Contents

1 Safety Norms and Regulations in Handling Fungal Specimens................................................................. 1
   Finola E. Cliffe

2 Methods of Cryopreservation in Fungi........................................ 9
   Ladislav Homolka

3 Long-Term Preservation of Fungal Cultures in All-Russian Collection of Microorganisms (VKM): Protocols and Results .................................................................................................................. 17
   Svetlana M. Ozerskaya, Natalya E. Ivanushkina, Galina A. Kochkina, Svetlana S. Eremina, Alexander N. Vasilenko, and Nadezhda I. Chigineva

4 Fungal Specimen Collection and Processing .................. 67
   Anthonia O’Donovan, Vijai Kumar Gupta, and Maria G. Tuohy

5 Chemical and Molecular Methods for Detection of Toxigenic Fungi and Their Mycotoxins from Major Food Crops .................................................................................................................. 73
   S. Chandra Nayaka, M. Venkata Ramana, A.C. Udayashankar, S.R. Niranjana, C.N. Mortensen, and H.S. Prakash

6 Identification Key for the Major Growth Forms of Lichenized Fungi ......................................................... 91
   Jeyabalan Sangeetha and Devarajan Thangadurai

7 Microscopic Methods for Analytical Studies of Fungi ...... 113
   De-Wei Li

8 Scanning Electron Microscopy for Fungal Sample Examination ........................................................................ 133
   Eduardo Alves, Gilvaine Ciavareli Lucas, Edson Ampélio Pozza, and Marcelo de Carvalho Alves

9 High-Resolution Imaging and Force Spectroscopy of Fungal Hyphal Cells by Atomic Force Microscopy ........ 151
   Biplab C. Paul, Hui Ma, Laelie A. Snook, and Tanya E.S. Dahms
## Contents

10 **Use of Fourier-Transform Infrared (FTIR) Microscopy Method for Detection of Phyto-Fungal Pathogens** ................................................................. 161
   Vitaly Erukhimovitch and Mahmoud Huleihel

11 **Diagnosis of Parasitic Fungi in the Plankton: Technique for Identifying and Counting Infective Chytrids Using Epifluorescence Microscopy** ......................................................... 169
   Télesphore Sime-Ngando, Serena Rasconi, and Mélanie Gerphagnon

12 **Fungal Cell Wall Analysis** ........................................................... 175
   Pilar Pérez and Juan C. Ribas

13 **Histopathological Technique for Detection of Fungal Infections in Plants** ............................................................. 197
   Vijai Kumar Gupta and Brejesh Kumar Pandey

14 **Development of Media for Growth and Enumeration of Fungi from Water** ................................................................. 201
   Segula Masaphy

15 **Sabouraud Agar for Fungal Growth** .......................................... 211
   Janelle M. Hare

16 **A Method for the Formation of Candida Biofilms in 96 Well Microtiter Plates and Its Application to Antifungal Susceptibility Testing** ........................................... 217
   Christopher G. Pierce, Priya Uppuluri, and Jose L. Lopez-Ribot

17 **Screening for Compounds Exerting Antifungal Activities** ...... 225
   Jean-Paul Ouedraogo, Ellen L. Lagendijk, Cees A.M.J.J. van den Hondel, Arthur F.J. Ram, and Vera Meyer

18 **Fluorescence In Situ Hybridization of Uncultured Zoosporic Fungi** ............................................................. 231
   Télesphore Sime-Ngando, Marlène Jobard, and Serena Rasconi

19 **Staining Techniques and Biochemical Methods for the Identification of Fungi** ............................................................. 237
   Jeyabalan Sangeetha and Devarajan Thangadurai

20 **Protocol for the In Vivo Quantification of Superoxide Radical in Fungi** ............................................................. 259
   Konstantinos Grintzalis, Ioannis Papapostolou, and Christos Georgiou

21 **Isolation of Intact RNA from Sorted S. cerevisiae Cells for Differential Gene Expression Analysis** ............................................................. 265
   Jeannette Vogt, Frank Stahl, Thomas Scheper, and Susann Müller

22 **Quantitative PCR Analysis of Double-Stranded RNA-Mediated Gene Silencing in Fungi** ............................................................. 279
   José J. de Vega-Bartol, Vega Tello, Jonathan Niño, Virginia Casado, and José M. Díaz-Mínguez
23 Semi-Nested PCR Approach to Amplify Large 18S rRNA Gene Fragments for PCR-DGGE Analysis of Soil Fungal Communities .................................................. 289
Miruna Oros-Sichler and Kornelia Smalla

24 Proteomic Protocols for the Study of Filamentous Fungi ........ 299
Raquel González Fernández and Jesús V. Jorrín Novo

25 Detection and Quantification of Endoprotease Activity Using a Coomassie Dye-Binding Assay .................................................................. 309
Anthony J. O’Donoghue and Cathal S. Mahon

26 Protocol of a LightCycler™ PCR Assay for Detection and Quantification of Aspergillus fumigatus DNA in Clinical Samples of Neutropenic Patients........................................... 315
Birgit Spiess and Dieter Buchheidt

27 Application of Polymerase Chain Reaction and PCR-Based Methods Targeting Internal Transcribed Spacer Region for Detection and Species-Level Identification of Fungi............. 321
K. Lily Therese, R. Bagyalakshmi, and H.N. Madhavan

28 Real-Time PCR Assay in Fungi .......................................................... 331
Naomichi Yamamoto

29 Quantitative Sampling Methods for the Analysis of Fungi: Air Sampling ................................................................................. 337
Mary C. O’Loughlin, Katherine D. Turner, and Kevin M. Turner

30 Transformation of Filamentous Fungi in Microtiter Plate........... 343
Bianca Gielesen and Marco van den Berg

31 Molecular Fingerprinting of Fungal Communities in Soil........... 349
Roberto A. Geremia and Lucie Zinger

32 Development of Microsatellite Markers from Fungal DNA Based on Shotgun Pyrosequencing ................................................. 357
Shaobin Zhong

33 Multiplex and Quantifiable Detection of Infectious Fungi Using Padlock Probes, General qPCR, and Suspension Microarray Readout ................................................................. 363
Magnus Jobs, Ronnie Eriksson and Jonas Blomberg

34 Rapid Deletion Plasmid Construction Methods for Protoplast and Agrobacterium-based Fungal Transformation Systems .......................................................... 375

35 Improved Transformation Method for Alternaria Brassicicola and Its Applications ........................................................................ 395
Yangrae Cho, Akhil Srivastava, and Christopher Nguyen
36 Methods for High-Quality DNA Extraction from Fungi.......... 403
Vijai Kumar Gupta, Maria G. Tuohy, and Rajeeva Gaur

37 Production of Recombinant Proteins from *Pichia pastoris*:
Interfacing Fermentation and Immobilized Metal Ion
Affinity Chromatography............................................................ 407
Berend Tolner, Gaurav Bhavsar, Bride Foster,
Kim Vigor, and Kerry Chester

38 Development of a Real-Time Quantitative PCR Assay
for the Assessment of Uncultured Zoosporic Fungi............... 421
Télesphore Sime-Ngando and Marlène Jobard

39 Nucleic and Protein Extraction Methods for Fungal
Exopolysaccharide Producers.................................................. 427
Jochen Schmid, Dirk Mueller-Hagen, Volker Sieber,
and Vera Meyer

40 Directed Evolution of a Fungal Xylanase for Improvement
of Thermal and Alkaline Stability ........................................... 435
Dawn Elizabeth Stephens, Suren Singh, and Kugen Permaul

41 Genome Shuffling Protocol for the Pentose-Fermenting
Yeast *Scheffersomyces stipitis* .................................................. 447
Paramjit K. Bajwa, Nicole K. Harner, Terri L. Richardson,
Sukhdeep Sidhu, Marc B. Habash, Jack T. Trevors,
and Hung Lee

42 Detection and Identification of Fungal Microbial
Volatile Organic Compounds by HS-SPME-GC–MS............... 455
Bernhard Kluger, Susanne Zeilinger, Gerlinde Wiesenberger,
Denise Schöfbeck, and Rainer Schuhmacher

43 Transformation Methods for Slow-Growing Fungi.......... 467
Suman Mukherjee and Rebecca Creamer

44 Enzymatic Saccharification of Lignocellulosic Biomass.... 475
Manimaran Ayyachamy, Vijai Kumar Gupta,
Finola E. Cliffe, and Maria G. Tuohy

45 Protoplast Fusion Techniques in Fungi................................. 483
Annie Juliet Gnanam

46 Large-Scale Production of Lignocellulolytic Enzymes
in Thermophilic Fungi.............................................................. 489
Manimaran Ayyachamy, Mary Shier, and Maria G. Tuohy

47 Panfungal PCR Method for Detection
of Aflatoxigenic Molds ............................................................ 495
Malik M. Ahmad, Pravej Alam, M.Z. Abdin, and Saleem Javed

48 Protocols for the Quantification of dsDNA
and Its Fragmentation Status in Fungi................................. 501
Ioannis Papapostolou, Konstantinos Grintzalis,
and Christos Georgiou
49 Rapid Identification and Detection of Pathogenic Fungi by Padlock Probes ............................................................. 505 Clement K.M. Tsui, Bin Wang, Cor D. Schoen, and Richard C. Hamelin

50 Drug-Induced Permeabilization in Fungi .................................. 519 Maria D. Mayan, Alexandra McAleenan, and Priscilla Braglia

51 Extraction and Characterization of Taxol: An Anticancer Drug from an Endophytic and Pathogenic Fungi ..................... 523 M. Pandi, P. Rajapriya, and P.T. Manoharan

52 Identification of Mycotoxigenic Fungi Using an Oligonucleotide Microarray ........................................................... 529 Eugenia Barros

53 DNA Microarray-Based Detection and Identification of Fungal Specimens ............................................................ 535 Minna Mäki

54 Bioinformatic Protocols and the Knowledge-Base for Secretomes in Fungi ........................................................... 545 Gengkon Lum and Xiang Jia Min

55 High-Throughput Functional Annotation and Data Mining of Fungal Genomes to Identify Therapeutic Targets .............. 559 Gagan Garg and Shoba Ranganathan

56 Application of Support Vector Machines in Fungal Genome and Proteome Annotation ............................................. 565 Sonal Modak, Shimantika Sharma, Prashant Prabhakar, Akshay Yadav, and V.K. Jayaraman

57 Bioinformatics Tools for the Multilocus Phylogenetic Analysis of Fungi ............................................................... 579 Devarajan Thangadurai and Jeyabalan Sangeetha

Index ...................................................................................................... 593
Laboratory Protocols in Fungal Biology
Current Methods in Fungal Biology
Gupta, V.K.; Tuohy, M.G.; Ayyachamy, M.; Turner, K.M.; O’Donovan, A. (Eds.)
2013, XXV, 604 p., Hardcover
ISBN: 978-1-4614-2355-3