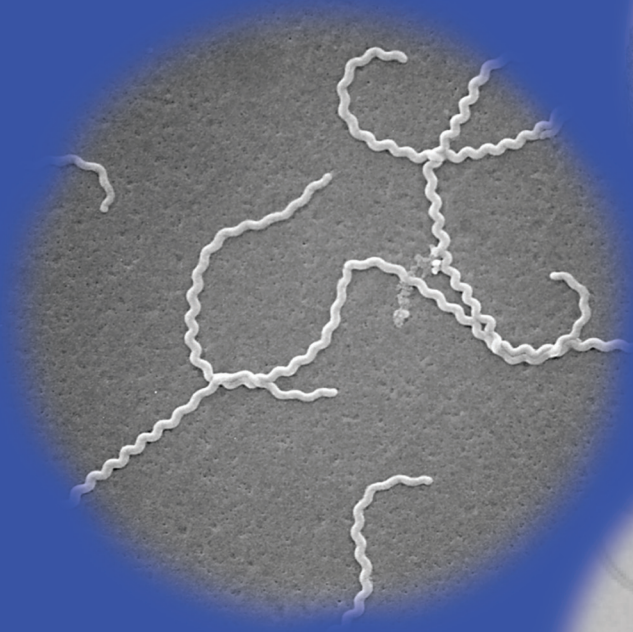
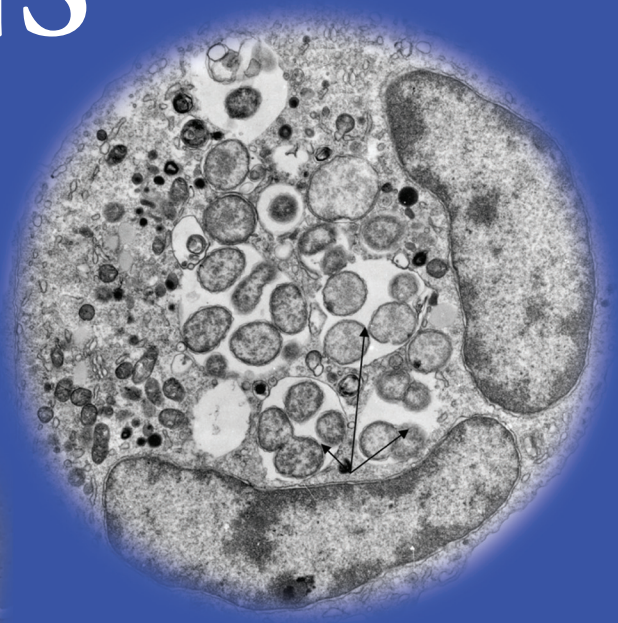
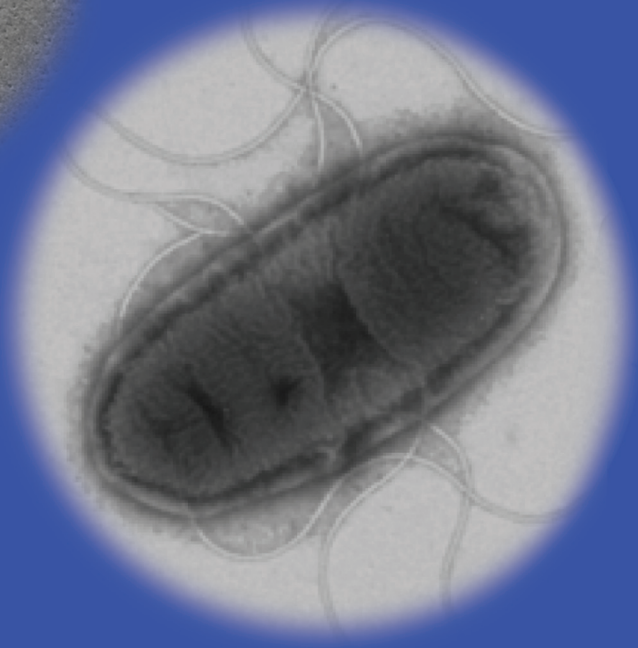
MOLECULAR DETECTION OF HUMAN BACTERIAL PATHOGENS







EDITED BY
DONGYOU LIU

CRC Press
Taylor & Francis Group

MOLECULAR DETECTION OF HUMAN BACTERIAL PATHOGENS

MOLECULAR DETECTION OF HUMAN BACTERIAL PATHOGENS

EDITED BY
DONGYOU LIU

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Version Date: 20110629

International Standard Book Number-13: 978-1-4398-1239-6 (eBook - PDF)

This volume is dedicated to a group of bacteriologists whose insight, knowledge, and expertise have made the all-inclusive coverage of major human bacterial pathogens a reality.

Contents

Clostridia

Mollicutes

SECTION III Bacteroidetes, Chlamydiae, and Fusobacteria

SECTION IV Proteobacteria

Alphaproteobacteria

Betaproteobacteria

Gammaproteobacteria

Epsilonproteobacteria

SECTION V Spirochaetes

SECTION VI Pan-Bacterial Detection

Preface

Bacteria are small, unicellular organisms that are invisible to the naked eye but are nonetheless present ubiquitously and abundantly in all environments. Although a majority of bacteria are free-living and symbiotic, some are capable of leading a parasitic life, inducing a range of disease syndromes in human and animal hosts during the process. Among the most devastating bacterial pathogens, *Yersinia pestis*, the causative agent of bubonic plague, was responsible for three major human pandemics in history, killing 200 million people prior to the advent of antibiotics. The current, most common fatal bacterial diseases are tuberculosis (caused by *Mycobacterium tuberculosis*), killing about 2 million people a year alone, and cholera (caused by *Vibrio cholerae*). Other globally important bacterial diseases include pneumonia (caused by *Streptococcus* and *Pseudomonas*), tetanus, typhoid fever, diphtheria, syphilis, and leprosy.

Traditionally, bacteria have been identified and diagnosed with the help of various phenotypic procedures, such as Gram stain, morphological, biochemical, and serological examination. Since the phenotypic techniques are often slow and lack desired specificity and reproducibility, nucleic acid amplification technologies such as polymerase chain reaction (PCR) have played an increasingly prominent role in the laboratory diagnosis of bacterial infections. Given their ability to specifically detect a single copy of bacterial nucleic acid template in a matter of hours, PCR-based assays offer unsurpassed sensitivity, specificity, accuracy, precision, and result availability for bacterial identification. The recent advances in instrumentation automation and probe chemistries have facilitated the development of real-time PCR that provides a convenient platform for high throughput detection and quantitation of bacterial pathogens in clinical specimens.

Considering that numerous original molecular protocols and subsequent modifications have been described and scattered in various journals and monographs, it has become difficult if not impossible for someone who has not been directly involved in the development of original or modified protocols to know which are most appropriate to adopt for accurate identification of bacterial pathogens of interest. The purpose of this volume is to address this issue, with international scientists in respective bacterial pathogen research and diagnosis providing expert summaries on current diagnostic approaches for major human bacterial pathogens. Each chapter consists of a brief review of the classification, epidemiology, clinical features, and diagnosis of an important pathogenic bacterial genus; an outline of clinical sample collection and preparation procedures; a selection of representative stepwise molecular protocols; and a discussion on further research requirements relating to improved diagnosis. This book represents a reliable and convenient reference on molecular detection and identification of major human bacterial pathogens; an indispensable tool for upcoming and experienced medical, veterinary, and industrial laboratory scientists engaged in bacterial characterization; and an essential textbook for undergraduate and graduate students majoring in bacteriology.

A comprehensive and inclusive book such as this is undoubtedly beyond an individual's capacity. I am fortunate and honored to have a large panel of bacteriologists as chapter contributors, whose detailed knowledge and technical insights on human bacterial pathogen detection have greatly enriched this book. In addition, the professionalism and dedication of executive editor Barbara Norwitz and senior project coordinator Jill Jurgensen at CRC Press have enhanced its presentation. Finally, without the understanding and support of my family, Liling Ma, Brenda, and Cathy, the compilation of this all-encompassing volume would have not been possible.

Editor

Dongyou Liu, PhD, undertook his veterinary science education at Hunan Agricultural University, China. Upon graduation, he received an Overseas Postgraduate Scholarship from the Chinese Ministry of Education to pursue further training at the University of Melbourne, Australia, where he worked toward improved immunological diagnosis of human hydatid disease. During the past two decades, he has crisscrossed between research and clinical laboratories in Australia and the United States of America, with focuses on molecular characterization and virulence determination of microbial pathogens such as ovine footrot bacterium (*Dichelobacter nodosus*), dermatophyte fungi (*Trichophyton, Microsporum,* and *Epidermophyton*), and listeriae (*Listeria* species). He is the senior author of more than 50 original research and review articles in various international journals and the editor of the recently released *Handbook of* Listeria monocytogenes, *Handbook of Nucleic Acid Purification*, *Molecular Detection of Foodborne Pathogens*, and *Molecular Detection of Human Viral Pathogens*, as well as the forthcoming *Molecular Detection of Human Fungal Pathogens* and *Molecular Detection of Human Parasitic Pathogens*, all of which are published by CRC Press.

Contributors

Martina Adamek
Karlsruhe Institute of Technology
Institute of Functional Interfaces
Karlsruhe, Germany

Catherine C. Adley
Microbiology Laboratory
Department of Chemical and Environmental Sciences
University of Limerick
Limerick, Ireland

Sascha Al Dahouk
Department of Internal Medicine III
RWTH Aachen University
Aachen, Germany

Rodrigo Alonso
Faculty of Pharmacy
Department of Immunology, Microbiology and Parasitology
University of the Basque Country
Vitoria-Gasteiz, Spain

Maria Silvana Alves
Faculdade de Farmácia e Bioquímica
Universidade Federal de Juiz de Fora
Minas Gerais, Brazil

Paula Lopes Alves
Instituto de Biologia Experimental e Tecnológica
Oeiras, Portugal

Emilio Aranda
Ciencia y Tecnología de los Alimentos
Escuela de Ingenierías Agrarias
Badajoz, Spain

Marco Arosio
USC Microbiology and Virology
A.O. Ospedali Riuniti
Bergamo, Italy

Aurora Fernández Astorga
Faculty of Pharmacy
Department of Immunology, Microbiology and Parasitology
University of the Basque Country
Vitoria-Gasteiz, Spain

Frank W. Austin
College of Veterinary Medicine
Mississippi State University
Starkville, Mississippi

Mario J. Avila-Campos
Department of Microbiology
Institute of Biomedical Science
University of São Paulo
São Paulo, Brazil

R. Aznar
Department of Microbiology and Ecology
University of Valencia
Valencia, Spain

Diprabhanu Bakshi
Syngene International
Bangalore, India

Stephan Bathe
German National Academic Foundation
Bonn, Germany

Harsh Vardhan Batra
Defence R & D Establishment
Gwalior, India

Asim Bej
Department of Biology
University of Alabama at Birmingham
Birmingham, Alabama

Scott C. Bell
Department of Thoracic Medicine
The Prince Charles Hospital
Brisbane, Australia

María J. Benito
Ciencia y Tecnología de los Alimentos
Escuela de Ingenierías Agrarias
Badajoz, Spain

Patrick Boiron
Research group on Bacterial Opportunistic Pathogens and Environment
Université de Lyon
Lyon, France

Dianna J. Bopp
Wadsworth Center
New York State Department of Health
Albany, New York

Alejandra Bosch
Facultad de Ciencias Exactas
Centro de Investigación y Desarrollo en Fermentaciones Industriales
Universidad Nacional de La Plata
La Plata, Argentina

Javier Enrique Botero
School of Dentistry
Grupo de Investigación Básica y Clínica en Periodoncia y Oseointegración
Universidad de Antioquia
Medellín, Colombia

Germán Bou
Servicio Microbiología
Complejo Hospitalario Universitario La Coruña
La Coruña, Spain

June Brown
Actinomycete Reference Laboratory
Bacterial Zoonoses Branch
Division of Foodborne, Bacterial and Mycotic Diseases
National Center for Zoonotic, Vector-Borne, and Enteric Diseases
Centers for Disease Control and Prevention
Atlanta, Georgia

Renato Corrêa Viana Casarin
Department of Oral Diagnostics
Division of Microbiology and Immunology
Piracicaba Dental School
University of Campinas
Campinas, Brazil

Nicholas Cassai
Department of Pathology and Laboratory Medicine
New York Harbor VA Medical Center
Brooklyn, New York

Marco Cassone
Sbarro Health Research Organization
College of Science and Technology
Temple University
Philadelphia, Pennsylvania

Bianca Castiglioni
Department of Veterinary Pathology, Hygiene and Public Health
University of Milan
Milan, Italy

Rita Castro
Instituto de Higiene e Medicina Tropical
Universidade Nova de Lisboa
Lisbon, Portugal

Tsung Chain Chang
Department of Medical Laboratory Science and Biotechnology
College of Medicine
National Cheng Kung University
Tainan, Taiwan

Rama Chaudhry
Department of Microbiology
All India Institute of Medical Sciences
New Delhi, India

Jianshun Chen
Institute of Preventive Veterinary Medicine
Zhejiang University
Zhejiang, China

Ameur Cherif
Faculté des Sciences de Tunis
Laboratoire Microorganismes et Biomolécules Actives
Campus Universitaire
Tunis, Tunisie

Bishwanath Kumar Chourasia
Department of Microbiology
All India Institute of Medical Sciences
New Delhi, India

Henrik Christensen
Faculty of Life Sciences
Department of Veterinary Pathobiology
Center for Applied Bioinformatics
Copenhagen University
Frederiksberg, Denmark

Deirdre L. Church
Departments of Pathology, Laboratory Medicine, and Medicine
University of Calgary
Alberta Health Services
and
Calgary Laboratory Services
Alberta, Canada

Lorenzo Ciceroni
Center for Research and Evaluation of Immunobiologicals
Istituto Superiore di Sanità
Rome, Italy

Alessandra Ciervo
Department of Infectious, Parasitic and Immune-Mediated Diseases
Istituto Superiore di Sanità
Rome, Italy

Lena Ciric
Microbial Diseases
UCL Eastman Dental Institute
London, United Kingdom

Ana Paula Vieira Colombo
Department of Medical Microbiology
Institute of Microbiology
Federal University of Rio de Janeiro
Rio de Janeiro, Brazil

Andréa Vieira Colombo
Institute of Biomedical Sciences
State University of São Paulo
Sao Paulo, Brazil

Adolfo Contreras
School of Dentistry
Grupo de Medicina Periodontal
Universidad del Valle
Cali, Colombia

Piet Cools
Department of Clinical Biology, Immunology and Microbiology
Ghent University Hospital
Ghent, Belgium

Juan J. Córdoba
Facultad de Veterinaria
Higiene de los Alimentos
Universidad de Extremadura
Cáceres, Spain

María G. Córdoba
Ciencia y Tecnología de los Alimentos
Escuela de Ingenierías Agrarias
Badajoz, Spain

Yvon Cormier
Research Unit
Laval Hospital Centre of Pneumology
University of Laval
Quebec, Canada

Paola Cremonesi
Department of Veterinary Pathology, Hygiene and Public Health
University of Milan
Milan, Italy

Maria Teresa Barreto Crespo
Instituto de Biologia Experimental e Tecnológica
Oeiras, Portugal

Daniele Daffonchio
Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche
Università degli Studi di Milano
Milan, Italy

Sharvari Dalal
Department of Pathology
SUNY Downstate Medical Center
Brooklyn, New York

Maria Dalamaga
Laboratory of Clinical Biochemistry
Attikon General University Hospital
Medical School, National & Kapodistrian University of Athens
Athens, Greece

George N. Dalekos
Department of Medicine, Medical School
University of Thessaly
Larissa, Greece

Anupam Das
Department of Microbiology
All India Institute of Medical Sciences
New Delhi, India

Gregory A. Dasch
Rickettsial Zoonoses Branch
Division of Vector-Borne Diseases
Centers for Disease Control and Prevention
Atlanta, Georgia

Jonathan J. Dennis
Department of Biological Sciences
University of Alberta
Alberta, Canada

Rubens Clayton da Silva Dias
Faculdade de Medicina
Universidade Federal do Rio de Janeiro
Rio de Janeiro, Brazil

Xiaoming Dong
Beijing Wantai Biological Pharmacy Enterprise
Beijing, China

Caroline Duchaine
Research Unit
Laval Hospital Centre of Pneumology
University of Laval
Quebec, Canada

Francis Dziva
Division of Microbiology
Institute for Animal Health
Berkshire, United Kingdom

P. Elizaquível
Department of Microbiology and Ecology
University of Valencia
Valencia, Spain

Marina E. Eremeeva
Rickettsial Zoonoses Branch
Division of Vector-Borne Diseases
Centers for Disease Control and Prevention
Atlanta, Georgia

Emenike Ribs K. Eribe
Norwegian Geotechnical Institute
Oslo, Norway

Klaus Eschrich
Institute for Biochemistry
School of Medicine
University of Leipzig
Leipzig, Germany

Weihuan Fang
Institute of Preventive Veterinary Medicine
Zhejiang University
Zhejiang, China

Seamus Fanning
Centre for Food Safety
School of Agriculture, Food Science and Veterinary Medicine
Veterinary Sciences Centre
University College Dublin
Dublin, Ireland

María Fiuza
Faculty of Biology
Section of Microbiology
Department of Molecular Biology
University of León
León, Spain

Maria Fredriksson-Ahomaa
Ludwig-Maximilian University
Munich, Germany

Elerson Gaetti-Jardim Jr.
Department of Oral Pathology
São Paulo State University
São Paulo, Brazil

Ana Cristina Gales
Departamento de Medicina
Universidade Federal de São Paulo
São Paulo, Brazil

María-Luisa García-López
Veterinary Faculty
Department of Food Hygiene and Food Microbiology
University of León
León, Spain

Maria del Mar Garcia-Suarez
Area de Microbiologia
Departamento de Biologia Funcional Instituto
Universitario de Biotecnologia de Asturias
Asturias, Spain

José A Gil
Faculty of Biology
Section of Microbiology
Department of Molecular Biology
University of León Campus de Vegazana
León, Spain

Cecilia Girbau
Faculty of Pharmacy
Department of Immunology, Microbiology and Parasitology
University of the Basque Country
Vitoria-Gasteiz, Spain

John I. Glass
J. Craig Venter Institute
Rockville, Maryland

Maryna Golovchenko
Faculty of Science
University of South Bohemia
Ceské Budějovice, Czech Republic

Reginaldo Bruno Gonçalves
Department of Oral Diagnostics
Division of Microbiology and Immunology
Piracicaba Dental School
University of Campinas
Campinas, Brazil

Keith Grimwood
Queensland Paediatric Infectious Diseases Laboratory
Royal Children's Hospital Queensland Children's Medical Research Institute
Queensland, Australia

Libor Grubhoffer
Biology Centre
Institute of Parasitology
Academy of Sciences of the Czech Republic
Ceské Budějovice, Czech Republic

Miguel Gueimonde
Department of Microbiology and Biochemistry of Dairy Products
Instituto de Productos Lácteos de Asturias
Consejo Superior de Investigaciones Científicas
Asturias, Spain

Saija Hallanvuo
Finnish Food Safety Authority Evira
National Public Health Institute
Helsinki, Finland

Rudy A. Hartskeerl
WHO/FAO/OIE
and
National Collaborating Centre for Reference and Research on Leptospirosis
Department KIT Biomedical Research
Royal Tropical Institute
Amsterdam, the Netherlands

John P. Hays
Department of Medical Microbiology and Infectious Diseases
Rotterdam, the Netherlands

Alexander M. Hirschl
Division of Clinical Microbiology
Department of Laboratory Medicine
Medical University Vienna
Vienna, Austria

Jeffrey Hoorfar
National Food Institute
Technical University of Denmark
Søborg, Denmark

Kurt Houf
Faculty of Veterinary Medicine
Department of Veterinary Public Health and Food Safety
Ghent University
Ghent, Belgium

Satoru Inagaki
Department of Microbiology
Oral Health Science Center
Tokyo Dental College
Chiba, Japan

Kazuyuki Ishihara
Department of Microbiology
Oral Health Science Center
Tokyo Dental College
Chiba, Japan

Nahed Ismail
Department of Pathology
Meharry Medical College
Nashville, Tennessee

Carol Iversen
Centre for Food Safety
School of Agriculture
Food Science and Veterinary Medicine
Veterinary Sciences Centre
University College Dublin
Dublin, Ireland

Jari Jalava
Department of Infectious Disease Surveillance and Control
National Institute for Health and Welfare
Turku, Finland

Judith Jansen
Medical Faculty
Institute of Immunology
RWTH Aachen University
Aachen, Germany

Adriana Jaramillo
School of Dentistry
Grupo de Medicina Periodontal
Universidad del Valle
Cali, Colombia

Mathilde H. Josefsen
National Food Institute
Technical University of Denmark
Søborg, Denmark

Estelle Jumas-Bilak
Universitaire de Montpellier
Hôpital Arnaud de Villeneuve
Laboratoire de Bactériologie
Montpellier, France

Bernhard Kaltenboeck
Molecular Diagnostics Laboratory
Department of Pathobiology
College of Veterinary Medicine
Auburn University
Auburn, Alabama

Mireille M. Kattar
Department of Laboratory Medicine and Pathology
University of Alberta Hospital
Alberta, Canada

Timothy J. Kidd
Queensland Paediatric Infectious Diseases Laboratory
Royal Children's Hospital
Queensland Children's Medical Research Institute
Queensland, Australia

Rickard Knutsson
Swedish National Veterinary Institute
Uppsala, Sweden

Eija Könönen
University of Turku
Institute for Dentistry
Turku, Finland

Hannu Korkeala
Faculty of Veterinary Medicine
Department of Food and Environmental Hygiene
University of Helsinki
Helsinki, Finland

Kiersten J. Kugeler
Centers for Disease Control and Prevention
National Center for Zoonotic Vector-Borne and Enteric Diseases
Division of Vector-Borne Infectious Diseases, Bacterial Diseases Branch
Fort Collins, Colorado

Susanne Thisted Lambertz
Research and Development Department
National Food Administration
Uppsala, Sweden

K.A. Lampel
Food and Drug Administration
Division of Microbiology
College Park, Maryland

Brent A. Lasker
Actinomycete Reference Laboratory
Bacterial Zoonoses Branch
Division of Foodborne, Bacterial and Mycotic Diseases
National Center for Zoonotic, Vector-Borne, and Enteric Diseases
Centers for Disease Control and Prevention
Atlanta, Georgia

Susanna K.P. Lau
Department of Microbiology
The University of Hong Kong
Hong Kong

Kevin B. Laupland
Departments of Medicine, Critical Care Medicine, Community Health Sciences, and Pathology and Laboratory Medicine, and Centre for Antimicrobial Resistance
University of Calgary, Alberta Health Services, and Calgary Laboratory Services
Alberta, Canada

Frédéric Laurent
Faculté de Médecine Laennec
Lyon, France

Marc Lehmann
SIRS-Lab GmbH
Jena, Germany

Angelika Lehner
Vetsuisse Faculty
Institute for Food Safety and Hygiene
University of Zurich
Zurich, Switzerland

Manuel L. Lemos
Department of Microbiology and Parasitology
Institute of Aquaculture
University of Santiago de Compostela
Santiago de Compostela, Galicia, Spain

Michal Letek
Faculty of Biology
Section of Microbiology
Department of Molecular Biology
University of León Campus de Vegazana
León, Spain

Dongyou Liu
BioSecurity Quality Assurance Program
Royal College of Pathologists of Australasia
New South Wales, Australia

Charlotta Löfström
National Food Institute
Technical University of Denmark
Søborg, Denmark

José Maria Luengo
Department of Molecular Biology
University of León
León, Spain

Karlene H. Lynch
Department of Biological Sciences
University of Alberta
Alberta, Canada

Athanasios Makristathis
Division of Clinical Microbiology
Department of Laboratory Medicine
Medical University Vienna
Vienna, Austria

Fabiola Mancini
Department of Infectious, Parasitic and Immune-Mediated Diseases
Istituto Superiore di Sanità
Rome, Italy

Chaysavanh Manichanh
Digestive System Research Unit
University Hospital Vall d'Hebron
Bioinformatics and Genomics Program
Center for Genomic Regulation
Barcelona, Spain

Angel Manteca
Area de Microbiologia
Departamento de Biologia Funcional
Instituto Universitario de Biotecnologia de Asturias
Universidad de Oviedo
Asturias, Spain

Hélène Marchandin
Faculté de Pharmacie
Université Montpellier 1
Laboratoire de Bactériologie-Virologie-Contrôle Microbiologique
Montpellier, France

Abelardo Margolles
Department of Microbiology and Biochemistry of Dairy Products
Instituto de Productos Lácteos de Asturias
Consejo Superior de Investigaciones Científicas
Asturias, Spain

Luis M. Mateos
Faculty of Biology
Section of Microbiology
Department of Molecular Biology
University of León Campus de Vegazana
León, Spain

Johanna Maukonen
VTT Technical Research Centre of Finland
Espoo, Finland

Jere W. McBride
Department of Pathology
University of Texas Medical Branch at Galveston
Galveston, Texas

Andrew McDowell
Centre of Infection & Immunity
School of Medicine, Dentistry and Biomedical Sciences
Queen's University
Belfast, Northern Ireland

Francisco J. Mendez
Area de Microbiologia
Departamento de Biologia Funcional
Instituto Universitario de Biotecnologia de Asturias
Universidad de Oviedo
Asturias, Spain

Kåre Mølbak
Statens Serum Institut
Copenhagen, Denmark

Jens Kjølseth Møller
Department of Clinical Microbiology
Institute of Regional Health Services Research
University of Southern Denmark
Odense, Denmark

Beatriz Meurer Moreira
Instituto de Microbiologia
Universidade Federal do Rio de Janeiro
Rio de Janeiro, Brazil

Paolo Moroni
Quality Milk Production Services
Cornell University
Ithaca, New York

Benjamin D. Moser
Actinomycete Reference Laboratory
Bacterial Zoonoses Branch
Division of Foodborne, Bacterial and Mycotic Diseases
National Center for Zoonotic, Vector-Borne, and Enteric Diseases
Centers for Disease Control and Prevention
Atlanta, Georgia

Dipalok Mukherjee
Biological Sciences
Mississippi State University
Starkville, Mississippi

Soma Mukherjee
Palli Shikha Bhavan
Sriniketan, Birbhum
West Bengal, India

Kimberlee A. Musser
Wadsworth Center
New York State Department of Health
Albany, New York

Beth Mutai
Global Emerging Infections Surveillance System
U.S. Army Medical Research Unit
Kenya Medical Research Institute
Narombi, Kenya

Germán Naharro
Department of Animal Health
University of León
León, Spain

Futoshi Nakazawa
Department of Oral Microbiology
School of Dentistry, Health Sciences
University of Hokkaido
Hokkaido, Japan

Elizabeth J. Nazarian
Wadsworth Center
New York State Department of Health
Albany, New York

Heinrich Neubauer
Friedrich-Loeffler-Institut
Institute of Bacterial Infections and Zoonoses
Jena, Germany

Juan P. Olano
Department of Pathology
University of Texas Medical Branch at Galveston
Galveston, Texas

James H. Oliver, Jr.
Georgia Southern University
The James H. Oliver, Jr. Institute of Arthropodology and Parasitology
Statesboro, Georgia

Ingar Olsen
Faculty of Dentistry
Institute of Oral Biology
University of Oslo
Oslo, Norway

Katharina E.P. Olsen
Statens Serum Institut
Copenhagen, Denmark

Efrén Ordoñez
Faculty of Biology
Section of Microbiology
Department of Molecular Biology
University of León
León, Spain

Andrés Otero
Veterinary Faculty
Department of Food Hygiene and Food Microbiology
University of León
León, Spain

Carlos R. Osorio
Department of Microbiology and Parasitology
Institute of Aquaculture
University of Santiago de Compostela
Compostela, Spain

Jie Ouyang
Department of Pathology
SUNY Downstate Medical Center
Brooklyn, New York

Vanya Paralanov
J. Craig Venter Institute
Rockville, Maryland

Sheila Patrick
Centre of Infection & Immunity
School of Medicine, Dentistry and Biomedical Sciences
Queen's University
Belfast, Northern Ireland

Biswajit Paul
Escorts Heart Institute and Research Centre
New Delhi, India

Debarati Paul
College of Veterinary Medicine
Mississippi State University
Starkville, Mississippi

Debosmita Paul
Jamia Millia Islamia
New Delhi, India

Ana Isabel Pelaez
Area de Microbiologia
Departamento de Biologia Funcional
Instituto Universitario de Biotecnologia de Asturias
Universidad de Oviedo
Asturias, Spain

Claire Pelletier
Laboratoire Départemental d'Analyses de Saône-et-Loire
Mâcon, France

J. Tony Pembroke
Molecular and Structural Biochemistry Laboratory
Department of Chemical and Environmental Sciences
University of Limerick
Limerick, Ireland

Filomena Martins Pereira
Instituto de Higiene e Medicina Tropical Universidade Nova de Lisboa
Lisbon, Portugal

Jeannine M. Petersen
Centers for Disease Control and Prevention
National Center for Zoonotic, Vector-Borne and Enteric Diseases
Division of Vector-Borne Infectious Diseases, Bacterial Diseases Branch
Fort Collins, Colorado

Efthimia Petinaki
Department of Microbiology
University of Thessaly Medical School
Larissa, Greece

Wolfgang Pfister
Institute for Medical Microbiology
University Hospital of Jena
Jena, Germany

Matthew R. Pincus
Department of Pathology
SUNY Downstate Medical Center
Brooklyn, New York

Giuliano Pisoni
Department of Veterinary Pathology, Hygiene and Public Health
University of Milan
Milan, Italy

Brunella Posteraro
Institute of Microbiology
Università Cattolica del Sacro Cuore
Rome, Italy

Patrizia Posteraro
Laboratory of Clinical Pathology and Microbiology
Ospedale San Carlo
Rome, Italy

Noura Raddadi
Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche
Università degli Studi di Milano
Milan, Italy

Peter Rådström
Department of Applied Microbiology
Lund University
Lund, Sweden

Annibale Raglio
USC Microbiology and Virology
A.O. Ospedali Riuniti
Bergamo, Italy

Derren Ready
Eastman Dental Hospital
UCLH NHS Foundation Trust
London, United Kingdom

D. Madhusudan Reddy
Palamuru University
Andhra Pradesh, India

Clara G. de los Reyes-Gavilán
Department of Microbiology and Biochemistry of Dairy Products
Instituto de Productos Lácteos de Asturias
Consejo Superior de Investigaciones Científicas
Asturias, Spain

Lothar Rink
Institute of Immunology
Medical Faculty
RWTH Aachen University
Aachen, Germany

Ignasi Roca
Department of Clinical Microbiology
School of Medicine
University of Barcelona
Barcelona, Spain

Camilla Rodrigues
P D Hinduja National Hospital & Medical Research Centre
Mumbai, India

Mar Rodríguez
Facultad de Veterinaria
Higiene de los Alimentos
Universidad de Extremadura
Cáceres, Spain

Veronica Rodriguez-Nava
Research group on "Bacterial Opportunistic Pathogens and Environment"
Université de Lyon
Lyon, France

Antoni Różalski
Department of Immunobiology of Bacteria
Institute of Microbiology and Immunology
University of Łódź
Łódź, Poland

Patricia Ruas-Madiedo
Department of Microbiology and Biochemistry of Dairy Products
Instituto de Productos Lácteos de Asturias
Consejo Superior de Investigaciones Científicas
Asturias, Spain

Pedro Rubio
Department of Animal Health
University of León
León, Spain

Nataliia Rudenko
Biology Centre
Institute of Parasitology
Academy of Sciences of the Czech Republic
České Budějovice, Czech Republic

Stefan Rupf
Clinic of Operative Dentistry
Periodontology and Preventive Dentistry
Saarland University Hospital
Homburg/Saar, Germany

Michael P. Ryan
Microbiology Laboratory
Department of Chemical and Environmental Sciences
University of Limerick
Limerick, Ireland

Maria Saarela
VTT Technical Research Centre of Finland
Espoo, Finland

Konrad Sachse
Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health)
Institute of Molecular Pathogenesis
Jena, Germany

Atsushi Saito
Oral Health Science Center
Tokyo Dental College
Chiba, Japan

Daniel Saito
Department of Oral Diagnostics
Division of Microbiology and Immunology
Piracicaba Dental School
University of Campinas
Campinas, Brazil

Borja Sánchez
Department of Microbiology and Biochemistry of Dairy Products
Instituto de Productos Lácteos de Asturias
Consejo Superior de Investigaciones Científicas
Asturias, Spain

Ester Sánchez
Microbial Ecophysiology and Nutrition Research Group
Institute of Agrochemistry and Food Technology
Spanish Council for Scientific Research
Valencia, Spain

G. Sánchez
Department of Biotecnology
Institute of Agrochemistry and Food Technology
Valencia, Spain

Maurizio Sanguinetti
Institute of Microbiology
Università Cattolica del Sacro Cuore
Rome, Italy

Guido Lopes dos Santos Santiago
Department of Clinical Biology, Immunology and Microbiology
Ghent University Hospital
Ghent, Belgium

Jesús A. Santos
Veterinary Faculty
Department of Food Hygiene and Food Microbiology
University of León
León, Spain

Yolanda Sanz
Microbial Ecophysiology and Nutrition Research Group
Institute of Agrochemistry and Food Technology
Spanish Council for Scientific Research
Valencia, Spain

Roland P.H. Schmitz
SIRS-Lab GmbH
Jena, Germany

K.R. Schneider
Food Science and Human Nutrition Department
University of Florida
Gainesville, Florida

Bo Segerman
Swedish National Veterinary Institute
Uppsala, Sweden

Teresa Semedo-Lemsaddek
Faculdade de Ciências
Universidade de Lisboa
Center for Biodiversity Functional and Integrative Genomics
Edifício ICAT, Campus da FCUL
Lisbon, Portugal

Diego Omar Serra
Facultad de Ciencias Exactas
Centro de Investigación y Desarrollo en Fermentaciones Industriales
Universidad Nacional de La Plata
La Plata, Argentina

Nidhi Sharma
Department of Microbiology
All India Institute of Medical Sciences
New Delhi, India

Shubhada Shenai
P D Hinduja National Hospital & Medical Research Centre
Mumbai, India

Han Shiong Siah
TPP, Lambells Lagoon
Northern Territory, Australia

Shoo Peng Siah
Human Genetic Signatures
New South Wales, Australia

Maria R.L. Simionato
Department of Microbiology
Institute of Biomedical Science
University of São Paulo
São Paulo, Brazil

Mikael Skurnik
University of Helsinki
and
Helsinki University Central Hospital Laboratory Diagnostics
Helsinki, Finland

Renata Martins do Souto
Department of Medical Microbiology
Institute of Microbiology
Federal University of Rio de Janeiro
Rio de Janeiro, Brazil

Paweł Stączek
Department of Immunobiology of Bacteria
Institute of Microbiology and Immunology
University of Łódź
Łódź, Poland

Paola Stefanelli
Department of Infectious, Parasitic and Immune-Mediated Diseases
Istituto Superiore di Sanità
Rome, Italy

Roger Stephan
Vetsuisse Faculty
Institute for Food Safety and Hygiene
University of Zurich
Zurich, Switzerland

L.K. Strawn
Department of Food Science
Cornell University
Ithaca, New York

Yi-Wei Tang
Departments of Pathology and Medicine
Vanderbilt University School of Medicine
Nashville, Tennessee

Marleen Temmerman
Department of Obstetrics and Gynaecology
Ghent University
Ghent, Belgium

Rogério Tenreiro
Faculdade de Ciências
Universidade de Lisboa
Center for Biodiversity Functional and Integrative Genomics
Edifício ICAT, Campus da FCUL
Lisbon, Portugal

Corinne Teyssier
Faculté de Pharmacie, BP 14491
Université Montpellier 1
Montpellier, France

María Mar Tomás
Servicio Microbiología
Complejo Hospitalario Universitario La Coruña
La Coruña, Spain

Herbert Tomaso
Friedrich-Loeffler-Institut
Institute of Bacterial Infections and Zoonoses
Jena, Germany

Martha E. Trujillo
Departmento de Microbiología y Genética
Edificio Departamental
Universidad de Salamanca
Salamanca, Spain

Sheng Kai Tung
Department of Medical Laboratory Science and Biotechnology
College of Medicine
National Cheng Kung University
Taiwan

Mario Vaneechoutte
Department of Clinical Biology, Immunology and Microbiology
Ghent University Hospital
Ghent, Belgium

A.C.M. Veloo
Department of Medical Microbiology
University Medical Center Groningen
University of Groningen
Groningen, the Netherlands

Suzanne J.C. Verhaegh
Department of Medical Microbiology and Infectious Diseases, Erasmus MC
Rotterdam, the Netherlands

Rita Verhelst
Department of Clinical Biology, Immunology and Microbiology
Ghent University Hospital
Ghent, Belgium

Hans Verstraelen
Department of Obstetrics and Gynaecology
Ghent University Hospital
Ghent, Belgium

Jordi Vila
Department of Clinical Microbiology
Hospital Clinic
University of Barcelona School of Medicine
Barcelona, Spain

Almudena F. Villadangos
Section of Microbiology
Department of Molecular Biology
Faculty of Biology
University of León
León, Spain

Georgia Vrioni
Department of Microbiology
National & Kapodistrian University of Athens Medical School
Athens, Greece

Ken B. Waites
Department of Pathology
University of Alabama at Birmingham
Birmingham, Alabama

Chengming Wang
Molecular Diagnostics Laboratory
Department of Pathobiology
College of Veterinary Medicine
Auburn University
Auburn, Alabama

B.R. Warren
Research, Quality and Innovation
ConAgra Foods, Inc.
Omaha, Nebraska

David M. Whiley
Queensland Paediatric Infectious Diseases Laboratory
Royal Children's Hospital
Queensland Children's Medical Research Institute
Queensland, Australia

Patrick C.Y. Woo
Department of Microbiology
The University of Hong Kong
Hong Kong

Li Xiao
Department of Pathology
University of Alabama at Birmingham
Birmingham, Alabama

Osvaldo Miguel Yantorno
Facultad de Ciencias Exactas
Centro de Investigación y Desarrollo en Fermentaciones Industriales
Universidad Nacional de La Plata
La Plata, Argentina

Ting Zhang
College of Veterinary Medicine
Mississippi State University
Starkville, Mississippi

1 Introductory Remarks

Dongyou Liu

CONTENTS

1.1 PREAMBLE

Bacteria (singular, bacterium) are small unicellular organisms that are classified taxonomically in the domain Bacteria (or Eubacteria), the kingdom Prokaryotae (or Prokaryota or Monera). The only other domain in the kingdom Prokaryotae covers Archaea (or Archaeobacteria for "ancient bacteria"). With sizes ranging from 10^{-7} to 10^{-4} mm, prokaryotes are bigger than viruses (10^{-8}–10^{-6} mm), but smaller than eukaryotes (10^{-5}–10^{3} mm). While both bacteria and archaea may have evolved independently from an ancient common ancestor, eukaryotes may have arisen from ancient bacteria entering into endosymbiotic associations with the ancestors of eukaryotic cells (possibly related to the archaea) to form either mitochondria or hydrogenosomes. A subsequent independent engulfment by some mitochondria-containing eukaryotes of cyanobacterial-like organisms may have led to the formation of chloroplasts in algae and plants.

In contrast to the organisms in the eukaryotic kingdoms Protista, Fungi, Plantae, and Animalia, those in the kingdom Prokaryotae lack nuclear membrane (with their DNA usually in a loop or coil), contain few independent membrane-bounded cytoplasmic organelles (e.g., vacuole, endoplasmic reticulum, Golgi apparatus, and mitochondria) apart from chromosome and ribosome, have no unique structures in their plasma membrane and cell wall, and do not undergo endocystosis and exocytosis. In other words, whereas eukaryotic chromosome resides within a membrane-delineated nucleus, bacterial chromosome is located inside the bacterial cytoplasm. This entails that all cellular events (e.g., translational and transcriptional processes, and interaction of chromosome with other cytoplasmic structures) in prokaryotes occur in the same compartment. Furthermore, while eukaryotic chromosome is packed with histones to form linear chromatin, bacterial chromosome assumes a highly compact supercoiled structure in circular form (and rarely in linear form).

Although archaea are similar to bacteria in most aspects of cell structure and metabolism, they differ from bacteria in that being extremophiles, they can live in extreme environments where no other life forms exist. This may be due to the unique structure in archaeal lipids in which the stereochemistry of the glycerol is the reverse of that found in bacteria and eukaryotes, possibly the result of an adaptation on the part of archaea to hyperthermophily. In addition, the archaeal cell wall does not contain muramic acid, which is commonly present in bacteria. The archaeal RNA polymerase core is composed of ten subunits in comparison with four subunits in bacteria. Besides possessing distinct tRNA and rRNA genes, archaea uses eukaryotic-like initiation and elongation factors in protein translation, and their transcription involves TATA-binding proteins and TFIIB as in eukaryotes.

Bacteria are ubiquitously distributed in virtually every habitat on earth, and are abundantly present in soil, fresh water, plants, and animals. With an estimated number of 5 nonillion (5×10^{30}), bacteria form much of the world's biomass. Bacteria play an essential role in chemical cycles,

environmental maintenance, food production, and human wellbeing. However, some bacteria are pathogenic and capable of causing infectious diseases in humans, animals, and plants. Cholera, syphilis, anthrax, leprosy, bubonic plague, and tuberculosis are some of the examples of the deadly human diseases that are attributable to bacteria. Correct identification and detection of bacterial pathogens is not only fundamental to the study of these microorganisms but also critical to the control and prevention of the diseases they cause.

1.2 BACTERIAL CHARACTERISTICS

1.2.1 Classification

Bacteria are classified on the basis of their differences in morphology (e.g., rod, cocci, spirilla, and filament), cell wall structure (e.g., gram-negative and gram-positive), growth characteristics (e.g., aerobic and anaerobic), biochemical properties (e.g., fatty acids), and genetic features (e.g., 16S and 23S rRNA). Currently, the domain Bacteria (Eubacteria) is divided into 26 phyla (Acidobacteria, Actinobacteria, Aquificae, Bacteroidetes, Chlamydiae, Chlorobi, Chloroflexi, Chrysiogenetes, Cyanobacteria, Deferribacteres, Deinococcus-Thermus, Dictyoglomi, Fibrobacteres, Firmicutes, Fusobacteria, Gemmatimonadetes, Lentisphaerae, Nitrospira, Planctomycetes, Proteobacteria, Spirochaetes, Tenericutes, Thermodesulfobacteria, Thermomicrobia, Thermotogae, and Verrcomicrobia), whereas the domain Archaea (Archaeobacteria) is separated into two phyla (Crenarchaeota and Euryarchaeota). Among the 26 phyla in the domain Bacteria, Proteobacteria, and Firmicutes contain the largest numbers of genera and species followed by Cyanobacteria, Bacteroidetes, Spirochaetes, and Flavobacteria. Bacteria from other phyla are comparatively rare, from which fewer genera and species have been described. Most of human pathogenic bacteria are found in the phyla Actinobacteria, Bacteroidetes, Chlamydiae, Firmicutes, Fusobacteria, Proteobacteria, Spirochaetes, and Tenericutes (Table 1.1).

1.2.2 Morphology

Bacteria usually measure from 0.2 to 2.0 μm in width and 2–8 μm in length, and are 10 times smaller than eukaryotic cells. On one extreme, a few bacterial species (e.g., *Thiomargarita namibiensis*, and *Epulopiscium fishelsoni*) measure up to half a mm long and are visible to the naked eye. On the other extreme, the smallest bacteria in the genus *Mycoplasma* are only 0.3 μm in size, which are as small as the largest viruses.

Bacteria typically assume four distinctive forms: rod-like bacilli, spherical cocci, spiral bacteria (also called spirilla), and filamentous bacteria. Occasionally, a small number of bacterial species may appear tetrahedral or cuboidal in shape. While many bacterial species exist as

TABLE 1.1
Classification and Characteristics of Major Human Bacterial Pathogens

Phylum	Class	Brief Description	Notable Human Pathogens
Actinobacteria	Actinobacteria	Gram-positive bacteria with high G + C ratio; classification may be assisted through analysis of ferric uptake regulator (fur) and glutamine synthetase	*Actinomyces, Corynebacterium, Mycobacterium, Nocardia*
Firmicutes	Bacilli	Gram-positive cocci or rods with low G + C ratio; presence of cell wall	*Bacillus, Enterococcus, Listeria, Staphylococcus, Streptococcus*
	Clostridia	Gram-positive cocci or rods with low G + C ratio; presence of cell wall; some species (e.g., *Veillonella*) are gram-negative	*Clostridium, Eubacterium, Peptostreptococcus*
Tenericutes	Mollicutes	Small bacteria (0.2–0.3 μm in size) with low G + C ratio; absence of cell wall (outer membrane)	*Mycoplasma, Ureaplasma*
Bacteroidetes	Bacteroidia	Gram-negative, anaerobic bacteria; opportunistic pathogens	*Bacteroides, Porphyromonas, Prevotella*
	Flavobacteria	Gram-negative, anaerobic bacteria; opportunistic pathogens	*Elizabethkingia, Flavobacterium*
Chlamydiae	Chlamydiae	Gram-negative bacteria; obligate intracellular pathogens	*Chlamydia, Chlamydophila*
Fusobacteria	Fusobacteria	Gram-negative, filamentous, anaerobic bacteria	*Fusobacterium, Leptotrichia*
Proteobacteria	Alphaproteobacteria	Gram-negative, phototrophic bacteria, with symbiotic properties	*Bartonella, Brucella*
	Betaproteobacteria	Gram-negative, aerobic or facultative bacteria some of which are chemolithotrophs, or phototrophs	*Bordetella, Burkholderia, Neisseria*
	Gammaproteobacteria	Gram-negative, facultatively or obligately anaerobic bacteria, some of which are highly pathogenic	*Aeromonas, Klebsiella, Pseudomonas, Salmonella, Vibrio, Yersinia*
	Epsilonproteobacteria	Gram-negative, curved to spirilloid bacteria, inhabiting digestive tract	*Arcobacter, Campylobacter, Helicobacter*
Spirochaetes	Spirochaetes	Gram-negative, long, helically coiled (spiral-shaped), chemoheterotrophic, anaerobic bacteria, with length-wise flagella	*Borrelia, Leptospira, Treponema*

single cells, others present characteristic patterns such as diploids (pairs), chains, and clusters ("bunch of grapes"). In addition, some bacteria may be elongated to form filaments, which are often surrounded by a sheath containing many individual cells. The elaborated, branched filaments formed by *Nocardia* may even resemble fungal mycelia in appearance. Frequently, bacteria use quorum sensing to detect surrounding cells, migrate toward each other, and attach to solid surfaces to form dense aggregations called biofilms (bacterial mats, or fruiting bodies), which may measure a few micrometers in thickness to up to half a meter in depth, and which comprise multiple species of bacteria, archaea, and protists (numbering approximately 100,000 cells). The formation of biofilms protects bacteria from host defense mechanisms and antibiotic therapy, contributing to chronic bacterial infections and infections relating to implanted medical devices.

Structurally, a bacterial cell is surrounded by a rigid layer (cell wall) that is located externally to the lipid membrane. The cell wall provides structural support and protection, and acts as a filtering mechanism. In addition to prokaryotae, fungi and plantae also possess a cell wall, but animalia and most protista do not. While the bacterial cell wall is made up of peptidoglycan (also called murein, which in turn is composed of polysaccharide chain cross-linked by peptides containing D-amino acids), the archaeal cell wall consists of surface layer proteins (also known as S-layer), pseudopeptidoglycan (pseudomurein), and polysaccharides. By contrast, the fungal cell wall includes chitin, the algal cell wall has glycoprotein and polysaccharides, and the plant cell wall often incorporates cellulose and proteins such as extensins.

Based on the ability of bacterial cell wall to retain Gram stain (consisting of crystal violet as primary stain and Gram's iodine and basic fuchsin as subsequent stain), bacteria are divided into gram-positive and gram-negative categories. The gram-positive bacterial cell wall is composed of several layers of peptidoglycan (which is responsible for retaining the crystal violet dyes during the Gram staining procedure, leading to its purple color) surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins. Located outside of cytoplasmic membrane, peptidoglycan is a large polymer (formed by poly-*N*-acetylglucosamine and *N*-acetylemuramic acid) that contributes to the structural integrity of the bacterial cell wall in addition to countering the osmotic pressure of the cytoplasm. Peptidoglycan is predominant in the cell walls of high and low percentage G + C gram-positive organisms (e.g., actinobacteria and firmicutes). Also imbedded in the gram-positive cell wall are teichoic acids, some of which are lipid linked to form lipoteichoic acids. On the other hand, the gram-negative cell wall has a thin peptidoglycan layer adjacent to the cytoplasmic membrane that contributes to its inability to retain the crystal violet stain upon decolonization with ethanol during the Gram staining procedure (leading to its red or pink color after restaining with basic fuchsin). Apart from the thin peptidoglycan layer, the gram-negative cell wall also has an outer membrane that is formed by phospholipids and lipopolysaccharides.

Within the gram-positive bacterial category, there is another distinct group of bacteria (i.e., acid-fast bacteria such as *Mycobacterium* and *Nocardia*) that can resist decolorization with an acid-alcohol mixture during the acid-fast (or Ziehl–Neelsen) staining procedure and retain the initial dye carbol fuchsin and appear red. The acid-fast cell wall of *Mycobacterium* includes a large amount of glycolipids, especially mycolic acids that make up approximately 60% of the acid-fast cell wall in addition to a thin, inner-layer peptidoglycan. The presence of the mycolic acids and other glycolipids impede the entry of chemicals, causing the organisms to grow slowly and be more resistant to chemical agents and lysosomal components of phagocytes than most bacteria.

Whereas a vast majority of bacteria possess the gram-negative cell wall, the firmicutes and actinobacteria (previously known as the low percentage G + C and high percentage G + C gram-positive bacteria, respectively) have the gram-positive structure, and the tenericutes (e.g., the genus *Mycoplasma*) are devoid of a cell wall in spite of their similarity in G + C ratio to the firmicutes. The differences in the cell wall often determine the susceptibility and resistance of bacteria to antibiotics and other therapeutic reagents. Given that *Mycoplasma* species lack a cell wall, they are unaffected by such commonly used antibiotics such as penicillin and streptomycin that target cell wall synthesis. With their small size (0.3 μm), *Mycoplasma* species are often identified as a source of contaminating infection in the cell culture (where penicillin and streptomycin are incorporated in the culture media), causing retarded growth of cultured cell lines. The cell wall of bacteria forms part of pathogen-associated molecular patterns (or PAMPs), which are recognized by pattern-recognition receptors (or PRRs) in mammalian hosts to initiate and promote innate and adaptive immune defenses against invading bacteria.

Several recognizable extracellular structures are present in bacteria. These include flagella, pili, and fimbriae, which protrude from bacterial cell wall and are involved in bacterial twitching movement as well as interaction with one another and other organisms. Bacterial flagellum (measuring 20 nm in diameter and up to 20 μm in length) is a long, whip-like, and helical projection made up of repeating flagellin protein. The numbers and arrangements of flagella vary among bacterial genera and species. Monotrichous bacteria have a single flagellum, amphitrichous bacteria contain a single flagellum on each of cell poles, lophotrichous bacteria include multiple flagella that are located at one cell pole, and peritrichous bacteria have multiple flagella that are situated at several locations. Flagella in bacteria are powered by a flow of H+ ions (sometimes Na+ ions), and those in archaea are powered by adenosine 5'-triphoshate (ATP). Despite having a similar appearance, eukaryotic flagella (called cilia or undulipodia) differ from prokaryotic flagella in both structure and evolutionary origin. A eukaryotic flagellum is a bundle of nine fused pairs of microtubule doublets surrounding two single microtubules. Eukaryotic flagella are often arranged

en masse at the surface of a stationary cell anchored within an organ, lashing back and forth and serving to move fluids along mucous membranes such as trachea. In addition, some eukaryotic cells (e.g., rod photoreceptor cells of eye, olfactory receptor cells of nose, and kinocilium in cochlea of ear) have immotile flagella that function as sensation and signal transduction devises.

Pilus and fimbria are proteinaceous, hair- or thread-like appendages in bacteria (particularly of gram-negative category) that are much shorter and thinner than flagellum. Bacteria have up to ten pili (typically 6–7 nm in diameter) whose main function is to connect the bacterium to another of the same or a different species to enable transfer of plasmids between the bacteria (i.e., conjugation). A fimbria (measuring 2–10 nm in diameter and up to several μm in length) is shorter than pilus. A bacterium possesses as many as 1000 fimbriae, which are deployed to attach to surface of another bacterium (to form a biofilm) or host cell (to facilitate invasion). Many pilin proteins are characteristic among bacterial species and subgroups, which have been exploited as targets for serological typing of bacteria (serotypes or serovars).

Many bacteria produce capsules or slime layers around their cells, which can protect cells from engulfment by eukaryotic cells (e.g., macrophages), act as antigens for cell recognition, and aid attachment to surfaces and the formation of biofilms. In addition, some gram-positive bacteria (e.g., *Bacillus, Clostridium*, and *Anaerobacter*) can form highly resistant, dormant structures called endospores, which contain a central core of cytoplasm with DNA and ribosomes surrounded by a cortex layer and protected by an impermeable and rigid coat. Endospores can survive extreme physical and chemical stresses (e.g., UV lights, γ-radiation, detergents and disinfectants, heat, pressure, and desiccation), and may remain viable for millions of years. Endospore-forming bacteria (e.g., *Bacillus anthrax* and *Clostridium tetanus*) are also capable of causing disease.

Underneath the lipid membrane is the cytoplasm, which is composed of nutrients (or nutrient storage granules such as glycogen, polyphosphate, sulfur, or polyhydroxyalkanoates), proteins, and other essential components. There is a notable absence of membrane-bound organelles (with the exception of chromosome and ribosome) in the bacterial cytoplasm, although certain subcellular compartments (prokaryotic cytoskeleton), such as carboxysome-containing polyhedral protein shells, have been detected. These polyhedral organelles compartmentalize bacterial metabolism, similar to the function performed by the membrane-bound organelles in eukaryotes. The bacterial chromosome consists of a single circular DNA molecule that is situated together with associated proteins and RNA in an irregularly shaped body called the nucleoid. The bacterial ribosomes are responsible for production of proteins.

1.2.3 Biology

Bacteria utilize many metabolic pathways (e.g., glycolysis, electron transport chains, chemiosmosis, cellular respiration, and photosynthesis) and thus virtually all carbon or energy supplies for their maintenance and growth. They are easily grown using either solid or liquid media (e.g., Luria Bertani broth). Solid growth media (e.g., agar plates) are useful for isolation of pure cultures of a bacterial strain, and liquid growth media are employed to generate bulk quantities of bacterial cells. In addition, selective media (containing specific nutrients and antibiotics) assist the isolation and identification of specific bacterial organisms.

As single-celled organisms, prokaryotes reproduce by asexual binary fission, which begins with DNA replication within the cell until the entire prokaryotic DNA is duplicated. The two chromosomes then separate as the cell grows and the cell membrane invaginates, splitting the cell into two daughter cells. This reproductive process is highly efficient and leads to exponential growth of bacteria. In fact, under optimal growth conditions, *Escherichia coli* cells can double every 20 min. Because bacteria are able to multiply rapidly with minimal nutritional requirements, they are abundant in virtually every habitat on earth. In soil, bacteria live by degrading organic compounds and assist in soil formation. In aquatic environments such as ponds, streams, lakes, rivers, seas, and oceans, bacteria such as cyanobacteria (sometimes called blue-green algae because of their color) utilize their chlorophylls to capture energy from the sunlight. In the depths of the sea, bacteria obtain energy from oxidizing or reducing naturally occurring sulfur compounds. In humans and animals, bacteria are found in large numbers on the skin, the respiratory and digestive tracts, and other parts of the body, constituting a normal microbiota in an essentially symbiotic relationship with mutual benefits. Although the vast majority of bacteria are harmless and sometimes even beneficial to their hosts, a few have the capacity to take advantage of temporary weakness in the host (e.g., injury and/or impaired immune function) to cause diseases of varying severity.

In a high-nutrient environment, the growth cycle of bacteria usually undergoes three phases. The first phase (the lag phase) is a period of slow growth with the bacterial cells adapting to the high-nutrient environment and preparing for fast growth. In the lag phase, the cell replicates its DNA and makes all the other molecules (e.g., ribosomes, membrane transport proteins) needed for the new cell. The second phase (the logarithmic phase or "log" phase, also known as the exponential phase) occurs when DNA replication stops, and is characterized by rapid cell division and exponential growth. The rate at which cells grow during this phase is known as the growth rate, and the time it takes the cells to double is known as the generation time. During the log phase, nutrients are metabolized at maximum speed until one of the nutrients is depleted, which poses a negative impact on growth. The final phase (the stationary phase) results from the depletion of nutrients. During the stationary phase, the cells decrease their metabolic activity and consume nonessential cellular proteins. As a transition from rapid growth to a stress response state, there is heightened expression of genes involved in DNA repair, antioxidant metabolism,

and nutrient transport. Although the entire cycle of bacterial growth takes about an hour, a rapidly growing bacterial cell carries out multiple rounds of replication simultaneously, which helps to shorten the doubling time for most bacteria to about 20 min.

1.2.4 Genetics

Bacteria have a single circular chromosome that ranges in size from only 160,000 bp (base pairs) (e.g., *Candidatus Carsonella ruddii*) to 12,200,000 bp (e.g., *Sorangium cellulosum*). However, *Borrelia burgdorferi*, the causal agent for Lyme disease, contains a single linear chromosome. In addition, bacteria may possess small extrachromosomal DNA called plasmid, which ranges from 1 to 400 kb in size and comprises genes or gene cassettes for antibiotic resistance or virulence factors. As plasmids have at least an origin of replication (or *ori*)—a starting point for DNA replication—they are capable of autonomous replication independent of the chromosomal DNA. A plasmid that integrates into the chromosomal DNA is called episome, which permits its duplication with every cell division of the host. Some viruses (bacteriophages or phages) may also exist in bacteria, with some merely infecting and lysing their host bacteria, while others inserting into the bacterial chromosome. Phages are usually made up of a nucleic acid core (e.g., ssRNA, dsRNA, ssDNA, or dsDNA measuring 5–500 bp in length) with an outer protein hull. A phage containing particular genes may contribute to its host's phenotype, as illustrated by the evolution of *Escherichia coli* O157:H7 and *Clostridium botulinum*, which are converted from harmless ancestral bacteria into lethal pathogens through the integration of phages harboring toxin genes.

Being the key component of the ribosome, ribosomal RNA molecules (rRNA) consists of two complex folded subunits of differing sizes (small and large), whose main functions are to provide a mechanism for decoding messenger RNA (mRNA) into amino acids (at center of small ribosomal subunit) and to interact with transfer RNA (tRNA) during translation by providing petidyltransferase activity (large subunit). Whereas the two rRNA subunits in eukaryotes have sedimentation coefficiency values of 40S (Svedberg units) and 60S, those in bacteria measure 30S and 50S, respectively. In virtually all organisms, the small rRNA subunit (40S in eukaryotes and 30S in bacteria) contains a single RNA species (i.e., 18S rRNA in eukaryotes and 16S rRNA in bacteria); the large rRNA subunit (60S) in eukaryotes comprises three RNA species (5S, 5.8S, and 25/28S rRNA), while that (50S) in bacteria contains two RNA species (5S and 23S rRNA).

Although bacteria do not undergo meiosis or mitosis and do not require cellular fusion to initiate reproduction (as bacteria are not diploid), many bacteria do involve a cell-to-cell transfer of genomic DNA by various mechanisms. These mechanisms may range from the uptake of exogenous DNA from their environment (a process called transformation) and the integration of a bacteriophage introduces foreign DNA into the chromosome (a process called transduction), to the acquisition of DNA through direct cell contact (a process called conjugation). The incorporation of genes and DNA from other bacteria or the environment into the recipient cell's DNA is also called horizontal gene transfer. While DNA transfer occurs less frequently per individual bacterium than that among eukaryotes involving obligate sexual reproduction, the much shorter generation times and high numbers associated with bacteria can make the DNA transfer a significant contributor to the evolution of bacterial populations. Gene transfer is vital to the development of antibiotic resistance in bacteria as it allows the rapid transfer of resistance genes between different pathogens.

Regardless of genome size, most organisms show a mutation rate on the order of one mutation per genome per generation. Given their very short generation times (<1 h in culture media and a few hours in the wild) and small genomes (which are a 1000 times smaller than most eukaryotes), prokaryotes generally display 1000 times more mutations per gene, per unit time, and per individual than eukaryotes. Furthermore, with greater population sizes resulting in the absolute amount of mutational variation entering the population, prokaryotes have enormous capacity to adapt to and invade new niches, which are the key factors contributing to the evolutionary success of prokaryotes.

Genomic diversity in bacteria comes in two forms: (i) genetic heterogeneity wherein different strains have different alleles of the same gene and (ii) genomic plasticity where different strains have different genes. Recent studies indicate that each strain (serovar) within a bacterial species receives a unique distribution of genes from a population-based supragenome that is many times larger than the genome of any given strain.[1] Through the autocompetence and autotransformation mechanisms, bacterial strains (or serovars) within the species may evolve and generate diversity in vivo to enable them to persist in the face of myriad host defense mechanisms and environmental stresses. In other words, the strain (serovar)-specific genes (e.g., contingency genes) may provide for an increased number of genetic characters that facilitate the population as a whole to adapt rapidly to environmental factors, such as those experienced in the host during chronic infectious processes. There is evidence that under arduous external conditions, many bacteria form biofilms that often exchange DNA at rates several orders of magnitude greater than planktonic bacteria and that are responsible for many chronic bacterial infections in human patients. For example, biofilm-associated growth of *Pseudomonas aeruginosa* has been implicated in several chronic suppurative otitis media. It appears that the possession of a distributed genome is a common host interaction strategy.

1.2.5 Ecological and Medical Importance

Bacteria play a number of beneficial roles in the ecological balance of our planet. Bacteria are involved in the recycling of carbon dioxide (CO_2) to oxygen via photosynthesis;

in the decomposition of dead plant and animal matter, improving soil fertility; and in the fixation of nitrogen into the nitrogen compound ammonia for plant growth. Bacteria can help clean up oil spills, pesticides, and other toxic materials. Some bacteria are able to remove (leach) the copper from the ores (copper sulfides), while others are useful for food production such as yogurt, cheese, cider, and vinegar. Some bacteria (e.g., *Bacillus thuringiensis* or BT, a soil dwelling gram-positive bacterium) can be used as pesticides (trade names Dipel and Thuricide) in the biological control of Lepidopteran pest. In addition, as fast growers with relatively low demands for nutrients, bacteria represent ideal hosts for mass production of certain plastics, enzymes used in laundry detergents, and antibiotics such as streptomycin and tetracycline, as well as pharmaceuticals and fine chemicals upon genetic modification. Furthermore, some bacteria (e.g., *Listeria monocytogenes*, *Mycobacterium bovis* Baille Calmette-Guerin or BCG, *Salmonella, Shigella*, and *Escherichia coli*) are useful carriers for delivering vaccine molecules against microbial diseases and cancers.[2]

Although many bacteria are harmless or beneficial, a few can exert detrimental effects on food and plant production, as well as human and animal health. Some bacteria can cause food spoilage (e.g., *Lactobacillus*) and foodborne diseases (e.g., *Shigella, Campylobacter, Salmonella*, and *Listeria*), and others can harm agriculture because of the major diseases of plants and farm animals they cause. For instance, *Brachyspira hyodysenteriae* causes a type of severe diarrhea in pigs that can have disastrous consequences for pig farmers. Some bacteria are involved in metal corrosion (wearing away) through the formation of rust, especially on metals containing iron.

There are approximately ten times as many bacterial cells as human cells in the human body, with large numbers of bacteria on the skin and in the digestive tract. The communities of bacteria and other organisms that inhabit the body are sometimes referred to as the normal microflora or microbiota. Some bacteria in human body produce essential nutrients (e.g., vitamin K) that the body cannot make itself. Nonetheless, some bacteria are highly pathogenic and deadly. *Yersinia pestis* was responsible for the most fatal and devastating bacterial disease in history—the bubonic plague—which killed an estimated 200 million people prior to the advent of antibiotics. Currently, the most common fatal bacterial diseases are respiratory infections, with tuberculosis (caused by *Mycobacterium tuberculosis*) alone killing about 2 million people a year. One of the world's deadliest bacterial diseases today is cholera, which is caused by foodborne *Vibrio cholerae*. Other globally important bacterial diseases include pneumonia caused by *Streptococcus* and *Pseudomonas*, tetanus, typhoid fever, diphtheria, syphilis, and leprosy. Another common bacterial disease is tooth decay, which results from the acids bacteria produce from sugar via fermentation, which dissolves the enamel of the teeth and create cavities (holes) in the teeth.

1.3 BACTERIAL IDENTIFICATION

1.3.1 Current Diagnostic Approaches

Identification and characterization of bacteria and their roles in disease processes represent a critical step in the effective management of bacterial infections.

Traditionally, bacteria have been identified and diagnosed through examination of their phenotypic characteristics such as Gram staining, cell and colony morphology, and biochemical and serological properties. As a key component in the phenotypic identification scheme, in vitro culture is often time consuming, and its performance is affected by the changing features of bacterial metabolisms. Given the limited variations in bacterial cell and colony morphology, it is impossible to differentiate most bacterial species on morphological criteria alone. Biochemical and serological techniques are helpful in bacterial identification. However, overreliance on these techniques not only adds to the cost of identification, but also creates delay in the result availability. Furthermore, the intrinsic variability of phenotypic procedures remains a potential source of misdiagnosis.

In recent decades, DNA- or RNA-based genotypic (molecular) procedures have been increasingly utilized for microbial identification.[3] Besides comparison at the genome level (e.g., G + C content determination and DNA–DNA hybridization), detection of nucleotide differences among shared and uniquely present gene regions provides a practical means for bacterial characterization. Due to its crucial roles in cellular function and maintenance, the ribosomal RNA (rRNA) gene is highly conserved (i.e., the least variable) and abundant (with each living cell containing 10^4–10^5 copies of the 5S, 16S, and 23S rRNA molecules). The rRNA gene (and its genomic coding sequence rDNA) therefore offers a valuable target for confirmation of an organism's taxonomic status and species identity, and for estimation of the rates of species divergence.[4–8] In addition, a range of housekeeping-, species-, group-, and virulence-specific genes provide alternative targets for improved determination of bacterial organisms.[9,10]

The early molecular methods are largely nonamplified, as exemplified by G + C content determination, DNA–DNA hybridization, fluorescence in situ hybridization (FISH), ribotyping, and so forth. Because these methods often require large amounts of starting materials and are cumbersome to perform, they are now rarely used in routine clinical setting, and their applications are limited to the characterization and description of novel bacterial species/subspecies. The more recent molecular methods often involve nucleic acid amplification. These include polymerase chain reaction (PCR), ligase chain reaction (LCR), nucleic acid sequence-based amplification (NASBA), transcription-mediated amplification (TMA), strand displacement amplification (SDA), rolling circle amplification (RCA), cycling probe technology (CPT), branched DNA (bDNA), and loop mediated isothermal amplification (LAMP), and so forth. Due to their efficiency, simplicity, and robustness, PCR and its derivatives have been widely adopted in research and clinical laboratories for

specific, sensitive and rapid identification and diagnosis of bacterial pathogens.[11]

In a standard (conventional) PCR, a pair of oligonucleotides (of about 20 bases in length) is typically used as primers to anneal and amplify a gene region of interest with the help of DNA polymerase. The resulting amplified products (or amplicons) are separated by agarose gel electrophoresis and visualized with a DNA-binding dye (e.g., ethidium bromide). Further refinements of standard PCR led to the development of nested PCR (in which two consecutive PCR are performed one after another), multiplex PCR (in which multiple pairs of primers are included for simultaneous amplification of several genes of interests from the same or different organisms), arbitrarily primed PCR (in which a single oligonucleotide of about 10 bases in length is used to amplify random regions in a genome), and reverse-transcriptase PCR (RT–PCR) (in which RNA instead of DNA is targeted).[12,13]

Increasingly, the formats of PCR product detection have moved away from gel electrophoresis to enzymatic signal detection (e.g., ELISA and flow cytometry), real-time detection (e.g., using intercalating fluorescent dye [SYBR® green], hydrolysis dual-labeled probes [TaqMan®], hybridization probes [LightCycler], molecular beacons, peptide nucleic acid [PNA] probes, TaqMan minor groove binding [MGB™] probes, locked nucleic acid [LNA®] primers and probes, and scorpions™), line probe assay (LiPA), microarray, sequencing analysis (e.g., pyrosequencing), mass spectrometry (MS), and so forth.[14] In particular, real-time PCR demonstrates superior sensitivity, rapidity, and broad dynamic range, eliminates postamplification handling steps (thus minimizing carryover contamination), and is amenable to automation for high throughput detection.[15] Furthermore, real-time PCR provides the option for melting curve analysis, permitting discrimination of the amplified product from nonspecific product or primer-dimmers.[14] For these reasons, real-time PCR is becoming a method of choice for microbial identification in clinical laboratories worldwide.

1.3.2 Performance Parameters

The performance of a diagnostic assay is often evaluated by using several key parameters, including detection limit, sensitivity, specificity, accuracy, intraassay precision, interassay precision, and linearity (as in the case of a quantitative assay). Detection limit (or limit of detection) is the lowest concentration or quantity of bacteria that can be detected by a given assay. Sensitivity is the percentage of samples containing bacteria of interest that are identified by the assay as positive for the bacteria. Specificity is the percentage of samples without bacteria of interest that are identified by the assay as negative for the bacteria. Accuracy (or trueness) is the degree of conformity of an assay's measurements to the actual (true) value. It is often estimated by analyses of reference materials or comparisons of results with those obtained by a reference method. The closer an assay's measurements to the accepted value, the more accurate the assay is. Precision is the degree of mutual agreement among a series of assay's individual measurements, values, or results. Usually characterized in terms of the standard deviation of the measurements, precision can be stratified into (i) repeatability (the variation arising using the same instrument and operator in a single rune—i.e., intraassay precision—or repeating during a short time period) and (ii) reproducibility (the variation arising using the same measurement process among different instruments and operators from one run to another—i.e., interassay precision—or over longer time periods). Linearity refers to the tendency of measurements by a quantitative assay to form a straight line when plotted on a graph. Data from linearity experiments may be subjected to linear regression analysis with an ideal regression coefficient of 1. In case of a nonlinear curve, other objective, statistically valid methods may be utilized.[16]

1.3.3 Result Interpretation

A positive result by a molecular assay for a given pathogen normally confirms the etiologic relationship if the clinical syndrome is compatible with the pathogen identified. Considering the sensitive nature of the amplified methods such as PCR, it is important to rule out the possibility of a false positive result. Occasionally, false positive results may originate from the low diagnostic specificity of the assay, in which primers bind to irrelevant sequences and occasionally a homologous sequence that is shared among related or unknown bacteria. More often, false positive results in the molecular testing come from contamination, which may arise during manual handling of the samples in the testing laboratory either at the pre or postextraction (while setting up the PCR) stages. This risk is heightened when a high copy number polynucleotide (or plasmid) is used as a quantification standard and distributed around the laboratory, contaminating reaction source. In addition, contamination may be attributable to samples referred from other laboratories that do not utilize manipulation techniques that are mandatory for the molecular testing. These may include the use of unplugged pipette tips, infrequent changing of gloves and using pipette for long periods without decontamination. Another cause of contamination is by amplification products from previous tests.[17–19] Contamination may also occur by leakage from tubes or microtiter plates with lids not tightly closed or by breakage of glass capillaries leading to spillage of the amplification mixture. Besides the adoption of stringent laboratory practice, the risk of contamination with PCR products may be reduced by replacing nucleotide dTTP with dUTP in PCR, and implementing a digestion step with Uracil-DNA-glycosylase (UNG) to remove previous PCR products containing dUTP prior to each amplification reaction. Furthermore, inclusion of multiple negative controls, such as no-template controls (NTC) and no-amplification controls (NAC) may help identify the likely source of contamination and prevent false positive results. Moreover, microbial DNA may come with PCR reagents.

Similarly, a negative result by a molecular assay for a given pathogen normally indicates the absence of the pathogen.

However, it is equally important to rule out the possibility of false negative results. One possible cause is due to the low sensitivity of the assay employed. Alternatively, an insufficient amount of bacteria may be present in the sample (due to sample degradation or prior antibiotic treatment). Another cause may be the impurity of the processed sample. Enzymes used in PCR and RT–PCR (e.g., DNA polymerase, reverse transcriptase) are impeded by components in blood and feces (e.g., heme, hemoglobulin, lactoferrin, immunoglobulin G, leukocyte DNA, polysaccharides, and urea), in foods (e.g., phenolics, glycogen, calcium ions, fat, and other organic substances), in environmental specimens (e.g., phenolics, humic acids, and heavy metals), and in added anticoagulants (e.g., EDTA and heparin) as well as nucleic acid purification reagents (e.g., detergents, lysozyme, NaOH, alcohol, EDTA, EGTA, phenol, and high salt concentrations).[20–24] Any impurities and contaminations present in the samples after nucleic acid isolation may contribute to false negative results. A useful way to determine the effectiveness of a nucleic acid purification procedure for removing inhibitory substances is to spike samples with well-defined DNA or RNA prior to and after sample preparation (as process and amplification internal controls). In light of the high sensitivity of PCR, the occurrence of false negative results is probably a truly underestimated problem.

Thus, before definitive diagnoses and treatment decisions are made, all molecular and immunological diagnostic results need be reviewed and critically interpreted in combination with the patient's clinical presentation.

1.3.4 Standardization, Quality Control, and Assurance

Molecular tests such as PCR offer improved sensitivity, specificity, accuracy, precision, and result availability for microbial identification and diagnosis. There is a clear trend toward the adoption and application of these methods in routine diagnostics. However, in view of the possibility of false positive and false negative results occurring in these highly sensitive tests, it is vital that molecular diagnostic methods are properly standardized and validated and appropriate quality control measures are put in place.

Standardization and Validation. Standardization of molecular tests addresses the need for standardized reagents and common units, contamination control mechanisms, inhibition control mechanisms, clinically relevant dynamic ranges and internal controls, and so forth. Validation helps to verify the sensitivity, specificity, accuracy, repeatability (intraassay precision), reproducibility (interassay precision), detection limit, and linearity (if quantitative) of molecular tests.[25]

Before validating a method, it is important to have all instruments calibrated and maintained throughout the testing process. The validation process may involve a series of steps, including (i) testing of dilution series of positive samples (or plasmid construct) to determine the limits of detection of the assay and their linearity over concentrations to be measured in quantitative test (using minimal number of reference calibrators such as previously tested patient samples or pooled sera); (ii) evaluating the sensitivity and specificity of the assay, along with the extent of cross-reactivity with other genomic material; (iii) establishing the day-to-day variation of the assay's performance; (iv) assuring the quality of assembled assays using quality control procedures that monitor the performance of reagent batches; and (v) aligning the in-house primer and probe sequences with a genome sequence databank to avoid extended specificity testing.[25–28]

Quality Control. Quality control strategies for nucleic acid–based tests include (i) designation of a "clean" area for reaction setup (e.g., room under negative air pressure, positive-displacement pipettes, aerosol-block pipette tips, UV-equipped PCR cabinet); (ii) use of personal protective equipment (PPE) (e.g., disposable gloves and lab coats to prevent introduction of contaminating DNA or nucleases); (iii) use of uracil-*N*-glycosylase (UNG) in real-time PCR (to eliminate crossover amplicon contamination); (iv) use of a "hot-start" method (to minimize false priming events by withholding a crucial reaction component until appropriate temperature is reached); (v) use of external positive and negative controls (to monitor reaction performance and contamination) and homologous or heterologous internal controls (to monitor presence of inhibitors).[29]

A variety of test controls may be considered for diagnostic PCR. These include (i) internal amplification control (IAC) (negative sample spiked with sufficient pathogen and processed throughout the entire protocol); (ii) processing positive control (PPC) (negative sample spiked with sufficient closely related, but nontarget, strain processed throughout the entire protocol); (iii) reagent control (blank) (containing all reagents, but no nucleic acid apart from the primers); (iv) Premises control (tube containing the master mixture left open in the PCR setup room to detect possible contaminating DNA in the environment [carried out at regular intervals as part of the quality assurance program]); (v) standard (3–4 samples containing a tenfold dilution series of known number of target DNA copies in a range).[29–32]

Quality Assurance. One way to assess preparedness of the diagnostic laboratories is through the conduct of an external quality assurance (EQA) program that provides characterized specimens containing pathogens of interest. The design of a quality assurance program has the following components: (i) internal quality control (IQC) materials are distributed every month and comprising three pools of clinical samples of known pathogen status (typically one negative, one positive containing 1 log10 over the lower limit of detection of the assay, and one low positive containing up to 1 $\log_{10}$ of the lower limit of detection of the assay). These are incorporated in test runs on a weekly basis. The purpose of IQC is to provide samples of known status for repeated testing in parallel with clinical samples to ensure reproducibility of the test system in an individual laboratory. (ii) EQA distributions of panels of five unknown samples distributed quarterly. Results are returned to the QA laboratory for assessment. EQA compares the performance of different testing sites using specimens of known but undisclosed content. (iii) Aliquots of all samples

sent from the reference laboratory are posted back to Site A for repeat testing to check for integrity of the pools and for transport problems. (iv) A final element of the pilot program involves Sites B, C, and D sending an aliquot of every 50th sample to Site A to check for reproducibility. (v) A detailed record of distributions is kept to provide an audit trail.[33,34]

1.4 CONCLUSION

Molecular assays have the potential to increase the speed and accuracy of bacterial identification and diagnosis in research and clinical laboratories. When selecting and implementing a molecular test, a number of factors may be considered. These include (i) cost assessment; (ii) high throughput and automation capability (e.g., 96 or 384 well real-time PCR); (iii) detection limit; (iii) multiple target detection (e.g., multiplex PCR and microarray); (iv) broad range detection (e.g., 16S or 23S rRNA gene sequencing to identify poorly characterized, fastidious or noncultivable bacteria); (v) antimicrobial resistance detection; (vi) ease of use (commercial assays); (vii) sample preparation protocol (commercial kits). To overcome the limitations of molecular tests such as potential false positive and false negative results that impact significantly on patient management, laboratory space must be dedicated for instruments and sample preparation, contamination must be minimized, technicians must have proper training, and quality control procedures must be incorporated into routine laboratory workflow.[14]

There is a continuing trend toward miniature devices for microbial testing. One such promising device is biosensor. A biosensor incorporates a biological material (e.g., tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, natural products, etc.), a biologically derived material (e.g., recombinant antibodies, engineered proteins, aptamers, etc.) or a biomimic (e.g., synthetic catalysts, combinatorial ligands and imprinted polymers) in a physicochemical transducer or transducing microsystem (e.g., optical, electrochemical, thermometric, piezoelectric, magnetic, or micromechanical), leading to enhanced detection and identification of microbial pathogens in clinical, food, and environmental specimens.[35,36]

REFERENCES

1. Hogg, J.S. et al., Characterization and modeling of the *Haemophilus influenzae* core and supragenomes based on the complete genomic sequences of Rd and 12 clinical nontypeable strains, *Genome Biol.,* 8, R103, 2007.
2. Liu, D., *Listeria*-based anti-infective vaccine strategies, *Rec. Patents Anti-infect. Drug Disc*., 1, 281, 2006.
3. Vaneechoutte, M., and Van Eldere, J., The possibilities and limitations of nucleic acid amplification technology in diagnostic microbiology, *J. Med. Microbiol*., 46, 188, 1997.
4. Jonasson, J., Olofsson, M., and Monstein, H.J., Classification, identification and subtyping of bacteria based on pyrosequencing and signature matching of 16S rDNA fragments, *Apmis,* 110, 263, 2002.
5. Clarridge, J.E.III, Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases, *Clin. Microbiol. Rev.,* 17, 840, 2004.
6. Barlaan, E.A. et al., Electronic microarray analysis of 16S rDNA amplicons for bacterial detection, *J. Biotechnol.,* 115, 11, 2005.
7. Cherkaoui, A. et al., Development and validation of a modified broad-range 16S rDNA PCR for diagnostic purposes in clinical microbiology, *J. Microbiol. Methods*, 79, 227, 2009.
8. Sontakke, S. et al., Use of broad range16S rDNA PCR in clinical microbiology, *J. Microbiol. Methods,* 76, 217, 2009.
9. Liu, D. et al., Characterization of virulent and avirulent *Listeria monocytogenes* strains by PCR amplification of putative transcriptional regulator and internalin genes, *J. Med. Microbiol.,* 52, 1066, 2003.
10. Liu, D. et al., A multiplex PCR for species- and virulence-specific determination of *Listeria monocytogenes*, *J. Microbiol. Methods*, 71, 133, 2007.
11. Lantz, P.G. et al., Biotechnical use of polymerase chain reaction for microbiological analysis of biological samples, *Biotechnol. Annu. Rev.,* 5, 87, 2000.
12. Keer, J.T., and Birch, L., Molecular methods for the assessment of bacterial viability, *J. Microbiol. Methods,* 53, 175, 2003.
13. Liu, D., Identification, subtyping and virulence determination of *Listeria monocytogenes,* in important foodborne pathogen, *J. Med. Microbiol.,* 55, 645, 2006.
14. Mothershed, E.A., and Whitney, A.M., Nucleic acid-based methods for the detection of bacterial pathogens: Present and future considerations for the clinical laboratory, *Clin. Chim. Acta*, 363, 206, 2006.
15. Smith, K., Diggle, M.A., and Clarke, S.C., Automation of a fluorescence-based multiplex PCR for the laboratory confirmation of common bacterial pathogens, *J. Med. Microbiol.,* 53, 115, 2004.
16. Hoorfar, J. et al., Practical considerations in design of internal amplification control for diagnostic PCR assays, *J. Clin. Microbiol.,* 42, 1863, 2004.
17. Millar, B.C., Xu, J., and Moore, J.E., Risk assessment models and contamination management: implications for broad-range ribosomal DNA PCR as a diagnostic tool in medical bacteriology, *J. Clin. Microbiol.,* 40, 1575, 2002.
18. Aslanzadeh, J., Preventing PCR amplification carryover contamination in a clinical laboratory, *Ann. Clin. Lab. Sci.,* 34, 389, 2004.
19. Borst, A., Box, A.T., and Fluit, A.C., False-positive results and contamination in nucleic acid amplification assays: Suggestions for a prevent and destroy strategy, *Eur. J. Clin. Microbiol. Infect. Dis.,* 23, 289, 2004.
20. Abu Al-Soud, W., and Rådström, P., Capacity of nine thermostable DNA polymerases to mediate DNA amplification in the presence of PCR-inhibiting samples. *Appl. Environ. Microbiol.,* 64, 3748, 1998.
21. Rådström, P. et al., Pre-PCR processing: strategies to generate PCR-compatible samples, *Mol. Biotechnol.,* 26, 133, 2004.
22. Wolffs, P. et al., Impact of DNA polymerases and their buffer systems on quantitative real-time PCR, *J. Clin. Microbiol.,* 42, 408, 2004.
23. Liu, D., Preparation of *Listeria monocytogenes* specimens for molecular detection and identification, *Int. J. Food Microbiol.,* 122, 229, 2008.
24. Handschur, M. et al., Preanalytic removal of human DNA eliminates false signals in general 16S rDNA PCR monitoring of bacterial pathogens in blood, *Comp. Immunol. Microbiol. Infect. Dis*., 32, 207, 2009.

25. Malorny, B. et al., Standardization of diagnostic PCR for the detection of foodborne pathogens, *Int. J. Food Microbiol.,* 83, 39, 2003.
26. Anon, Microbiology of food and feeding stuffs—protocol for the validation of alternative methods. ISO 16140, first edition, ISO, Geneva, Switzerland, 2003.
27. Josefsen, M.H. et al., Validation of a PCR-based method for detection of foodborne thermotolerant campylobacters in a multi-center collaborative trial. *Appl. Environ. Microbiol.,* 70, 4379, 2004.
28. Hoorfar, J., Wolffs, P., and Radstrom, P., Diagnostic PCR: Validation and sample preparation are two sides of the same coin, *Apmis*, 112, 808, 2004.
29. Hoorfar, J. et al., Making internal amplification control mandatory for diagnostic PCR, *J. Clin. Microbiol.,* 41, 5835, 2003.
30. Rodriguez-Lazaro, D. et al., Construction strategy for an internal amplification control for real-time diagnostic assays using nucleic acid sequence-based amplification: Development and clinical application, *J. Clin. Microbiol.,* 42, 5832, 2004.
31. Trapmann, S. et al., Development of a novel approach for production of dried genomic DNA standards for quantitative PCR testing of food-borne pathogens, *Accred. Qual. Assur.,* 9, 695, 2004.
32. Burggraf, S., and Olgemoller, B., Simple technique for internal control of real-time amplification assays, *Clin. Chem.,* 50, 819, 2004.
33. Raggi, C.C. et al., External quality assurance program for PCR amplification of genomic DNA: An Italian experience, *Clin. Chem.,* 49, 782, 2003.
34. Cubie, H.A. et al., The development of a quality assurance programme for HPV testing within the UK NHS cervical screening LBC/HPV studies, *J. Clin. Virol.,* 33, 287, 2005.
35. Uslu, F., et al., Label-free fully electronic nucleic acid detection system based on a field-effect transistor device, *Biosens. Bioelectron.,* 19, 1723, 2004.
36. Lazcka, O., Del Campo, F.J., and Munoz, F.X., Pathogen detection: A perspective of traditional methods and biosensors, *Biosens. Bioelectron.*, 22, 1205, 2007.

Section I

Actinobacteria

2 *Actinomadura*

Martha E. Trujillo

CONTENTS

2.1 INTRODUCTION

The term actinomycete is an informal designation for filamentous gram-positive bacteria with high G + C content in their genome and that belong to the order *Actinomycetales* in the class *Actinobacteria*.[1,2] Most aerobic actinomycetes are soil saprophytes and are rarely encountered in clinical practice, but some are serious pathogens of humans and animals causing a number of diseases that include actinomycetoma, actinomycosis, and nocardiosis. The clinical manifestations and severity of the disease and the prognosis in an infected host are extremely variable and may be determined by factors such as the route of infection and the presence or absence of a properly functioning immune system.[3] The diagnosis of actinomycete infections has been hindered by a combination of clinical and microbiologic difficulties, including their often nonspecific clinical presentation, a requirement for invasive diagnostic biopsy procedures, difficulty in isolation, and incorrect identification.

Strategies to improve outcome for infected patients include a heightened awareness of clinicians and clinical microbiology personnel, which may enable the earliest possible diagnosis; standardization of antimicrobial susceptibility testing methods; and the evaluation of newer effective drug therapies for these patients. In addition, new developments in the classification of these microorganisms should serve as a framework for the identification of clinically significant species.

Actinomycetoma is a localized chronic, destructive, and progressive infection of the skin and subcutaneous tissues caused by aerobic actinomycetes.[3–5] The main species involved include *Actinomadura madurae*, *Actinomadura latina, Actinomadura pelletieri, Nocardia brasiliensis, Nocardia otitidiscaviarum, Nocardia transvalensis*, *Streptomyces somaliensis*, and *Streptomyces sudanensis*.[6,7] The disease is endemic in certain tropical and subtropical regions, where it has a devastating effect on patients as it frequently leads to deformities, disabilities, and eventually amputation of the affected organs.[7] Actinomycetoma can become dangerous to health, or even life, when treatment is inadequate or delayed.

This chapter will focus on the genus *Actinomadura* and its significance as a pathogen of actinomycetomas and nonmycetomic infections.

2.1.1 Taxonomy

The genus *Actinomadura* contains aerobic, gram-positive, nonacid-fast, nonmotile, and chemo-organotrophic actinomycetes that produce well-developed, nonfragmenting vegetative mycelia and aerial hyphae that differentiate into surface-ornamented spore chains of various lengths (10–50 spores).

Members of the genus *Actinomadura* are characterized chemotaxonomically by the presence of *meso*-diaminopimelic acid and madurose in their cell wall with peptidoglycan structures of the acetyl type, major proportions of hexahydrogenated menaquinones with nine isoprene units, complex fatty acid profiles, including hexadecanoic, 14-methylpentadecanoic, and 10-methyloctadecanoic acids as predominant components, and diphosphatidylglycerol and phosphatidylinositol as major phospholipids.[8]

The systematics of the genus *Actinomadura* has been significantly improved by the application of modern taxonomic methods.[8–14] On the basis of 16S and 23S rRNA gene sequence analyses, the genus is phylogenetically related to members of the family *Thermomonosporaceae*,[1,14] which also includes the genera *Actinocorallia*, *Spirillospora*, and *Thermomonospora*. The family *Thermomonosporaceae* belongs to the suborder *Streptosporangineae* in the order *Actinomycetales*.[2]

A. madurae was first described in 1894 by Vincent,[15] based on several strains isolated from an Algerian case of Madura foot, as "*Streptothrix madurae*." The organism was subsequently classified in the genus *Nocardia*,[16] then in the genus *Streptomyces*.[17] The taxonomic status of this microorganism remained controversial until Becker et al.[18] found that whole-organism hydrolysates of representative strains contained *meso*-diaminopimelic acid (*meso*-DAP) and a characteristic sugar identified as madurose.[19] In 1983, compelling evidence that the genus *Actinomadura* was heterogeneous was provided by Fischer et al.[10] when these authors assigned representative strains to two aggregate groups defined on the basis of chemical and nucleic acid pairing data—*A. madurae* group and *Actinomadura pusilla* group. The division of the genus *Actinomadura* into two aggregate groups was formally recognized by Kroppenstedt et al.,[8] who proposed that the genus *Actinomadura* be retained for *A. madurae* and related species, and that the *Actinomadura pusilla* group be reclassified in the genus *Microtetraspora*.

Taxonomic work carried out with a group of clinically significant *Actinomadura* strains received either as *A. madurae* or *A. pelletieri* revealed that a third species, *A. latina* should be officially recognized.[12] The genus currently comprises more than 40 validly described species (http://www.bacterio.net),[20] but only three—*A. latina*, *A. madurae*, and *A. pelletieri*—are considered pathogens. However, it is becoming increasingly evident that additional pathogenic actinomadurae need to be formally described as new species.[6,12,21]

2.1.2 Cell Morphology, Physiology, and Isolation

Actinomadura strains characteristically form nonfragmenting, extensively branched, substrate mycelia and aerial hyphae, which carry from 1 to 50 arthrospores. Spores are borne in curled, hooked, spiral, or straight chains and the spore surface may be folded, irregular, rugose, smooth, shiny, or warty.

Some strains, notably those from clinical sources, mostly lack aerial mycelium, with colonies exhibiting a cartilaginous or leathery appearance (Figure 2.1). However, members of most species form a spore-bearing, powdery aerial mycelium on media such as inorganic salts-starch (ISP 4), oatmeal (ISP 3), and yeast extract-malt extract agars (ISP 2) after cultivation for 10–14 days.[22] At maturity, aerial mycelia may be blue, cream, gray, green, pink, yellow, or white, thereby differing little from streptomycetes. Superficial similarity to streptomycetes is also reinforced by the morphology of the sporophores. Actinomadurae and streptomycetes can be distinguished by direct microscopic comparison of cultures. Most *Actinomadura* strains are conspicuous for the size of their spores. The diameter of spores noticeably exceeds the diameter of the hyphae, whereas in streptomycetes spores and hyphae are of similar diameter.

In general, actinomadurae are slow-growing microorganisms. These bacteria generally grow well on modified Bennett's agar,[23] glucose-yeast extract agar,[24] and on formulations used for the cultivation of *Streptomyces*.[22] *A. latina*, *A. madurae*, and *A. pelletieri* show moderate-to-good growth on Czapek-dox casamino acids.[25,26]

Actinomadura strains are strictly aerobic with an oxidative metabolism. Most species grow well between 25°C and 40°C but species such as *A. formosensis*, *A. rubrobrunea*, and *A. viridilutea* are thermophilic and grow between 45°C and 65°C. Actinomadurae can metabolize a wide range of sugars and amino acids as carbon sources for growth. Most strains hydrolyse aesculin, arbutin, casein, elastin, gelatine, testosterone, and Tweens 60 and 80.[6,27] Pectinase activity has been reported for *A. mexicana*, *A. napariensis*, and *A. verrucosispora*.[6,28] Pathogenic strains of *A. madurae* produce collagenolytic enzymes.[29] Most actinomadurae grow in the presence of NaCl 4% (w/v), but *A. atramentaria* can grow in NaCl concentrations of 7% (w/v).

Diverse culture media have been employed for the isolation of *Actinomadura* strains, especially from soil samples. The

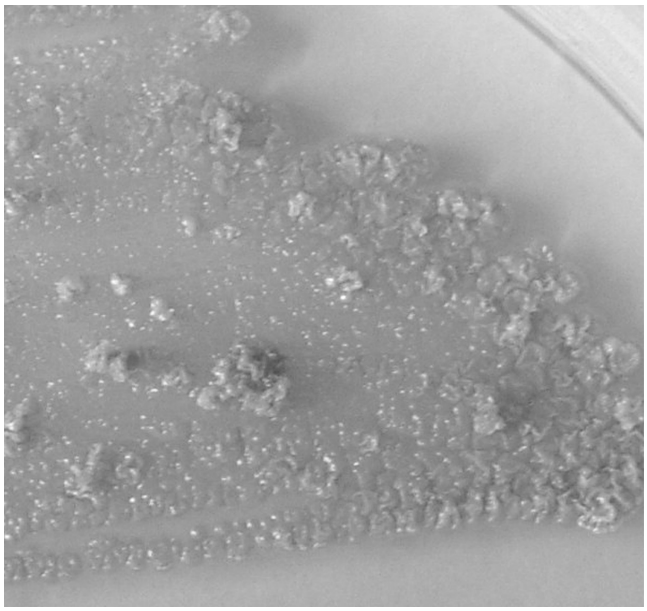


FIGURE 2.1 *Actinomadura* sp. grown on ISP 2 agar for 10 days. The strain does not produce aerial mycelium or diffusible pigments.

media that have proved most suitable include oatmeal agar (ISP 3), yeast extract-malt extract agar (ISP 2), starch-mineral salts agar (ISP 4), Bennett-sucrose agar, and glycerol-asparagine agar (ISP 5). Strains may be isolated from agar plates by dilution techniques after incubation for up to 6 weeks.

Trujillo and Goodfellow[6] successfully isolated new *Actinomadura* species from environmental samples collected in Hong Kong, Kenya, Mexico, South Africa, and Venezuela using the selective isolation procedure recommended by Athalye et al.[24]

Pathogen *Actinomadura* species can be isolated from clinical samples, including pus and biopsy material, using brain heart infusion agar,[30] Sabouraud dextrose agar,[31,32] and Columbia agar supplemented with 5% sheep blood.[33] All samples should be incubated aerobically between 27°C and 37°C for up to 3 weeks and examined both macroscopically and microscopically for growth every 2 days.

Actinomadurae can be recognized by their filamentous appearance, leathery colonies, and by the production of red prodiginine pigments. *A. madurae* and *A. pelletieri* produce prodigiosin-like pigments[34,35] that are similar to those of *Serratia marcescens*. Members of these species isolated from patients produce prodiginines characterized by a tripyrrole skeleton and identified as cyclononylprodiginine, nonylprodiginine, and undecylprodiginine.

A. latina, *A. madurae*, and *A. pelletieri* strains are mainly isolated from clinical material, though there is some evidence that members of *A. madurae* are widespread in organically rich soils. *A. madurae* strains isolated from environmental samples tend to lack the red endopigment of clinical isolates and sporulate more readily.[34–36]

2.1.3 Differentiation of Pathogen *Actinomadura* Species

Published descriptions of *Actinomadura* species are often incomplete since different investigators emphasize some phenotypic features and omit others, thereby making identification difficult. Nevertheless, most species can be separated using a combination of morphological and physiological properties, though in most cases only the type strain has been studied. However, even when several strains have been studied (e.g., *A. madurae*, *A. pelletieri*), the results tend to be variable or inconsistent when those from the literature are compared.

Enzymatic substrates based on the fluorophores 4-methylumbelliferone (4-MU) and 7-amino-methylcoumarin (7-AMC) were carried out by Trujillo and Goodfellow[6,12,36] to differentiate *Actinomadura* species. Encouraging results were obtained for differentiating between pathogenic *Actinomadura* species (Table 2.1).[6,36] Enzymatic tests were highlighted by Wink et al.[37] to differentiate *A. kijaniata* from *A. namibiensis*, as they share a DNA homology higher than 70%.

2.1.4 Pathogenicity and Clinical Features

Actinomycetoma. Actinomycetoma is a localized chronic, destructive infection of the skin and subcutaneous tissues caused by aerobic actinomycetes, *Actinomadura* being one of the main causal agents. The disease is characterized by progressive swelling of the infected area, distortion of the normal anatomy, and multiple draining sinuses and fistulae.[3–5,38–40] Purulent discharge containing the causative agent in the form of grains is characteristic of advanced stages of the disease. The grains vary in size and consistency and may be white, yellow, brown, red, or black depending on the causative agents. Almost 80% of the infections are through the lower extremities of the body; this probably explains the etiology of "Madura foot" described by Gill of Madura in South India in 1842. In areas where actinomycetoma is endemic, it is a common habit to walk barefooted, exposing the skin to thorns and splinters on the soil; as a result, natural infection appears to be more frequent. Other sites of the human body may also be affected such as the back, head, knee, arm, and neck.[41,42] In Mexico, the back is the second most common location.[43]

The clinical picture of actinomycete mycetoma is almost uniform irrespective of the causal agent. In contrast to eumycetoma (caused by eumycetes), infections caused by actinomycetes are more aggressive and destructive and involve both muscle and bone at an early stage.[5,41] In general, actinomycetoma is painless, even when the disease is at an advanced stage.[41] However, respiratory, neurologic, or other symptoms may be present when the disease affects the chest, head, and neck, or spine. The invasion of the periosteum and adjacent bones may lead to osteomyelitis.[42]

The infection remains localized and constitutional disturbances are rare, but when they do occur they are generally due to septicemia or to immune depression. Actinomycetoma can produce many disabilities, distortion, and deformity. It can be fatal especially if it affects the skull.[41,44]

Nonmycetomic Infections. Nonmycetomic infections produced by *A. madurae* have been reported, including one involving an immunocompromised patient.[45] These observations suggested that *A. madurae* could play a role in pneumonitis or bronchitis. Although no detailed epidemiological studies have been carried out, Bar et al.[46] reported a growing number of patients with pneumonia caused by *A. madurae*. Most of the patients had been infected as a consequence of impaired immunity. These studies suggested that *A. madurae* should also be considered as a causative agent for nonmycetomic infections such as pneumonia. Since that time, no epidemiological studies on *Actinomadura* strains from nonmycetomic regions or clinical specimens such as sputum, bronchoalveolar lavage, and blood samples have been reported. However, in Japan between 1996 and 2004, 21 actinomycete strains were isolated from sputum and broncho alveolar lavage.[21] Molecular identification using 16S rRNA gene sequencing indicated that all strains belonged to the genus *Actinomadura* but were unrelated to *A. madurae* or *A. pelletieri*. Ninety-five percent of the clinical isolates showed a close phylogenetic relationship with the species *Actinomadura cremea* and *Actinomadura nitritigenes*, while the remaining strains could not be related to any other species due to their moderate 16S rRNA gene sequence similarity.

TABLE 2.1
Differential Characteristics That Separate Pathogenic *Actinomadura* Species

Species	*A. latina*	*A. madurae*	*A. pelletieri*
Enzyme tests			
Cleavage of 7-amino-4-methylcoumarin (7AMC-) substrates			
Glutaryl-L-phenylalanine-7AMC	−	+	+
L-pyroglutamide-7AMC	+	+	−
Cleavage of 4-methylumberlliferone (4MU-) substrates			
4MU-*N*-acetyl-D-galactosoamide	−	d	+
4MU-α-L-arabinopyranoside	+	+	−
4MU-β-D-cellopyranoside	+	+	−
4MU-β–D-galactoside	−	+	−
4MU-β-D-glucoside	+	+	−
4MU-β-D-glucuronide	+	+	−
4MU-β-D-lactoside	−	+	−
4MU-sulfate	−	+	−
4MU-xyloside	+	+	−
Growth on sole carbon sources (1%, w/v)			
Adonitol	−	+	−
Fructose	+	+	−
Galactose	+	+	−
Glycerol	+	+	−
Mannitol	+	+	−
Melezitose	+	−	−
Degradation tests			
Arbutin	+	+	−
Esculin	+	+	−
Starch	+	−	+

Source: Based on data from Trujillo, M.E., and Goodfellow, M., *Antonie van Leeuwenhoek*, 84, 39, 2003 and Trujillo, M.E., and Goodfellow, M., *Zbl. Bakt*., 285, 212, 1997.

Epidemiology. It is not possible to state with any certainty the prevalence of *Actinomadura* infections (mainly actinomycetoma) in any part of the world, including the endemic areas, since there have not been any international surveillance efforts to ascertain the incidence of the disease. At most, information is limited to a few studies that have been made by individual researchers.[21,40,41,47]

Actinomycetomas have a worldwide distribution but occur mainly in the tropical and subtropical regions in the area between the latitudes of 15°S and 30°N. This area includes countries such India, Mali, Mexico, Senegal, Somalia, Sudan, and much of Central and South America.[5] Sudan appears to have the highest number of actinomycetomas in the world followed by Mexico with an average of 70 cases per year.[40] In these countries, the actinomycetoma infections are a major health problem, commonly affecting farmers and herdsmen. In general, the disease is four times more frequent in males than females and mainly affects adults between the ages of 16 and 40 years. Since these are the most active members of the society, the disease has a socioeconomic effect on dependent family members in underdeveloped countries.[48] Nevertheless, cases involving children and elderly men do occur.[49–51] Actinomycetoma infections have also been reported in the United States and Europe although in most cases, the patient contracted the disease in a different country.[38,52]

It is interesting to note that actinomycetoma becomes more active and aggressive in pregnant women. Hormonal changes and decreased immune response during pregnancy may be the explanation for this observation.[41]

There is evidence that climatic conditions may play a major role in the distribution of actinomycetomas. In the case of Mexico and Sudan, these countries have a rainy season from June to October; a dry, cool season from October to March; and hot and dry weather without rainfall from March to June. In Venezuela, most cases of actinomycetoma have been reported from semiarid areas colonized by trees, bushes, and cacti with thorns. The microorganisms are usually present in the soil. Traumatic inoculation of the subcutaneous tissues caused by sharp objects such as thorns or splinters is thought to be the route of entry. However, this theory has been recently disputed, as many patients have no history of trauma at the infection site.[41]

2.1.5 Diagnosis

The clinical diagnosis of actinomycetoma becomes more apparent in advanced disease with the development of characteristic sinuses and discharging grains; thus, the disease is often at an advanced stage when diagnosed.

2.1.5.1 Conventional Techniques

Microscopic Observation and Isolation. The diagnosis of the actinomycetoma is currently based on the isolation and identification of the causal microorganisms. In practice, the isolation of the causal agent is not always possible or can take a long time.

Direct microscopic examination of the pus from the lesions in the actinomycetomas with 10% KOH or saline reveals the presence of granules. The size, form, and color, together with the presence or absence of clubs or pseudoclubs, gives a clue to the identity of the etiologic agent. In the case of *A. madurae*, the granules can be seen without the aid of a microscope; in other species, the granules are smaller.[42]

Isolation of the microorganisms can be achieved by culture of the pus, granules, or tissue samples using various culture media such as Sabouraud, mycobiotic, or blood agar media.[33,42] The culture technique is often cumbersome and time consuming, and possible sample contamination may give a false positive result. This technique also requires experience to identify the causative microorganisms.

Fine Needle Aspiration Cytology. Actinomycetoma can be accurately diagnosed by fine needle aspiration cytology.[53–55] Mycetoma lesions have a distinct appearance in a cytology smear and are characterized by the presence of polymorphous inflammatory cells such as neutrophils, histiocytes, lymphocytes, plasma cells, macrophages, and foreign body giant cells. This allows differentiation from artifacts and inflammatory lesions caused by other bacteria and fungi. Diagnosis of actinomycetoma using fine needle aspiration cytology has proved to be as accurate as histopathological observations when the grains are present.[54]

This technique is fast, inexpensive, and easy to apply, and it is well tolerated by patients. It can be used in routine diagnosis and as an effective means of collection of material for culture and immunological studies. Due to the simplicity of the technique, it can be used in epidemiological surveys of mycetoma and for detection of early cases when radiological and serological techniques may not be useful.[41]

Radiology. The radiological features of mycetoma are complex. As the disease spreads to the bone, the earliest changes include periosteal erosion and adjacent sclerosis. There may be a soft tissue mass with obliteration of fascial planes. Bone cortex may be compressed from outside by the mass, producing scalloping, which is followed by variable amount of periosteal reaction. Sunray appearance and Codman's triangle may be present, producing a picture similar to that of osteogenic sarcoma. There may be multiple cavities within normal-density bone; these cavities are small and numerous and their edges not well defined. The cavity size is directly related to the size of the grains. Osteosporosis is common in advanced mycetoma due to compression of the bone and insufficient blood irrigation caused by pressure on the blood vessels.

Radiographs are helpful to determine the extent of the infection, although they are not diagnostic. Computed tomography or magnetic resonance imaging can also be helpful to determine the full extent of bone involvement and to delineate soft tissue involvement.[39]

Immunodiagnosis. Enzyme-linked immunosorbent assays have been used to screen for antibodies against *S. somaliensis* and *A. madurae* in two regions of Sudan.[56] These results indicated that a relatively large proportion of clinically asymptomatic individuals in one region were infected with *S. somaliensis* and, to a lesser degree, *A. madurae*. To date, however, there are no specific and reliable serologic or immunologic tests useful in the diagnosis of actinomycetoma. This is in part because of the lack of specific antigens that do not cross-react with antibodies of infections caused by other related microorganisms.[42] Attempts have been made to develop a delayed-type hypersensitivity test in mycetoma using microbial agents, but in most cases the assays have not been sensitive enough, or represented, as in the case of aerobic actinomycetic infection, cross-reactions with tuberculosis, and leprosy.[42]

2.1.5.2 Molecular Techniques

PCR-RFLP Analysis. Use of PCR coupled with restriction endonuclease and/or probe hybridization analyses of PCR products has been the focus of recent interest for the separation of mycobacteria from nocardiae as well as for the recognition of species within the genera *Mycobacterium* and *Nocardia*.[57] The method has proven to be sensitive, less time consuming, and less labor intensive than traditional biochemical methods for the identification of the clinically significant species.

This technique can be directly applied to clinical isolates provided that sufficient biomass is available for genomic DNA extraction. A 439-bp fragment corresponding to the 65-kDa heat shock protein gene is then amplified by PCR using primers TB11 and TB12,[57] and the PCR product is then digested using a combination of five commercially available restriction endonucleases (*Bst*EII, *Hae*III, *Msp*I, *Hinf*I, and *Bsa*HI). After digestion, the restriction fragments are electrophoresed on 3% Metaphor agarose containing ethidium bromide at 95 V for 1.5–2 h to obtain the RFLP band patterns. This PCR-RFLP methodology distinguished clinical isolates of aerobic actinomycetes, including *A. madurae*, with 96.8% accuracy. Thus, identification of clinical isolates can be accomplished within 24–48 h of receipt of pure cultures and therefore this system can be readily and economically implemented for routine clinical use.[57]

16S rRNA Gene Sequencing. Identification of *Actinomadura* actinomycetes from nonmycetomic clinical samples has also been carried out successfully using 16S rRNA gene sequencing. In this case, Hafany et al.[21] isolated *Actinomadura* strains from the sputa or the bronchoalveolar lavage fluid of patients with pulmonary infections.