METHODS IN MOLECULAR BIOLOGY™

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Microbial Carotenoids from Bacteria and Microalgae

Methods and Protocols

Edited by

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Humana Press

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ISSN 1064-3745 e-ISSN 1940-6029
ISBN 978-1-61779-878-8 e-ISBN 978-1-61779-879-5
DOI 10.1007/978-1-61779-879-5
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2012937073

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Printed on acid-free paper

Humana Press is part of Springer Science+Business Media (www.springer.com)

Preface

Carotenoids are a family of yellow to orange-red terpenoid pigments synthesized by photosynthetic organisms and by many bacteria and fungi. They have beneficial health effects, protecting against oxidative damage, and may be responsible for the colors associated with plants and animals. Carotenoids are also desirable commercial products used as colorants, feed supplements, and nutraceuticals in the food, medical, and cosmetic industries. Only a few of the more than 600 identified carotenoids are produced industrially, with β -carotene (a popular additive for butter, ice cream, orange juice, candies, etc.) the most prominent.

Commercial production of natural carotenoids from microorganisms is a new approach more eco-friendly than synthetic manufacture by chemical procedures. Despite the availability of a variety of natural and synthetic carotenoids, there is currently renewed interest in microbial sources. Due to its increasing importance, industrial biotechnological methods of carotenoids production have been developed with the algae *Dunaliella salina* and *Haematococcus pluvialis*, the fungus *Blakeslea trispora*, and the heterobasidiomycetous yeast *Xanthophyllomyces dendrorhous*.

This book is intended to provide practical experimental laboratory procedures for a wide range of carotenoids producing microorganisms. Although not an exhaustive treatise, it provides a detailed “step-by-step” description of the most recent developments in applied biotechnological processes useful for screening and selection of carotenoids producing microorganisms, construction of new carotenoids biosynthetic pathways, and extraction and analysis of carotenoids. The detailed protocols are cross-referenced in the Notes section, providing special details, little problems, troubleshooting, and safety comments that may not normally appear in journal articles and can be particularly useful for those not familiar with specific techniques.

The lead chapter of this volume represents an overview on the pathways of carotenoids biosynthesis in bacteria and microalgae. Next chapters show comprehensive experimental methods for the selection of new carotenoids producing bacteria and the engineering of several bacteria (including *Escherichia coli*, *Bradyrhizobium* sp., *Methylobionas* sp., *Bacillus subtilis*, and halophilic bacteria) for carotenoids production. Furthermore, methods for canthaxanthin production, construction of carotenoid reporter systems, directed evolution of carotenoid synthases, and improvement of β -carotene hydroxylase catalytic activity are described. Additionally, the book includes methods of DNA fingerprinting for the identification of carotenogenic *Dunaliella* species, ketocarotenoid biosynthesis in microalgae expressing the β -C-4-carotene oxygenase gene, characterization of carotenogenesis genes in *Anabaena* sp., obtaining lutein from microalgal biomass, NMR-based isotopologue profiling of microbial carotenoids, and analysis of diapocarotenoids.

This book has been written by outstanding experts in their fields and provides a reference source for laboratory and industrial professionals, as well as for graduate students in a number of biological disciplines (biotechnology, microbiology, genetics, molecular biology, etc.).

I am indebted to the authors that, in spite of their professional activities, agreed to participate in this book, to Dr. J. Walker, Series Editor, for his encouragement and advice in reviewing the manuscripts, and to the rest of the staff of The Humana Press, Inc., for their assistance in assembling this volume and their efforts in keeping this project on schedule. Last but not least, I warmly acknowledge my wife Natalia and our children Diego, José-Luis, Álvaro, and Gonzalo for their patience and support.

León, Spain

José-Luis Barredo

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