiments exclusively without the daily medical duties.

In Berlin, KOCH first continued his investigations to improve bacteriological methods which he began at Wollstein. KOCH, who was an inspired expert in carrying out experiments, did pioneering work by creating the fundamentals of classical microbiology. KOCH's main achievements were:

- the comprehensive use of the microscope in microbiology especially for diagnostic purposes
- the development of new staining methods in bacteriology, especially by the successful application of aniline dyes
- the extensive application of microphotographical technique for the documentation of microscopical preparations and above all
- the introduction of solid media for the isolation of microorganisms and the production of pure cultures.

The methods and principles established by KOCH provided a great impetus in the areas of medical microbiology and also plant pathology in this century.

In his second year in Berlin, KOCH started studies on the causes of tuberculosis, which in that time was considered one of the most dangerous diseases. Half a year later, in March 1882, he was able to present his sensational results in a memorable lecture which was arranged by the "Berliner Physiologische Gesellschaft". Already three weeks later the lecture was published in the "Berliner Klinische Wochenblatt". That publication finally established the world-wide reputation of ROBERT KOCH and in 1883, KOCH was appointed the head of the German cholera expedition to Egypt and to India, where he finally succeeded in clearly identifying the cause and manner of transmission of cholera. In this way, the fundamentals of prevention and control of this terrible disease were established.

The library hall in the Physiological Institute of the Berlin University in the former Dorotheenstraße, where the lecture took place, survived the last war and is to be seen there.

KOCH as University Professor

In 1885, when at the Berlin University a chair of hygiene was established, KOCH was appointed as professor (Fig. 2). The new institute at that time accommodated in the Klosterstraße, was his second domain in Berlin. During his time as university teacher KOCH attracted many students from far and near. However, his work as a lecturer was very laborious and time-consuming, allowing little time for experiments. Thus, in 1890 KOCH requested to be released from this task. Simultaneously, he suggested the foundation of a National Institute for Infectious Diseases, and after passing through the parliament this Royal Institute containing a research and a clinical department was established in 1891.

Fig. 2: Portrait of R. KOCH in the age of 45 years (1889)

The Foundation of the Royal Institute for Infectious Diseases

This new institution became KOCH's third and invariably last domain in Berlin. From the beginning to 1904 he worked as the head of the Royal Institute for Infectious Diseases which now carries his name. It is located in a Northern district of Berlin.

In that time he continued his studies on tuberculosis and its control. He worked hard to improve the preparation "tuberkulin" which he had developed some years before not only as a diagnostic, but also as medicine against this disease. In this case he did not achieve remarkable success, as the only possibilities to control this public disease remained at this time the improvement of population living conditions, and to prevent the transmission of the causal agent by strict isolation of the infected persons.

The time of the big expeditions

During his activities at the Royal Institute he also made several long expeditions to tropical countries to study on the etiology of some dreadful diseases attacking the inhabitants or their animals. The following list shows a synopsis of his expeditions:

- After the already mentioned cholera expedition to Egypt and India
- he travelled to South Africa because of a cattle plague outbreak,
- to India and Eastern Africa because of the plague (the pathogen his pupil KITASATO and the French physician YERSIN had detected before),
- to Italy, Java and New Guinea because of malaria,
- once more to South Africa because of the African coast fever of cattle and
- finally to Uganda because of the sleeping sickness.

In all these cases he succeeded in finding the causal agents that were responsible and in clearing up the epidemiology of these diseases (Fig. 3).

The last years

In 1904 KOCH retired from his position as the head of the Royal Institute, but he remained a member of the institution and continued his scientific activities,

especially concerning the epidemiology of tuberculosis.

On May 27th, 1910, he died while undergoing a treatment at Baden-Baden.

Fig. 3: ROBERT KOCH in his field laboratory at Kimberley / South Africa (ca. 1905)

Influence on the posterity and his significant pupils

In the course of his splendid career ROBERT KOCH obtained numerous honours because of his extraordinary merits for the scientific and medical progress before the first world war. In 1890 he became honorary citizen of Berlin, and in 1905 he won the Nobel prize for Medicine. However, KOCH's influence on the development of the bacteriology, microbiology and control of infectious diseases of the world did not end with his death. Further progress was made by his numerous excellent pupils, particularly

FRIEDRICH LOEFFLER (diphtheria, MKS-virus)
 GEORG GAFFKY (typhus)
 RICHARD PFEIFFER (cholera control)
 SHIBASABURO KITASATO (plague, tetanus, bacillary dysentery)
 EMIL v. BEHRING (diphtheria immunization) and
 PAUL EHRLICH (chemotherapy)

Even today research on infectious diseases of plants would be inconceivable and impossible without the methods developed and postulates layed down by ROBERT KOCH in Berlin more than 100 years ago.

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