



Martin Rühl (Autor)

**Laccases and other ligninolytic enzymes of the basidiomycetes
Coprinopsis cinerea and *Pleurotus ostreatus***

*- submerged and solid state fermentation, morphological studies of
liquid cultures and characterisation of new laccases*

Martin Rühl

Laccases and other ligninolytic enzymes
of the basidiomycetes *Coprinopsis cinerea*
and *Pleurotus ostreatus*



- submerged and solid state fermentation,
morphological studies of liquid cultures
and characterisation of new laccases.



Cuvillier Verlag Göttingen
Internationaler wissenschaftlicher Fachverlag

<https://cuvillier.de/de/shop/publications/873>

Copyright:

Cuvillier Verlag, Inhaberin Annette Jentsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen, Germany
Telefon: +49 (0)551 54724-0, E-Mail: info@cuvillier.de, Website: <https://cuvillier.de>

Table of contents

<i>Summary</i>	vii
<i>Zusammenfassung</i>	ix
<i>Abbreviations</i>	xii

Chapter **1**

Introduction	1
I. Laccases: sources and applications	3
A. Occurrence and functions of laccases in nature	4
B. Scientific and commercial applications	9
1. Lignin modification: degradation and polymerisation.....	9
a) Laccases and laccase mediator systems (LMS) in pulp processing.....	9
b) Laccase in the wood composite industry.....	11
c) Alteration of wood properties with laccases.....	12
2. Bioremediation	12
3. Immobilisation of laccase for different applications	15
4. Commercial laccase products	16
5. Other potential applications	18
II. Basidiomycetes: higher fungi with a great potential	19
A. Applications of living basidiomycetes on lignocellulosic material	19
B. In vivo bioremediation and dye decolourisation.....	20
C. Mushrooms as a valuable food.....	21
III. Aim of the thesis	26
IV. References.....	28

Chapter 2

Laccases and other ligninolytic enzymes of basidiomycetes	45
I. Production of laccase and other enzymes for the wood industry	47
II. Ligninolytic enzyme activities alternate with mushroom production during industrial cultivation of <i>Pleurotus ostreatus</i> on wheat-straw-based substrate.....	87

Chapter 3

Morphology of <i>Coprinopsis cinerea</i> in submerged cultures	103
I. Introduction: Fermentation of basidiomycetes in liquid cultures	105
A. Important parameters of fermentation on fungal morphology and on production yields of enzymes and other metabolites.....	107
1. Inoculum.....	107
2. Oxygen.....	108
3. Vessel and impeller	110
4. Mode of cultivation	112
B. Conclusion.....	113
II. Tools for analysis of biomass and morphology of organisms	114
A. General biomass determination	114
B. Approaches to determine morphological characteristics of free hyphae and mycelial pellets	115
1. General image analysis.....	117
2. Automated image analysis	117
3. Methods for a more precise image analysis	118
4. Conclusion	121
III. Automated image analysis to observe pellet morphology in liquid cultures of filamentous fungi such as the basidiomycete <i>Coprinopsis cinerea</i>	122
A. Abstract.....	122
B. Introduction	122
C. Material and Methods.....	124

1.	Fungal cultures.....	124
2.	Imaging	125
3.	Analysis of the images	125
4.	Data processing	128
D.	Results.....	129
E.	Discussion	133
IV.	Fermentation of <i>C. cinerea</i> in shake flasks and bioreactors.....	136
A.	Abstract.....	136
B.	Introduction	137
C.	Material and Methods	138
1.	Fungal cultures.....	138
2.	Determination of the dry weight (DW) in gram per litre [g/l].....	139
3.	Laccase assay.....	140
4.	Glucose determination	140
5.	C/N determination of culture supernatant.....	140
6.	Morphological observation	140
7.	Embedding of mycelium pellets into Roti [®] -Plast (Fa. Roth, Karlsruhe, Germany)	141
D.	Results.....	141
1.	Cultural growth and laccase production at 25 °C and 37 °C.....	141
2.	Pellet morphology in shake flask cultures	144
3.	Micro-morphological analysis of <i>C. cinerea</i> pellets.....	149
4.	Laccase production in a stirred bioreactor.....	152
5.	Pellet morphology in stirred bioreactor cultures	154
E.	Discussion	156
1.	Shake flask cultures: laccase and biomass production	156
2.	Shake flask cultures: pellet morphology	157
3.	Fermentation in a stirred bioreactor.....	161
V.	References.....	163

Native and recombinant laccase production with <i>Coprinopsis cinerea</i>	171
I. Secretion of natural laccases in <i>Coprinopsis cinerea</i>	173
A. Abstract	173
B. Introduction	173
C. Material and methods	175
1. <i>C. cinerea</i> strains and culture conditions	175
2. Laccase activity assay	175
3. SDS-PAGE	176
4. Staining	176
5. Protein identification	176
D. Results	177
1. Laccase secretion depends on medium and temperature	177
2. Different isoenzymes account for the laccase activity of different strains	178
E. Discussion	182
1. Temperature and medium effect	182
2. Isoenzyme	183
II. Optimisation of recombinant laccase production in <i>Coprinopsis cinerea</i>	186
A. Abstract	186
B. Introduction	187
C. Material and Methods	188
1. DNA transformation and <i>C. cinerea</i> strains used	188
2. Fungal cultures with spore inoculum	188
3. Glucose determination	189
4. Ergosterol measurement	189
5. Determination of the mycelial dry weight (DW)	190
6. Enzyme assays	190
D. Results	192
1. Media supporting growth for recombinant laccase Lcc1 production	192
2. Temperature as an important growth parameter	198
E. Discussion	203

1. Media effects on laccase production	204
2. Temperature effects on laccase secretion.....	208
3. Protease production and effect on laccase activities.....	209
III. References	210

Chapter 5

Purification and characterisation of three new laccases from the basidiomycete <i>Coprinopsis cinerea</i>	217
I. Abstract.....	219
II. Introduction	220
III. Material and Methods	221
A. Fungal culture	221
B. Purification.....	221
C. Molecular characterisation.....	223
1. Electrophoresis.....	223
2. Isoelectric focusing (IEF).....	223
3. Staining	223
4. Protein identification	224
D. Biochemical characterisation	225
1. Optimal reaction conditions	226
2. Enzyme kinetics	226
3. Stability of laccases against inhibitors and in organic solvents	226
4. Dye decolourisation.....	227
5. Redox potential determination.....	227
IV. Results	228
A. Purification.....	228
B. Molecular characteristics	232
C. Biochemical characterisation using standard laccase substrates	234
D. Stability against inhibitors and organic solvents	239
E. Reactions with industrial dyes.....	241
F. Structural characteristics	242

V. Discussion	243
A. Molecular structure of enzymes produced by the different <i>C. cinerea</i> laccase genes	243
B. Efficiency of the laccase production by the different laccase genes...	246
C. Stability of recombinantly produced enzymes	247
D. Enzymatic kinetics with different substrates	249
E. Conclusions.....	252
VI. References	253

Chapter 6

Final discussion and prospects	259
I. Solid state fermentation (SSF) versus submerged fermentation (SmF) for production of laccases	260
II. Improvement of recombinant laccase production in <i>Coprinopsis cinerea</i>	262
A. Optimisation of laccase production.....	263
B. Changing biochemical properties of <i>C. cinerea</i> laccases	265
III. General conclusions.....	266
IV. References	267
 <i>Curriculum vitae</i>	 271
 <i>Publications</i>	 273