METHODS IN MOLECULAR BIOLOGY

Series Editor
John M. Walker
School of Life and Medical Sciences
University of Hertfordshire
Hatfield, Hertfordshire, AL10 9AB, UK

For further volumes:
http://www.springer.com/series/7651

Microbial Lipid Production

Methods and Protocols

Edited by

Venkatesh Balan

Department of Engineering Technology, Biotechnology Program, College of Technology, University of Houston, Houston, TX, USA

Humana Press

Editor
Venkatesh Balan
Department of Engineering Technology
Biotechnology Program
College of Technology
University of Houston
Houston, TX, USA

ISSN 1064-3745 ISSN 1940-6029 (electronic)
Methods in Molecular Biology
ISBN 978-1-4939-9483-0 ISBN 978-1-4939-9484-7 (eBook)
https://doi.org/10.1007/978-1-4939-9484-7

This Humana imprint is published by the registered company Springer Science+Business Media, LLC, part of Springer Nature.
The registered company address is: 233 Spring Street, New York, NY 10013, U.S.A.

Preface

Lipids derived from conventional sources such as oil seeds include building blocks of cellular membranes. They are utilized to make substances used as energy storage, insulation, a method of cellular communication, and protection. With population growth and increasingly limited farmland, there is an ever-growing demand to develop alternate sources of lipids to meet our food and energy needs. Oleaginous microorganisms such as bacteria, algae, yeast, fungi, and thraustochytrids are a promising, largely untapped, resource for lipid production. Microbial lipids offer some unique advantages, such as high content of unique polyunsaturated fatty acids that are widely used for dietary purposes and of chemically modified lipids that confer desirable physical properties. Lipid content in microorganisms depends upon genetic constituents and environmental conditions such as pH, temperature, exposure to natural light, and nitrogen content in the media. There are several advantages of producing lipids in microorganisms such as lower cultivation costs, ability to produce a diverse range of lipids using genetic manipulation, and the possibility of producing lipids year-round with limited space and infrastructure without the need for agricultural land.

The 24 chapters of this book provide comprehensive routinely used methods for isolation and characterization of lipids and for their production using various oleaginous organisms. The methods presented consider sugars derived from different substrates including chemically pretreated agricultural residues, industrial residues containing lignin, food wastes, and industrial waste water in an approachable format. The authors have also provided detailed applications using oleaginous organisms to transform substrates into a variety of products including bio-crude, high-value fatty acids, biofuels such as biodiesel, neutral lipids, volatile fatty acids, and surfactants. Protocols are presented in a basic standard outline format conducive to adaptation to suit specific application needs. This book is aimed at the novice, and therefore each technique is complete and assumes no prior knowledge. Novel screening protocols to identify oleaginous organisms with exceptional lipid yields, genetic engineering approaches to increase microbial lipid content, methods to judge fuel quality from microbial lipids, and life cycle analysis are some of the unique topics that are covered in this book.

Much of the success of the series is due to the "Notes" section, which describes where common procedural problems are identified, and solutions are discussed along with alternative procedures. It is in this section where important, practical details are presented that are rarely included in other published works. This is how the authors have passed along their practical experience to help mentor readers. Most of the chapters are focused on specific laboratory methods, but the first chapter provides a comprehensive review about lipids derived from plants and various microorganisms, an overview of available analytical techniques to characterize lipids, and their applications in various processes.

It has been inspiring to see the natural diversity of lipid molecules with varying properties and functions synthesized by microorganisms using complex genetic and enzymatic machinery. Also, it is equally inspiring to see the numerous analytical techniques and protocols now available for lipid researchers to use to analyze structure and function and to pursue diverse applications. Though it was challenging to organize such a range of chapter topics to fit together in a common theme, all the contributing authors are commended for their expertise, ensuring each chapter was on topic, and for writing in a clear and

direct style. I take this opportunity to thank both my wife and two daughters for sacrificing family time and motivating me to complete the book at times when my morale ebbed. I thank all my colleagues in the College of Technology and administrator at the University of Houston for their support and encouragement that made this book possible. Also, I thank Dr. Patricia Slinger and Dr. Bruce Dien from USDA ARS, Peoria, for providing the cover picture and also helping me to critically review several chapters in this book.

Houston, TX, USA ***Venkatesh Balan***

Cover Illustration Caption

Shown in the figure are cells of Saitoella coloradoensis strain NRRL YB-2330 containing numerous lipid granules. This strain was discovered to tolerate and produce abundant lipid when cultivated on ammonia fiber expansion (AFEX)-pretreated enzyme saccharified hydrolyzates of corn stover (Slininger et al. 2016; Dien et al. 2016). Saitoella, NRRL YB-2330, was isolated by L. J. Wickerham from insect frass collected in 1950 by staff of the U.S. Forest Service from an Engelmann spruce (Picea engelmannii) growing in the White River National Forest, Meeker, CO, USA. Taxonomically described by Kurtzman and Robnett (2012), NRRL YB-2330 is recognized as a member of the genus Saitoella and as a new species distinct from NRRLY-17804, type strain of S. complicata, the only other Saitoella species in collection.

- Kurtzman CP and Robnett CJ (2012) Saitoella coloradoensis sp. nov., a new species of the Ascomycota, subphylum Taphrinomycotina. Antonie van Leeuwenhoek 101:795–802.
- Slininger PJ, Dien BS, Kurtzman CP, Moser BR, Bakota EL, Thompson SR, O'Bryan PJ, Cotta MA, Balan V, Jin M (2016) Comparative lipid production by oleaginous yeasts in hydrolyzates of lignocellulosic biomass and process strategy for high titers. Biotechnology and Bbioengineering 113:1676–1690
- Dien BS, Slininger PJ, Kurtzman CP, Moser BR, O'Bryan PJ (2016a) Identification of superior lipid producing Lipomyces and Myxozyma yeasts. AIMS Environmental Science, 3:1–20.

Contents

Contributors

Venkatesh Balan • *Department of Engineering Technology, Biotechnology Program, College of Technology, University of Houston, Houston, TX, USA*

G. Florin Barla • *Faculty of Food Engineering, University of Suceava, Suceava, Romania; Tyton Biosciences, Danville, VA, USA*

Tyler J. Barzee • *Department of Biological and Agricultural Engineering, University of California, Davis, Davis, CA, USA*

Eboibi Blessing • *Department of Chemical Engineering, Delta State University, Oleh, Nigeria*

Kyria L. Boundy-Mills • *Phaff Yeast Culture Collection, Department of Food Science and Technology, University of California, Davis, Davis, CA, USA*

Tom Bradley • *Narec Distributed Energy, CPTC, High Quay, Blyth, UK*

Tomas Cajka • *West Coast Metabolomics Center, University of California, Davis, Davis, CA, USA; Department of Metabolomics, Institute of Physiology CAS, Prague, Czech Republic*

Senthil Chinnasamy • *Biotechnology Division, Aban Infrastructure Limited, Chennai, India*

Jacob D. Crowe • *Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI, USA*

Farha Deeba • *Department of Polymer and Process Engineering, IIT Roorkee, Saharanpur, India*

Goksel N. Demirer • *Central Michigan University, School of Engineering & Technology, Mount Pleasant, MI, USA*

Bruce S. Dien • *Bioenergy Research Unit, National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL, USA*

Irene Fakankun • *Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB, Canada*

Oliver Fiehn • *West Coast Metabolomics Center, University of California, Davis, Davis, CA, USA*

Annaliese K. Franz • *Department of Chemistry, University of California, Davis, Davis, CA, USA; Agricultural and Environmental Chemistry Graduate Group, University of California, Davis, CA, USA*

Antonio L. Garay • *Phaff Yeast Culture Collection, Department of Food Science and Technology, University of California, Davis, Davis, CA, USA; Pepsico, Plano, TX, USA*

Naseem A. Gaur • *Yeast Biofuel Group, DBT-ICGEB Centre for Advanced Bioenergy Research, International Centre for Genetic Engineering and Biotechnology, New Delhi, India*

Manickam Gayathri • *Department of Microbiology, Centre of Excellence in Life Sciences, Bharathidasan University, Tiruchirappalli, TN, India*

Eduardo J. Gudiña • *CEB–Centre of Biological Engineering, University of Minho, Braga, Portugal*

Fatemeh Heidari • *Department of Bioproducts and Biosystems Engineering, University of Minnesota, Saint Paul, MN, USA*

David B. Hodge • *Department of Chemical and Biological Engineering, Montana State University, Bozeman, MN, USA; Department of Civil, Environmental, and Natural Resources Engineering, Luleå University of Technology, Luleå, Sweden*
Bo Hu • *Department of Bioproducts and Biosystems Engineering, University of Minnesota, Saint Paul, MN, USA*
Singaram Jayanthi • *Government College of Technology, Coimbatore, India*
Umakanta Jena • *Department of Chemical and Materials Engineering, New Mexico State University, Las Cruces, NM, USA*
Mingjie Jin • *School of Environmental and Biological Engineering, Nanjing University of Science and Technology, Nanjing, China*
A. Daniel Jones • *Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA; Department of Chemistry, Michigan State University, East Lansing, MI, USA*
Sandeep Kumar • *Department of Civil and Environmental Engineering, Old Dominion University, Norfolk, VA, USA*
Priya Kumari • *Yeast Biofuel Group, DBT-ICGEB Centre for Advanced Bioenergy Research, International Centre for Genetic Engineering and Biotechnology, New Delhi, India*
Cletus P. Kurtzman • *Mycotoxin Prevention and Applied Microbiology Research Unit, National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL, USA*
Rosemary K. Le • *Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville, TN, USA*
David B. Levin • *Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB, Canada*
Muyang Li • *Department of Agricultural and Biological Engineering, Michigan State University, East Lansing, MI, USA*
Wei Liao • *Anaerobic Digestion Research and Education Center (ADREC), Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI, USA*
Tongjun Liu • *Department of Bioengineering, Qilu University of Technology, Jinan, China*
Balakrishna Maddi • *Suganit Bio-Renewables LLC, Toledo, OH, USA*
Daniel Maga • *Fraunhofer Institute for Environmental, Safety and Energy Technology UMSICHT, Oberhausen, Germany*
Kristina M. Mahan • *Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville, TN, USA*
Schonna R. Manning • *UTEX Culture Collection of Algae, Department of Molecular Biosciences, The University of Texas at Austin, Austin, TX, USA*
Maryam Mirzaei • *Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB, Canada*
Sima Modiri • *Department of Energy and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran*
Gangatharan Muralitharan • *Department of Microbiology, Centre of Excellence in Life Sciences, Bharathidasan University, Tiruchirappalli, TN, India*
Yuvraj S. Negi • *Department of Polymer and Process Engineering, IIT Roorkee, Saharanpur, India*
David R. Nobles Jr. • *UTEX Culture Collection of Algae, Department of Molecular Biosciences, The University of Texas at Austin, Austin, TX, USA*

Kambiz Akbari Noghabi • *Department of Energy and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran*
Vikas Pruthi • *Department of Biotechnology, IIT Roorkee, Roorkee, India*
Joshua C. Quarterman • *Bioenergy Research Unit, National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL, USA*
Arthur J. Ragauskas • *Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville, TN, USA; Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA; Department of Forestry, Wildlife and Fisheries, Center of Renewable Carbon, Institute of Agriculture, University of Tennessee, Knoxville, TN, USA*
Lígia R. Rodrigues • *CEB–Centre of Biological Engineering, University of Minho, Braga, Portugal*
Kayla M. Rude • *Department of Chemistry, University of California, Davis, Davis, CA, USA; Agricultural and Environmental Chemistry Graduate Group, University of California, Davis, CA, USA*
Sumathy Shunmugam • *Department of Microbiology, Centre of Excellence in Life Sciences, Bharathidasan University, Tiruchirappalli, TN, India*
Irnayuli R. Sitepu • *Phaff Yeast Culture Collection, Department of Food Science and Technology, University of California, Davis, Davis, CA, USA*
Patricia J. Slininger • *Bioenergy Research Unit, National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL, USA*
Alex D. Smith • *Department of Chemical and Biological Engineering, University of Wisconsin, Madison, WI, USA*
Xiao-Ling Tang • *Zhejiang University of Technology, Hangzhou, Zhejiang, China*
Arun Kumar Thalla • *Department of Civil Engineering, National Institute of Technology Karnataka, Mangalore, Karnataka, India*
Stephanie R. Thompson • *Bioenergy Research Unit, National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL, USA*
Bryan Ubanwa • *Department of Engineering Technology, Biotechnology Program, College of Technology, University of Houston, Houston, TX, USA*
Sibel Uludag-Demirer • *Anaerobic Digestion Research and Education Center (ADREC), Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI, USA*
Agasteswar Vadlamani • *Department of Chemical Engineering, The University of Toledo, Toledo, OH, USA*
Sasidhar Varanasi • *Department of Chemical Engineering, The University of Toledo, Toledo, OH, USA*
Sridhar Viamajala • *Department of Chemical Engineering, The University of Toledo, Toledo, OH, USA*
Zhiqiang Wen • *School of Environmental and Biological Engineering, Nanjing University of Science and Technology, Nanjing, China*
Daniel L. Williams • *Element Materials Technology, Plymouth, MI, USA*
Zhaoxian Xu • *School of Environmental and Biological Engineering, Nanjing University of Science and Technology, Nanjing, China*
Ya-Ping Xue • *Zhejiang University of Technology, Hangzhou, Zhejiang, China*
Yan Yang • *Department of Bioproducts and Biosystems Engineering, University of Minnesota, Saint Paul, MN, USA*
Cody Yothers • *Department of Chemistry, University of California, Davis, Davis, CA, USA*

Farnaz Yusuf • *Yeast Biofuel Group, DBT-ICGEB Centre for Advanced Bioenergy Research, International Centre for Genetic Engineering and Biotechnology, New Delhi, India*
Hossein Shahbani Zahiri • *Department of Energy and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran*
Rui Zhai • *School of Environmental and Biological Engineering, Nanjing University of Science and Technology, Nanjing, China*