Characterization of incompatible and compatible *Camellia-Ciborinia camelliae* plant-pathogen interactions

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Abstract

Many *Camellia* species and cultivars are susceptible to infection by the host-specific fungal phytopathogen *Ciborinia camelliae* L. M. Kohn (Sclerotiniaceae). This necrotrophic pathogen specifically infects floral tissue resulting in rapid development of host-cell death and premature flower fall. *C. camelliae* is considered to be the causal agent of '*Camellia* flower blight' and is an economically significant pest of both the *Camellia* floriculture and *Camellia* oil seed industries. This study sought to identify molecular components that contribute to incompatible and compatible interactions between *C. camelliae* and *Camellia* petal tissue.

Microscopic analyses of incompatible *C. camelliae-Camellia lutchuensis* interactions revealed several hallmarks of induced plant resistance, including papillae formation, H_2O_2 accumulation, and localized cell death. Extension of resistance analyses to an additional 39 *Camellia* spp. identified variable levels of resistance within the *Camellia* genus, with *Camellia lutchuensis, Camellia transnokoensis and Camellia yuhsienensis* exhibiting the strongest resistance phenotypes. Collectively, *Camellia* species of section Theopsis showed the highest levels of incompatibility. Based on this observation, a total of 18 *Camellia* interspecific hybrids with section Theopsis species in their parentage were tested for resistance to *C. camelliae*. The majority of hybrids developed disease symptoms, although the speed and intensity of disease development varied. Hybrids containing high genetic dosages of *C. lutchuensis* within their parentage were the most effective at resisting *C. camelliae* infection. Therefore, introgression of genetic information from *Camellia lutchuensis* into hybrids of *Camellia* is likely to be a valid approach for breeding *Camellia* hybrids with increased *C. camelliae* resistance. A comparison between transcriptomes of mock-infected and infected *C. lutchuensis* samples identified plant genes that may contribute to *C. camelliae* resistance, including two putative transcription factors.

C. camelliae growth in compatible tissue demonstrated a switch from biotrophy to necrotrophy, evident from the simultaneous development of secondary hyphae and

necrotic lesions. The initial biotrophic-like period of *C. camelliae* growth *in planta* was asymptomatic; leading to the hypothesis that *C. camelliae* may secrete fungal effectors during infection. A bioinformatic approach was taken to identify putative effectors of *C. camelliae*. To facilitate this approach, a 40.7 MB draft genome of *C. camelliae* was assembled and validated. Genomic and transcriptomic data were used to predict a total of 14711 *C. camelliae* protein coding genes of which 749 were predicted to form the *C. camelliae* secretome. The secretome of *C. camelliae* was compared with the predicted secretomes of the closely related species, *Botrytis cinerea* (Sclerotiniaceae) and *Sclerotinia sclerotiorum* (Sclerotinaceae). Comparative analysis of the secretomes of these three species identified protein conservation within CAZyme (carbohydrate active enzyme) and protease categories. In contrast, the oxidoreducatase and small secreted protein (SSP) categories were less conserved. Further analysis of SSPs revealed a conserved family of putative effector proteins that appear to have undergone lineage-specific expansion within the host-specific pathogen *C. camelliae* (*n* = 73), but remain as single genes in the two host-generalists *B. cinerea* and *S. sclerotiorum*. Members of this *Ciborinia camelliae*-like small secreted protein (CCL-SSP) family share 10 conserved cysteine residues, are expressed during the early stages of infection and have homologs in other necrotrophic fungal phytopathogens. Functional assays of native recombinant CCL-SSPs indicate that the *B. cinerea* and *S. sclerotiorum* single gene homologs are able to induce necrosis when infiltrated into *Camellia* 'Nicky Crisp', *Camellia lutchuensis* and *Nicotiana benthamiana* host tissue. In comparison, nine native recombinant CCL-SSP homologs of *C. camelliae* were unable to replicate the necrosis phenotype when infiltrated into the same host tissue. This work identifies a previously uncharacterized family of fungal necrosis-inducing proteins that appear to have contrasting functions in related host-specific and host-generalist phytopathogens.

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Table of Contents

Abstract i
Acknowledgements iii
List of Figures xi
List of Tables xiii
Abbreviations xiv
1. Introduction 1
1.1. Background 2
1.2. Plant resistance 3
1.2.1. Preformed plant resistance 3
1.2.2. Induced plant resistance 5
1.3. Fungal phytopathogens 15
1.3.1. The biotrophs and the hemibiotrophs 15
1.3.2. The necrotophs 18
1.4. *Botrytis cinerea* and *Sclerotinia sclerotiorum* 21
1.5. *Ciborinia camelliae* 23
1.6. Research Aims 27
2. Materials and Methods 31
2.1. Plant and fungal material 32
2.2. *Ciborinia camelliae* infection assays 32
2.3. Microscopy 33
2.3.1. Light microscopy 33
2.3.2. Confocal laser scanning microscopy 34
2.3.3. Scanning electron microscopy 34
2.4. Image analysis 35
2.4.1. Petal lesion area quantification using ImageJ 35
2.4.2. Chemiluminescence quantification using ImageJ 35
2.5. DNA and RNA manipulations 35
2.5.1. Genomic DNA extraction 35
2.5.2. Total RNA extraction 36
2.5.3. Nucleic acid quantification 37
2.5.4. Restriction endonuclease digests 37

2.5.5. General PCR 38
2.5.6. Colony PCR 38
2.5.7. Quantitative Real-Time PCR (qRT-PCR) 38
2.5.8. DNA and cDNA sequencing 40
2.6. Bioinformatic analyses 40
2.6.1. Post sequencing quality control 41
2.6.2. Draft genome assembly and validation 41
2.6.3. Transcriptome assembly and validation 42
2.6.4. Transcriptome quantification 43
2.6.5. *Ciborinia camelliae* EST identification and validation 44
2.6.6. *Ciborinia camelliae* protein prediction and validation 45
2.6.7. Fungal secretome prediction and validation 45
2.6.8. Secretome annotation 46
2.6.9. Comparative secretome analyses 47
2.6.10. Phylogenetic trees 47
2.6.11. Small secreted protein characterization 48
2.7. Cloning and recombinant protein expression 49
2.7.1. Host strains and growth conditions 49
2.7.2. *E. coli* plasmid DNA isolation 50
2.7.3. Construct and vector design 50
2.7.4. Site-directed mutagenesis 51
2.7.5. *E. coli* and *P. pastoris* transformation 52
2.7.6. Recombinant protein expression 53
2.8. Recombinant protein manipulations 54
2.8.1. *In planta* functional protein assays 54
2.8.2. Protein separation via SDS-PAGE 55
2.8.3. Western blotting 56
2.8.4. Chemiluminescence detection 56
2.9. Statistical analysis 57
2.9.1. *Ciborinia camelliae* host-resistance screening. 57
2.9.2. *Ciborinia camelliae* genome assembly calculations. 57
2.9.3. Statistical comparisons of secretome category raw counts. 58
3. *Ciborinia camelliae* plant resistance 59
3.1. Introduction 60

3.2. Results 62
3.2.1. *Camellia* genotype influences macroscopic disease development. 62
3.2.2. Compatible interactions involve cuticle penetration, intercellular growth and secondary hypha development. 62
3.2.3. Incompatible interactions induce characteristic plant defense responses. 67
3.2.4. Levels of incompatibility to *Ciborinia camelliae* vary within the *Camellia* genus..... 68
3.2.5. Interspecific hybrid resistance is influenced by parent genotype 71
3.3. Discussion 76
3.3.1. *Ciborinia camelliae* establishment and growth in compatible tissue 76
3.3.2. *Ciborinia camelliae* resistance in incompatible *Camellia lutchuensis* tissue 78
3.3.3. *Ciborinia camelliae* resistance within *Camellia* species 79
3.3.3. *Ciborinia camelliae* resistance within *Camellia* interspecific hybrids 81
3.4. Conclusions 83
4. The development of genomic and transcriptomic resources for the *Camellia-Ciborinia camelliae* interaction 85
4.1. Introduction 86
4.2. Results 87
4.2.1. *Ciborinia camelliae* draft genome sequencing and assembly..... 87
4.2.2. *Ciborinia camelliae* shares genomic characteristics with close relatives *Botrytis cinerea* and *Sclerotinia sclerotiorum* 88
4.2.3. The *Ciborinia camelliae* MAT locus exhibits synteny with the heterothallic *Botrytis cinerea* B05.10 *MAT1-1* mating locus 88
4.2.4. *Camellia* 'Nicky Crisp' infected samples produce mixed origin transcriptomes..... 91
4.2.5. Fungal ESTs make up 10% of the *Camellia* 'Nicky Crisp' non-redundant transcriptome..... 96
4.2.6. Large changes in host-defense gene expression are induced in compatible *Camellia 'Nicky Crisp-Ciborinia camelliae* interactions..... 98
4.3. Discussion 101
4.3.1. RNA sequencing bias and *de novo* assembly limitations 101
4.3.2. Comparative genome analysis reveals potential heterothallism in *Ciborinia camelliae* 102
4.3.3. The *Ciborinia camelliae* transcriptome is estimated to be 94% complete 103
4.3.4. Compatible *Camellia* 'Nicky Crisp'-*Ciborinia camelliae* interactions induce dramatic changes in host gene expression 104
4.4. Conclusions 107
5. Comparative analysis of the secretomes of *Ciborinia camelliae*, *Botrytis cinerea* and *Sclerotinia sclerotiorum* 109

5.1. Introduction 110
5.2. Results 112
5.2.1. The *Ciborinia camelliae* genome is predicted to contain 14711 non-redundant proteins 112
5.2.2. The secretomes of *Ciborinia camelliae*, *Botrytis cinerea* and *Sclerotinia sclerotiorum* are estimated to be more than 80% complete. 112
5.2.3. Scatterplot analysis reveals differences in secretome composition between *Ciborinia camelliae*, *Botrytis cinerea* and *Sclerotinia sclerotiorum*. 113
5.2.4. Secretome annotation identifies conserved and divergent protein categories 115
5.2.5. Phylogenetic analysis reveals a conserved family of small secreted proteins that appear to be under diversifying selection in *Ciborinia camelliae*. 119
5.3. Discussion 124
5.3.1. Validating the secretomes of *Ciborinia camelliae*, *Botrytis cinerea* and *Sclerotinia sclerotiorum* 124
5.3.2. Two-dimensional scatterplot analysis enables visual comparison of related fungal secretomes. 126
5.3.3. CAZymes and proteases are highly conserved in all three secretomes 127
5.3.4. Diversity exists within the oxidoreductase and small secreted protein categories of each fungal secretome. 129
5.3.5. Half of the small secreted proteins are homologous. 130
5.4. Conclusions 132
6. *In silico* and functional characterization of a conserved family of small secreted proteins from *Ciborinia camelliae*, *Botrytis cinerea* and *Sclerotinia sclerotiorum* 133
6.1. Introduction 134
6.2. Results 135
6.2.1. *In silico Ciborinia camelliae*-like small secreted protein (CCL-SSP) characterization reveals conserved primary protein structure 135
6.2.2. CCL-SSP homologs are present across fungal classes but are confined to species with necrotrophic lifestyles 135
6.2.3. Members of the *CCL-SSP* family share genomic loci. 138
6.2.4. *Ciborinia camelliae CCL-SSP* family members are expressed during pre-lesion development 141
6.2.5. Recombinant *Botrytis cinerea* and *Sclerotinia sclerotiorum* CCL-SSP family members induce host-cell necrosis 144
6.2.6. *BcSSP2* and *SsSSP3* are expressed in *Pichia pastoris* post-induction and are highly stable 145
6.2.7. Tagged recombinant CCL-SSPs are compromised in their ability to induce host-cell necrosis 145

6.3. Discussion 150
6.3.1. CCL-SSP family members share a robust, heat tolerant structure. 150
6.3.2. CCL-SSP family members share characteristics with other necrosis-inducing fungal effectors. 152
6.3.3. *Ciborinia camelliae CCL-SSP* family members are likely to have undergone gene duplication. 154
6.3.4. *Ciborinia camelliae CCL-SSP* gene expression precedes host-cell necrosis 155
6.3.5. There is no evidence to suggest *Ciborinia camelliae* CCL-SSPs are functional homologs of BcSSP2 and SsSSP3. 156
6.4. Conclusions 158
7. General discussion 159
7.1. Breeding for *Ciborinia camelliae* resistance 160
7.2. Utilizing bioinformatics for fungal gene discovery 161
7.3. Putative necrosis-inducing mechanisms of BcSSP2 and SsSSP3 164
7.4. Adaptive evolution of *Ciborinia camelliae CCL-SSP* genes correlates with a loss of necrosis-inducing activity 166
7.5. Future directions 170
7.5. Concluding remarks 173
8. References 175
9. Appendices 199
Appendix 9.1A. Taxonomy, distribution and accession number information for *Camellia* species harvested from Auckland Botanic Gardens, New Zealand 200
Appendix 9.1B. *Camellia* species and interspecific hybrids harvested from Heartwood Nursery, CA 202
Appendix 9.2. Primers used in this study 203
Appendix 9.3. Aerial secondary hyphae of *Ciborinia camelliae* during infection of *Camellia* 'Nicky Crisp' 205
Appendix 9.4. Comparisons of host-resistance between *Camellia* sections using Tukey's range test. 206
Appendix 9.5. A phylogenetic tree of the Sclerotiniaceae 207
Appendix 9.6. Determination of the 'best' k-mer length for *Ciborinia. camelliae* genome assembly (Velvet) 208
Appendix 9.7. Transcriptome validation results using BLASTN 209
Appendix 9.8. Defense gene homologs identification using BLASTN 212
Appendix 9.9. BLASTN alignments of the *Camellia* 'Nicky Crisp' non-redundant transcriptome to the *Ciborinia camelliae* genome. 213

Appendix 9.10A. Validation of a subset of *Ciborinia camelliae* proteins (*n* = 1472) using BLASTP and the Genbank non-redundant protein database 214

Appendix 9.10B. Validation of a subset of *Ciborinia camelliae* proteins (*n* = 1472) using BLASTP and the Genbank non-redundant protein database - top hit E-value distribution. . 214

Appendix 9.11. Secretome validation using previously published *Botrytis cinerea* secreted proteome data..... 215

Appendix 9.12. Secretome annotation categories and protein counts. 216

Appendix 9.13. Secretome subcategory annotation and scatterplot analyses..... 218

Appendix 9.14. A phylogenetic tree of the conserved CCL-SSP family highlighting CCL-SSPs chosen for recombinant analysis..... 219

Appendix 9.15. CCL-SSP family statistics 220

Appendix 9.16. An alignment of all CCL-SSP family members showing the conserved cysteine residues. 222

Appendix 9.17. Fungal gDNA accumulation *in planta* correlates with fungal NAD and TUB mRNA accumulation. 224

Appendix 9.18. Recombinant CCL-SSP construct sequences optimized for *Pichia pastoris* codon usage (note: these are not endogenous nucleotide sequences). 225

Appendix 9.19. Vector maps..... 227

Appendix 9.20. Functional protein assays using native recombinant CCL-SSPs infiltrated into *Camellia lutchuensis* petal tissue..... 229

Appendix 9.21. Functional protein assays using culture filtrate aliquots of BcSSP2 and SsSSP3 collected at different time-points post-induction. 230

Appendix 9.22. Western blot of tagged recombinant CCL-SSPs. 231

Appendix 9.23. Isoelectric point values of native and tagged recombinant proteins, not including the signal peptide. 232

Appendix 9.24. Coding sequences of all conserved CCL-SSPs from *Ciborinia camelliae, Botrytis cinerea* and *Sclerotinia sclerotiorum* (*n* = 75). 233

10. Publication 241

List of Figures

Figure 1.1. The two-branched plant immune system…… 6

Figure 1.2. A fungal phylogeny based on 82 complete fungal genomes……. 16

Figure 1.3. The lifecycle of *Ciborinia camelliae*……. 24

Figure 3.1. Macroscopic assessment of *Ciborinia camelliae* infection on petals of *Camellia* 'Nicky Crisp' and *Camellia lutchuensis*…….. 63

Figure 3.2. The development and interaction of *Ciborinia camelliae* with host epidermal cells…….. 64

Figure 3.3. Subcuticular development of *Ciborinia camelliae* with host petal tissue……. 65

Figure 3.4. Analysis of macroscopic and microscopic parameters in 40 species and one interspecific hybrid of *Camellia*…… 70

Figure 3.5. A comparison of *Ciborinia camelliae* resistance within 18 interspecific hybrids and 1 species of *Camellia*…… 73

Figure 3.6. Examples of blooms from interspecific *Camellia* hybrids that are highly susceptible or semi-resistant to *Ciborinia camelliae* infection…….. 74

Figure 4.1. Mating (MAT) locus synteny comparisons between *Ciborinia camelliae* *Sclerotinia sclerotiorum* and *Botrytis cinerea*…… 90

Figure 4.2. Validation of the origin of *Ciborinia camelliae* transcriptome ESTs using BLASTX and the Genbank non-redundant protein database…….. 94

Figure 4.3. *Camellia* 'Nicky Crisp' and *Camellia lutchuensis* infection-associated gene expression changes of ESTs homologous to 9 *Camellia* defense-related genes…….. 97

Figure 5.1. The *Ciborinia camelliae*, *Botrytis cinerea* and *Sclerotinia sclerotiorum* secretome prediction pipeline…….. 114

Figure 5.2. Scatterplot analysis of secretome proteins from *Ciborinia camelliae*, *Botrytis cinerea* and *Sclerotinia sclerotiorum*…… 116

Figure 5.3. A comparison of secretome composition and conservation between *Ciborinia camelliae*, *Botrytis cinerea* and *Sclerotinia sclerotiorum*…….. 117

Figure 5.4. Analysis of the composition of the four most common secretome protein categories…….. 120

Figure 5.5. Analysis of secretome subcategory conservation within the four most common secretome protein categories…… 121

Figure 5.6. A cladogram of the 149 small secreted proteins (SSPs) from *Ciborinia camelliae*, *Botrytis cinerea* and *Sclerotinia sclerotiorum*…… 123
Figure 6.1. *In silico* characterization of the conserved *Ciborinia camelliae*-like small secreted protein (CCL-SSP) family…….. 136
Figure 6.2. Clustering of 73 homologous *Ciborinia camelliae CCL-SSP* gene loci within the *Ciborinia camelliae* genome …….. 139
Figure 6.3. Quantitative real-time PCR data for a subset of 9 of the 73 *Ciborinia camelliae CCL-SSP* genes during host infection …….. 140
Figure 6.4. Functional assays using native recombinant *Ciborinia camelliae*-like small secreted proteins (CCL-SSPs)…….. 142
Figure 6.5. Functional stability of the BcSSP2 and SsSSP3 proteins…… 143
Figure 6.6. Tagged recombinant *Ciborinia camelliae*-like small secreted protein (CCL-SSP) expression and functional assays…….. 149
Figure. 7.1. Backcrossing interspecific *Camellia* hybrids with *Camellia lutchuensis*…… 162
Figure 7.2. Hypothesized molecular mechanisms of BcSSP2, SsSSP3 and the *Ciborinia camelliae* CCL-SSP homologs (CcSSPs). …….. 167
Figure 7.3. A comparison of the native BcSSP2, SsSSP3 and CcSSP92 primary protein sequences…… 171

List of Tables

Table 3.1. Quantification of microscopic parameters associated with *Ciborinia camelliae* development in *Camellia* 'Nicky Crisp' and *Camellia lutchuensis* petal tissue..... 66

Table 4.1. Comparative analysis of the *Ciborinia camelliae* draft genome with the *Botrytis cinerea* and *Sclerotinia sclerotiorum* genomes..... 89

Table 4.2. Transcriptome assembly statistics..... 92

Table 4.3. Comparative analysis of the *Camellia* 'Nicky Crisp' non-redundant transcriptome with the putative *Ciborinia camelliae* transcriptome..... 93

Table 4.4. The top 10 *Camellia* 'Nicky Crisp' and *Camellia lutchuensis* genes undergoing gene expression increases in response to *Ciborinia camelliae* infection..... 99

Table 6.1. Characterization of CCL-SSP family homologs across different taxonomic classes of the fungal kingdom..... 137

Abbreviations

°C	Degrees Celsius
µl	Microlitre
µm	Micrometre
µmol m-2 s-1	Micromoles per square metre per second
AA	Amino acid
ACC	1-aminocyclopropane-1-carboxylate synthase
Avr	Avirulence
Avr2	Avirulence 2 protein of *Cladosporium fulvum*
BMGY	Buffered glycerol-complex medium
BMMY	Buffered methanol-complex medium
bp	Base pair
Ca^{2+}	Calcium ions
CAZyme	Carbohydrate active enzyme
CCL-SSPs	*Ciborinia camelliae*-like small secreted proteins
cDNA	Complementary deoxyribonucleic acid
Cf-2 R	*Cladosporium fulvum* 2 resistance protein
cm	Centimetre
CO_2	Carbon dioxide
Cq	Quantification cycle
CsCl	Caesium chloride
CTAB	Hexadecyltrimethylammonium bromide
CWDE	Cell wall degrading enzyme
DAB	3,3'-Diaminobenzidine
DAMP	Damage-associated molecular pattern
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
DTT	Dithiothreitol

EDTA	Ethylenediaminetetraacetic acid
Eff	Primer efficiency
EST	Expressed sequence tag
ET	Ethylene
ETI	Effector-triggered immunity
g	Gram
GABA	γ-aminobutyric acid
GB	Gigabase (1 billion bases)
GC	Guanine and cytosine
gDNA	Genomic deoxyribonucleic acid
h	Hour(s)
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
hpi	Hours postinoculation
HR	Hypersensitive response
HRP	Horseradish peroxidase
HST	Host-selective toxin
ICMP	International Collection of Microorganisms from Plants
JA	Jasmonic acid
Kb	Kilobase
L	Litre
LB	Luria-Bertani broth
LM	Light microscopy
LOV1	*Locus orchestrating victorin effects 1 gene*
LRR	Leucine rich repeat domain
M	Molar
MAMP	Microbe-associated molecular pattern
MAP	Mitogen-activated protein
MAT	Mating locus
MB	Megabase (1 million bases)
mg	Milligram
mins	Minutes

ml	Millilitre
mm	Millimetre
mM	Millimolar
MPa	Megapascal
mRNA	Messenger ribonucleic acid
N	Normal
$Na_2S_2O_5$	Sodium metabisulfite
NADPH	Nicotinamide adenine dinucleotide phosphate
NBS	Nucleotide binding site domain
NCBI	National Center for Biotechnology Information
NEP1	Necrosis and ethylene-inducing peptide 1
ng	Nanogram
NGS	Next generation sequencing
NLP	Necrosis and ethylene-inducing peptide 1-like protein
nm	Nanometre
NO	Nitric oxide
O^{2-}	Superoxide anion
OAH	Oxaloacetate acetylhydrolase
OD	Optical density
PAMP	Pathogen-associated molecular pattern
PDB	Potato dextrose broth
PCD	Programmed cell death
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PRR	Pathogen recognition receptor
PTI	PAMP-triggered immunity
pv.	Pathovar
PVDF	Polyvinylidene fluoride
qRT-PCR	Quantitative real-time polymerase chain reaction
RCR3	*Required for full Cladosporium fulvum resistance 3 gene*
R gene	Resistance gene
RIN	RNA integrity number

RNA	Ribonucleic acid
RNAi	RNA interference
RNase	Ribonuclease
RNA-seq	Ribonucleic acid sequencing
ROS	Reactive oxygen species
rpm	Revolutions per minute
RT	Room temperature
S/TPK	Serine/threonine protein kinase
SA	Salicylic acid
SAR	Systemic acquired resistance
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
secs	Seconds
SEM	Scanning electron microscopy
Snn1	*Stagonospora nodorum toxin 1 sensitivity gene*
Snn3	*Stagonospora nodorum toxin 3 sensitivity gene*
SnTox1	*Stagonospora nodorum* toxin 1
SnTox3	*Stagonospora nodorum* toxin 3
SSITL	*Sclerotinia sclerotiorum integrin-like gene*
SSP	Small secreted protein
^{T}	Tagged
TBS	Tris buffered saline
TE	Tris-EDTA buffer
ToxA	Toxin A
Tris	Tris(hydroxymethyl) aminomethane
Tsn1	*ToxA sensitivity locus 1gene*
UV	Ultraviolet
w/v	Weight/volume
YPD	Yeast peptone dextrose medium
YPDS	Yeast peptone dextrose sorbitol medium