

Topics in Current Genetics

J. H. de Winde
(Ed.)

2

Functional Genetics of Industrial Yeasts



Springer

Table of contents

1 Functional genetics of industrial yeasts; of ancient skills and modern applications	1
Han de Winde	1
Abstract	1
1.1 Introduction	1
1.1.1 History	1
1.1.2 Yeast diversity	2
1.1.3 Yeast biotechnology and yeast genetics.....	3
1.1.4 Set-up of this book	4
1.2 From classical genetics to modern genomics	5
1.3 Improving ancient skills: <i>Saccharomyces cerevisiae</i> in food and beverage	6
1.3.1 Baker's yeast.....	6
1.3.2 Wine yeast	7
1.3.3 Brewer's yeast.....	8
1.4 Of apples and pears: new food yeast	8
1.4.1 <i>Kluyveromyces lactis</i>	9
1.4.2 <i>Torulaspora delbrueckii</i>	9
1.5 Yeast as model system	10
1.5.1 <i>Saccharomyces</i> yeast in signal transduction and cell wall biosynthesis.....	10
1.5.2 <i>Kluyveromyces lactis</i>	10
1.5.3 <i>Hansenula</i> , <i>Pichia</i> , and <i>Yarrowia</i> yeasts	11
1.6 Novel biotechnological tools and applications	11
1.6.1 Yeast in biocontrol.....	11
1.6.2 Metabolic pathway engineering	11
1.7 Perspectives, rules and regulations.....	13
Acknowledgements	13
References	14
2 Genetics and classical genetic manipulations of industrial yeasts.....	17
Paul V. Attfeld and Philip J. L. Bell	17
Abstract	17
2.1 Introduction	17
2.2 Genetic properties of industrial yeasts.....	19
2.2.1 An overview of the genome and functional genetic analysis of laboratory <i>S. cerevisiae</i> strains: a basis for comparison with industrial yeasts	19
2.2.2 Genomes of industrial <i>Saccharomyces</i> and non- <i>Saccharomyces</i> yeasts	24
2.2.3 Functional analyses of industrially relevant yeasts.....	26

2.2.4 Classical genetic features of laboratory and industrial yeasts.....	28
2.3 Industrial yeast strain improvement	30
2.3.1 Issues affecting the choice of genetic strategy.....	30
2.3.2 Mating.....	31
2.3.3 Mutagenesis.....	35
2.3.4 Protoplast / spheroplast fusion.....	37
2.3.5 Cytofusion.....	38
2.3.6 Screening strains for improvements.....	38
2.4 Improvements to strains via classical genetics.....	39
2.4.1 Improved maltose utilization in industrial <i>S. cerevisiae</i> : a union of functional analysis and classical genetics in strain improvement.....	40
2.5 Conclusions and prospects	46
References.....	46
3 Baker's yeast: challenges and future prospects.....	57
Francisca Randez-Gil, Jaime Aguilera, Antonio Codón, Ana M. Rincón, Francisco Estruch and Jose A. Prieto.....	57
Abstract.....	57
3.1 Introduction.....	57
3.2 Genetic characteristics of baker's yeast strains.....	59
3.2.1 Nuclear genome.....	59
3.2.2 Sporulation.....	62
3.3 Important traits for baking applications.....	62
3.3.1 Fermentative characteristics.....	62
3.3.2 Osmotolerance and Na ⁺ toxicity resistance.....	67
3.3.3 Cryoresistance.....	75
3.4 non- <i>Saccharomyces</i> baker's yeast: <i>Torulaspora delbrueckii</i>	83
3.4.1 Morphological and genetic characteristics.....	83
3.4.2 Baking applications.....	83
3.4.3 <i>T. delbrueckii</i> as a model system.....	84
3.5 Conclusions.....	85
Acknowledgements.....	85
References.....	85
4 The genetic analysis and tailoring of wine yeasts.....	99
Isak S. Pretorius.....	99
Abstract.....	99
4.1 Introduction.....	99
4.2 The need for improved wine yeast strains.....	100
4.2.1 The advantages and disadvantages of spontaneous and inoculated fermentations.....	100
4.2.2 The development of active dried wine yeast starter culture strains.....	101
4.3 The genetic features, analysis and modifications of wine yeasts.....	102
4.3.1 The morphology, reproduction and genetic constitution of wine yeasts.....	102

4.3.2 The genetic methods for the analysis and modification of wine yeasts	104
4.4 Strategies and targets for the improvement of wine yeasts	109
4.4.1 Improvement of fermentation performance	111
4.4.2 Improvement of processing efficiency.....	116
4.4.3 Improvement of wine wholesomeness.....	120
4.4.4 Improvement of wine flavour and other sensory qualities	125
4.5 Conclusions and future perspectives	130
Acknowledgements	133
References	134
5 Brewer's yeast: genetic structure and targets for improvement	143
Jørgen Hansen and Morten C. Kielland-Brandt.....	143
Abstract	143
5.1 The role of yeast in beer production.....	143
5.2 Brewer's yeast: a chimera in service	145
5.3 How to breed brewer's yeast	149
5.4 Targets and approaches in breeding of brewer's yeast.....	151
5.4.1. Feeding the beast: carbohydrate fermentation	151
5.4.2 Flavour components: too little and too much.....	153
5.4.3. Flavour stability: a way to increased shelf life.....	156
5.4.4 Diacetyl and maturation: how to speed things up	157
5.4.5 Sedimentation and filtration.....	159
5.5 Brewer's yeast in the post-genomic era.....	160
5.6 Concluding remarks	160
Acknowledgements	162
References	162
6 <i>Kluyveromyces lactis</i>: genetics, physiology, and application	171
Karin D. Breunig and H. Yde Steensma	171
Abstract	171
6.1 Introduction	171
6.2 Genetics.....	172
6.2.1 Taxonomy of <i>Kluyveromyces</i> ssp. and phylogenetic relationship to <i>S. cerevisiae</i>	172
6.2.2 Chromosomes and extrachromosomal genetic elements.....	173
6.2.3 Mitochondrial DNA	176
6.2.4 Mating types	176
6.3 Physiology.....	177
6.3.1 Carbon and energy metabolism.....	177
6.3.2 Lactose utilization.....	188
6.3.3 The petite-negative phenotype.....	190
6.3.4 Killer Strains.....	191
6.4. Industrial applications	192
References	193

7 The methylotrophic yeasts <i>Hansenula polymorpha</i> and <i>Pichia pastoris</i>: favourable cell factories in various applications	207
Meis van der Heide, Marten Veenhuis and Ida van der Klei.....	207
Abstract.....	207
7.1 Introduction.....	207
7.2 Tools for the production of heterologous proteins in <i>Hansenula polymorpha</i> and <i>Pichia pastoris</i>	209
7.2.1 Transformation and vectors.....	209
7.2.2 Expression cloning strategies.....	209
7.2.3 Promoter systems for protein production.....	210
7.3 Sorting of heterologous proteins to specific subcellular locations.....	212
7.3.1 Protein sorting to peroxisomes.....	213
7.3.2 Protein secretion.....	218
7.4 Concluding remarks.....	219
Acknowledgements.....	219
References.....	219
8 Functional genetics of <i>Yarrowia lipolytica</i>	227
Gerold Barth, Jean-Marie Beckerich, Angel Dominguez, Stefan Kerscher, David Ogrzydziak, Vladimir Titorenko and Claude Gaillardin.....	227
Abstract.....	227
8.1 Introduction.....	227
8.2 Genetics and genetic tools.....	228
8.2.1 Genetics.....	228
8.2.2 Genetic engineering.....	229
8.2.3 Genomics.....	231
8.3 <i>Yarrowia lipolytica</i> as a model for protein secretion.....	231
8.3.1 Predominance of a cotranslational translocation pathway from cytoplasm to ER.....	232
8.3.2 Sls1p, prototype of a new ADP/ATP exchange factor family for eukaryotic Hsp70p.....	232
8.3.3 Other new components affecting secretion.....	234
8.3.4 Function of the <i>S. cerevisiae</i> homologues of <i>SEC</i> genes.....	235
8.4 <i>Yarrowia lipolytica</i> as a morphogenetic model.....	236
8.4.1 Morphogenesis in <i>Yarrowia lipolytica</i>	236
8.4.2 Experimental approaches for the molecular cloning of genes involved in the yeast-hypha transition in <i>Yarrowia lipolytica</i>	236
8.4.3 Identification and characterization of <i>Y. lipolytica</i> morphogenetic genes.....	236
8.4.4 Pathway conservation in <i>Y. lipolytica</i> , <i>S. cerevisiae</i> , and <i>C. albicans</i>	236
8.5 <i>Yarrowia lipolytica</i> as a model for mitochondrial complex I study.....	240
8.5.1 Mitochondrial metabolism.....	240
8.5.2 Mitochondrial respiratory chain.....	242
8.5.3 Mitochondrial genome.....	243
8.5.4 Respiratory chain complex I.....	244
8.5.5 The hydrogenase model for the catalytic core of complex I.....	246

8.6 Carbon metabolism in <i>Yarrowia lipolytica</i>	247
8.6.1 Utilisation of carbohydrates by <i>Yarrowia lipolytica</i>	247
8.6.2 Utilisation of alcohols	248
8.6.3 Utilisation of monocarboxylic acids	248
8.6.4 Accumulation of storage carbohydrates.....	249
8.6.5 Utilization of hydrocarbons as carbon source	249
8.6.6 Hydrolysis of fats.....	250
8.6.7 Fatty acids biosynthesis and degradation.....	251
8.7 Peroxisome assembly in the yeast <i>Yarrowia lipolytica</i>	251
8.7.1 Metabolic functions and biogenesis of peroxisomes.....	252
8.7.2 Peroxisome biogenesis as it is presented in cell-biology textbooks.....	253
8.7.3. A revision of the peroxisome biogenesis paradigm: peroxisomes assemble by a multistep pathway.....	253
8.7.4 Peroxisome fusion.....	254
8.7.5 The endoplasmic reticulum plays an essential role in peroxisome assembly	256
8.7.6 Folded, oligomeric proteins are imported into peroxisomes.....	257
8.8 Future prospects	258
Acknowledgements	258
References	259
9 Yeasts and food spoilage	273
S. Brul, J. van der Vossen, A. Boorsma, and F.M. Klis.....	273
Abstract	273
9.1 Introduction to microbial and yeast food spoilage	273
9.2 The ecology of food spoilage yeasts.....	275
9.2.1 Diversity of yeasts	275
9.2.2 Growth characteristics	275
9.2.3 Growth physiology	276
9.2.4 Preservation strategies	276
9.3 The yeast envelope, antifungal targets, and (preservation) stress resistance	277
9.3.1 Cell wall sugars and their synthesis	279
9.3.2 Membrane localised transporters of low molecular weight compounds.....	280
9.3.3 Cell wall proteins and stress response.....	282
9.3.4 Sensing the extracellular environment and signalling stress.....	284
9.4. A new method for the analysis of gene expression profiles	285
9.5 Concluding remarks and future prospects	287
Acknowledgements	288
References	288
10 Non-conventional yeasts in antifungal application	297
Volkmar Passoth and Johan Schnürer.....	297
Abstract	297
10.1 Introduction	297

10.2 Antifungal yeasts and their modes of inhibition.....	299
10.2.1 Phyllosphere yeasts.....	299
10.2.2 Biocontrol of postharvest diseases.....	300
10.2.3 Non-conventional killer yeasts in biocontrol.....	305
10.3 Available information about genome organisation in non-conventional antifungal yeasts.....	306
10.3.1 Sexuality, genome structure, and extrachromosomal elements.....	306
10.3.2 Genetics of the killer character in non-conventional biocontrol yeasts.....	309
10.4 Available genetic methods for biocontrol yeasts.....	310
10.4.1 Molecular methods for identification and phylogenetic analysis of biocontrol yeasts.....	311
10.4.2 Methods for the manipulation of biocontrol yeasts.....	314
10.5 The use of genetic methods for the manipulation of biocontrol activity.....	316
10.5.1 Application of mutants in biocontrol systems.....	316
10.5.2 Application of genetically engineered strains in biocontrol systems.....	317
10.6 Conclusions and outlook.....	318
Acknowledgements.....	318
References.....	319

11 Yeast functional genomics and metabolic engineering: past, present and future.....

Christoffer Bro, Birgitte Regenberg, and Jens Nielsen.....	331
Abstract.....	331
11.1 Introduction.....	331
11.2 Examples of metabolic engineering.....	333
11.2.1 Extension of substrate range.....	335
11.2.2 Heterologous protein production.....	335
11.2.3 Improvement of fluxes.....	336
11.3 Challenges in metabolic engineering.....	338
11.4 Functional genomics.....	340
11.4.1 Comparative sequence analysis.....	341
11.4.2 Transcriptome analysis.....	342
11.4.3 Global mutant analysis.....	343
11.4.4 Proteome analysis.....	344
11.4.5 Interactome analysis.....	345
11.4.6 Metabolome analysis.....	346
11.4.7 Fluxome analysis.....	346
11.5 Functional genomics in metabolic engineering.....	347
11.5.1 Need for integrated approach.....	350
11.6 Future prospects.....	351
Acknowledgements.....	352
References.....	352