Woodhead Publishing in Food Science and Technology

Yeasts in food

Edited by T. Boekhout and V. Robert

WOODHEAD PUBLISHING LIMITED

BEHR'S...VERLAG

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Beneficial and detrimental aspects

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T. Boekhout and V. Robert

CRC Press
Boca Raton Boston New York Washington, DC

WOODHEAD PUBLISHING LIMITED
Cambridge England

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Published by Woodhead Publishing Limited, Abington Hall, Abington
Cambridge CB1 6AH, England
www.woodhead-publishing.com

Published in North America by CRC Press LLC, 2000 Corporate Blvd, NW
Boca Raton FL 33431, USA

First published 2003, Woodhead Publishing Ltd and CRC Press LLC

This edition is published by arrangement with B. Behr's Verlag GmbH & Co. KG, Hamburg, Germany.

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library.

Library of Congress Cataloging in Publication Data
A catalog record for this book is available from the Library of Congress.

Woodhead Publishing ISBN 1 85573 706 X (book) 1 85573 711 6 (e-book)
CRC Press ISBN 0-8493-1926-9
CRC Press order number: WP1926

Printed by Bayerlein GmbH, 86356 Neusäss, Germany

Dedicated to the memory of

Prof. Dr. Herman Phaff

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Editors preface

The production and maintenance of good quality food products contribute to the quality of life. Yeasts and food are intimately related since the early days of human civilization. Early humans discovered that fermented foods and drinks had added nutritional value and, in various cases, could be better preserved. Consequently, fermented foods contributed to human survival during historical times. The workhorse among the yeasts, *Saccharomyces cerevisiae*, which is rare in natural environments, may be considered as a domesticated microbe. Since the discovery of yeasts by Antonie van Leeuwenhoek, the recognition of the biological nature of fermentation reactions by Pasteur, and the isolation of pure yeast cultures by Hansen, our knowledge of yeast biodiversity has increased enormously. About 800 species of yeast are presently known, and several play significant roles in the food, brewing, wine and beverage industries. This is clearly illustrated by the various chapters of this book. The contribution of yeast to the food industry can be either beneficial or detrimental. In many cases the relationship between these two aspects is a fragile balance, which depends on the interplay between various biotic and abiotic factors. In this sense, the study of yeast-food interactions can be really seen as applied ecology. Considerable progress has been made in the detection and identification of yeasts from food, due to the introduction of various molecular methods, and the development of extensive genome databases and advanced identification tools. Various protocols have been developed to selectively isolate yeasts from different sources of food and drinks, because of the increased knowledge on the ecology of food-related yeasts and the physico-chemical properties of the various foods. The genomics era already yielded significant progress in our understanding of the effects of the preservation of food on the yeast transcriptome. New insights will arise in the near future, and we are happy to present a comprehensive overview of the first genomic studies in this field. The physiological background of spoilage by yeasts, and the detection and management of spoilage incidents require utmost attention in the food industry. Yeasts cause a spoilage risk as many species are able to grow at low temperatures and low pH values. Only a few years ago a new yeast species was discovered, which was found to be resistant to commonly used preservatives in the food industry, and thus poses a serious spoilage threat.

The second part of this volume is dedicated to the various foods, fermented drinks and beverages. It is noteworthy that so many yeast species are involved in the manufacturing of the various foods and drinks. The diversity of foods and drinks involved is impressing as well. In many cases, yeasts interact with other microbes, such as filamentous fungi and bacteria, in temporarily and spatially differentiated, but balanced, physiological processes. This is the case in the production of e. g., soy sauce, coffee, cocoa, cheeses, kefyr, and the various traditional fermented products discussed. The production of wine, beer and bread are among the best-understood fermentation processes. Yeasts do not only contribute by the production of ethanol or CO_2, but are responsible for the production of a huge variety of olfactory and gustatory important compounds. These largely contribute to the value of the existing, and appreciated variety of wines, beers and breads occurring worldwide. Soft drinks present a niche for a specific yeast flora, which in most cases is detrimental to the product quality.

Due to the studies performed on this specific environment, the spoilage problem of beverages can be controlled in most cases.

Various authors emphasized the differences between yeast populations of processed and non-processed foods, in particular of dairy-, fruit- and meat-related products. The introduction of environmental yeasts into the food chain poses a potential spoilage risk, e. g., in products such as fruit yogurts. In contrast, environmental yeasts are indispensable in other fermentation processes.

We hope that many students of yeast biology, fermentation biology, food processing, brewing, viniculture and beverage industries will use this book, both educationally and professionally. Finally, we want to thank all authors for their pleasant and cooperative collaboration in the preparation of the book.

Teun Boekhout and Vincent Robert (Utrecht, November 26, 2002)

Editors and authors

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1 Yeast biodiversity

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1.1 Introduction

Identifying, naming and placing yeasts in their proper evolutionary framework is of importance to many areas of science, including agriculture, medicine, the biological sciences, biotechnology, food industry, and for determining industrial-property rights. At present, approximately 750 yeast species are recognized, but only a few are frequently isolated. Relatively few natural habitats have been thoroughly investigated for yeast species. Consequently, we can assume that many additional species await discovery. Because yeasts are widely used in traditional and modern biotechnology, the exploration for new species should lead to additional novel technologies.

Several definitions have been used to describe the yeast domain. According to GUILLIERMOND [53] and LODDER [88], yeasts are fungi reproducing unicellularly by budding or fission. In this sense only true unicellular fungi are regarded as yeasts. However, many yeast species are dimorphic and produce pseudohyphae and hyphae in addition to unicellular growth. Similarly, many hyphal fungi are dimorphic and are usually referred to as yeast-like. Because of the overlap in morphological appearance, some authors regard yeasts merely as fungi that produce unicellular growth, but that otherwise are not different from filamentous fungi [42], or as unicellular fungal growth forms which have resulted as a response to a commonly encountered set of environmental pressures [67]. OBERWINKLER [102] placed the yeasts in a phylogenetic framework and defined them as unicellular, ontogenetic stadia of either asco- or basidiomycetes [140]. In summary, yeasts are ascomycetous or basidiomycetous fungi that reproduce vegetatively by budding or fission, with or without pseudohyphae and hyphae, and forming sexual states that are not enclosed in fruiting bodies.

Some yeasts may reproduce sexually, resulting in an alternation of generations with the formation of characteristic cells in which reduction division (meiosis) takes place. In ascomycetous yeasts this cell is the ascus, in which ascospores are formed. In basidiomycetous yeasts the site of meiosis is called a basidium, on which basidiospores are exogenously formed.

Asexually reproducing yeasts are referred to as imperfect, mitosporic or anamorphic yeasts (e. g., *Cryptococcus neoformans*, *Candida utilis*), and sexually reproducing yeasts are called perfect, meiosporic or teleomorphic yeasts (e. g., *Filobasidiella neoformans*, *Pichia jadinii*). The combination of both states is called the holomorph, and for this the name of the sexual stage (teleomorph) is being used (in these examples *F. neoformans* and *P. jadinii*).

Molecular comparisons show the ascomycetous yeasts to be phylogenetically distinct from the filamentous Ascomycetes [78, 80, 81], whereas the basidiomycetous yeasts belong to

the three main classes of Basidiomycetes, namely the Urediniomycetes, Ustilaginomycetes and Hymenomycetes [39].

1.2 Developments in yeast systematics

Three main periods can be discerned in yeast taxonomy in which new concepts were developed, largely based on technological and scientific innovations. The first period (until approximately 1960) was characterized by a thorough study of morphology, comparative nutritional physiology, and conventional genetics. Important workers in this period were M. REESS (morphology), E. C. HANSEN (application of pure cultures and physiology), A. J. KLUYVER (physiology), L. J. WICKERHAM (physiology, genetics, ecology), and A. GUILLIERMOND, Ö. WINGE and C. C. LINDEGREN (genetics). Comparative taxonomic studies performed at the CBS Yeast Division [31, 87, 127], resulted in a series of monographs, which created the 'Delft School'.

Initially, responses on only a limited number of carbon and nitrogenous compounds were used for taxonomic purposes. WICKERHAM [150] extended this series, and today approximately 60 tests are being performed routinely, including fermentation and assimilation of carbon compounds, assimilation of nitrogenous compounds, vitamin requirements, resistance to cycloheximide, temperature requirements, etc. (see Chapter 3).

Genetic studies revealed the presence of different sexual strategies. Sexual cycles of ascomycetous yeasts may be haplontic, diplontic or diplohaplontic. Yeast species were found to be homothallic, heterothallic, or a combination of these. Incompatibility systems of basidiomycetous yeasts are bipolar, tetrapolar, or modified tetrapolar, and mating factors biallelic or multiallelic [7, 34–36, 84, 155].

The second period of yeast systematics (from 1960 until c.2000) was characterized by an extension of morphological characteristics because of the introduction of the electron microscope, the application of biochemical criteria, and the introduction of molecular studies. Transmission electron microscopy revealed differences between ascomycetous and basidiomycetous yeasts. Ascomycetous yeasts have electron-transparent cell walls and a thin electron-dense outer layer, whereas basidiomycetous yeasts have lamellate and electron-dense cell walls [70]. Bud formation is also different in these two groups of yeasts. Ascomycetous yeasts show holoblastic budding, i. e., the entire cell wall seems to be involved in the formation of the newly formed wall of the bud, while basidiomycetous yeasts have enteroblastic budding in which only the inner cell wall layer is involved in this process.

Septal ultrastructure shows important differences between the two groups of yeasts. Septa of many ascomycetous yeasts have one or several micropores. These are very thin electron-dense connections between two adjacent cells. Additionally, diaphragm-like pores occur as well, and Woronin bodies may be present. Pores of *Ambrosiozyma* species are swollen around the pore, thus resemble somewhat the dolipores of basidiomycetes. Basidiomycetous yeasts show a greater variation in septal ultrastructure. In the cytoplasm, a structure

made up of modified endoplasmic reticulum, the parenthesome or septal pore cap (SPC) may be present. The parenthesome can have different morphologies. Hymenomycetous yeasts usually have dolipores in which the septum is swollen around a central pore. *Filobasidiella* and *Bulleromyces*, have a parenthesome made up of U-shaped vesicles (Tremellales-type). Other basidiomycetous yeasts, currently classified in the order Cystofilobasidiales [37] lack such a parenthesome. The urediniomycetous yeasts have diaphragm-like pores reminiscent of those found in the higher ascomycetes, but without Woronin bodies. The ustilaginomycetous yeasts may have micropore-like structures [17]. The fine structure of septa is in full accordance with phylogenies based on ribosomal DNA (rDNA) data [39].

Biochemical characteristics, such as carbohydrate composition of cell walls and capsules [112, 146, 147, 131], proton magnetic resonance spectra of cell walls [125, 126], number of isoprene units of the coenzyme Q [156, 157, 159], cytochromes [23, 41, 98], fatty acid composition [25,145, 148], and isozyme patterns [160, 161] have been used for taxonomic purposes as well.

The introduction of comparative DNA studies in the late sixties of the last century provided, in principle, an objective parameter for estimating evolutionary distances between taxa. Different methods offer resolution at different taxonomic levels. The taxonomic value of nucleic acid base composition (mol% G+C) is mainly exclusionary (see Chapter 3). Phenotypically similar strains differing by more than ca. 2–3 % in their base composition are usually regarded as different species [72, 76, 107], while strains with the same base composition do not necessarily represent one and the same species. The range of nucleic acid base compo-

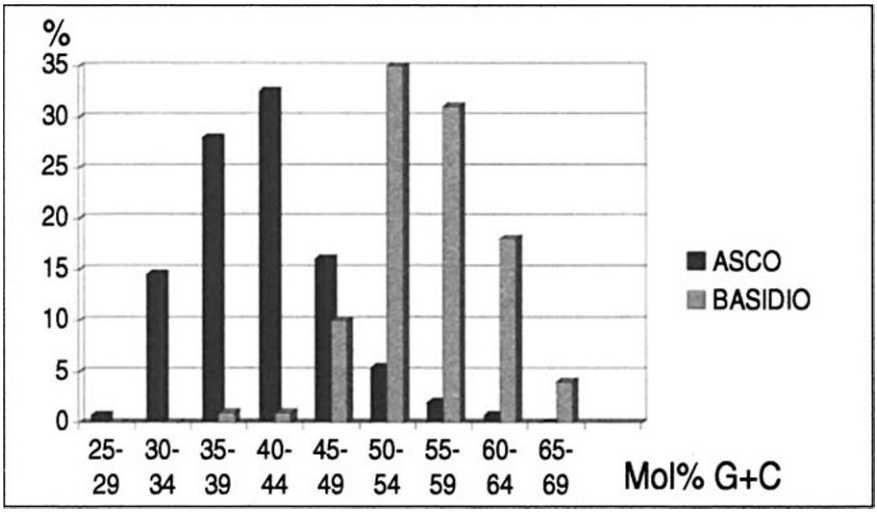

Fig.1.2-1 Distribution of percentage Guanine plus Cytosine (Mol% G+C) of the DNA among asco- and basidiomycetous yeasts