

Department of Plant Biology and Biotechnology

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

Department of Plant Biology and Biotechnology

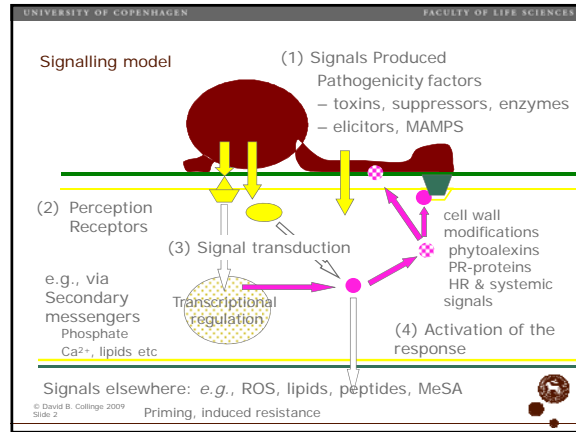
Plant defence responses in cereals

David B. Collinge

Plant Pathology, Dept. Plant Biology

dbc@life.ku.dk <http://www.plbio.kvl.dk/>

© David B. Collinge 2009
Slide 1



UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

The cereal group at LIFE

“Angie” Yan-Jun Chen, David B. Collinge, Jens Due Jensen, Hans J.L. Jørgensen, Chatchai Kosawang, Michael Lyngkjær, Bolette Lind Mikkelsen, Lisa Munk, “Kevin” Khoa D. Nguyen, Gowda Rayapuram.

Understanding the mechanisms of disease resistance in cereals

Hydrogen peroxide H₂O₂ in the wheat-*Septoria tritici* interaction.

Barley (*Hordeum vulgare*)-*Blumeria graminis* interaction

- Genes expressed in barley epidermis, e.g.:
 - DUF26 receptor-like protein kinases
 - NAC domain proteins (also in Arabidopsis)
- Determining their role in disease resistance

Rice-*Rhizoctonia solani*: induced resistance.

Barley-*Fusarium graminearum*: defence mechanisms and toxin accumulation.

Climate change effects on plant health

Utilisation of disease resistance

© David B. Collinge 2009
Slide 3

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

Genomics in barley

- EST - expression sequence tags in barley:
- NCBI listed 525,775 (21/10-09) in public data bases
 - IPK Gatersleben, has over 206,633
 - Clemson University, South Carolina, USA 60,000
- So there are plenty of relevant sequences to search!
- Filter membranes with 10500 spotted cDNAs (EST's)
- Affymetrix chip with 22,500 “Unigene” sequences (23,595 in NCBI, 21/10-09)

<http://www.gabi.de/>
<http://www.genome.clemson.edu/projects/barley/>
http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html

© David B. Collinge 2009
Slide 4

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

Genetics in barley

- Synchronised development of inoculum.
- Well characterised cytology.
- Possible to do studies at the single cell level.
 - Analysis of cell content
 - Altered gene expression
- VIGS being developed.
- Availability of good genetic material.
- TILLING populations.
- Map based cloning achieved

HR: Resistance

Susceptibility

papilla Resistance

DAB staining for H₂O₂

Photo: Per Gregersen

H. Thordal-Christensen, Z. G. Zhang, Y. D. Wei, and D. B. Collinge. Subcellular localization of H₂O₂ in plants. H₂O₂ accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. *Plant J.* 11: 1187-1194, 1997.

© David B. Collinge 2009
Slide 5

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

Tissue distribution of transcripts

- “Reverse northern blots” illustrate spatial differences in transcript distribution.
- Primitive arrays @

Stripping epidermis

Epidermis Mesophyll Total leaf

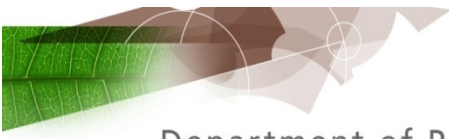
24 h after inoculation

Incompatible interaction Ooestryphodora

Peroxidase (Prx)
Oxalate oxidase-like
GRP94
14-3-3a
p816-12
PBI protein
β-1,3-glucanase
Chitinase
PR4 protein
PR5 protein (acidic)
PR5 protein (basic)
p8172-4Q5
Oxalate oxidase

Gregersen et al PMPP 51: 85 (1997)

© David B. Collinge 2009
Slide 6



Department of Plant Biology and Biotechnology

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

Plant sequences from differential display of mRNA isolated from inoculated epidermis

DD band#	Identical barley sequences	Homology	Confirmed expression
1-1, 10-26	Idi1- Iron deficiency Induced gene: EST from albino plants	putative acylreductone dioxygenase (ARD)	Max 3x
16-56	-	NAC domain proteins, OsNAC6 from rice	Max 3x
17-25	Barley SAM-synthetase (acc. D63835)	SAM-synthetases	Max 3x & Microarray
c2.16	-	Receptor-like kinases	Max 40 fold
23 bands	1: EST from albino plants (acc:023993) 22: unidentified	-	Student Projects!

© David B. Collinge 2009 Slide 7 Per L. Gregersen/Jesper Rung

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

Department of Plant Biology and Biotechnology

Part III: NAC domain proteins

N: NAC (from the *no apical meristem* mutant, *Petunia*)
 A: ATAF (*A. thaliana* proteins, activate CaMV 35S promoter)
 C: CUC (from the *cup-shaped cotyledon* mutant, *A. thaliana*)

DNA binding domain

A B C D E Transcriptional activation region

NAC domain 300 aa

- Highly conserved N-terminal (NAC domain)
- Large gene family ca 100 hits in Arabidopsis
- Some have a nuclear localization sequences
- Interact with factors for virus replication
- Transcription factors where studied
- Pathogen-induced in potato-Phytophthora & barley-*Bgh*
- Only found in plants

Per L. Gregersen
Michael Krogh Jensen

Angie Chen

© David B. Collinge 2009 Slide 8

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

NAC proteins

Activate the transcription of genes in the nucleus

pBD-HvNAC6₁₋₃₀₄ GAL4BD NAC domain TAR
 pBD-HvNAC6₁₋₁₆₄ GAL4BD NAC domain
 pBD (empty vector) GAL4BD

+ HIS - HIS β-GAL

pBD-HvNAC6₁₋₃₀₄
 pBD-HvNAC6₁₋₁₆₄
 pBD (empty vector)

"Yeast one-hybrid" assay demonstrates transcriptional activation of reporter gene expression
 Confocal microscopy demonstrates nuclear localisation of a GFT fusion

Jensen, et al. *Plant Mol Biol.* 65: 137-150, 2007.
 © David B. Collinge 2009 Slide 9

GFP - green fluorescent protein
 GFP-NAC fusion

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

The Barley "NAC-ome"

27 unigene sets among est's

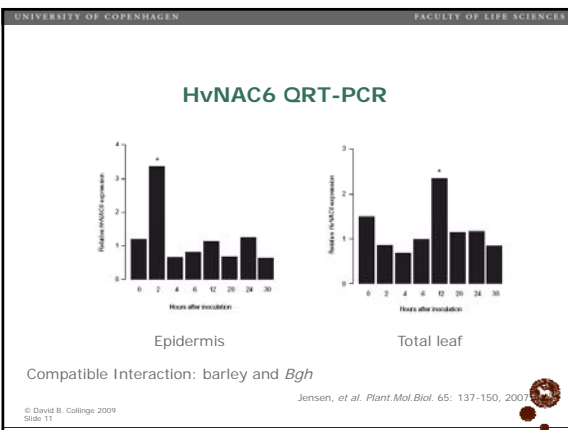
UniGene	Transcripts	cDNA Source
Hv4550	16	<i>Bgh</i> inoc. leaf, seed, stem, root
Hv1425	90	<i>Bgh</i> inoc. leaf, seed, callus, root
Hv984	6	Root
Hv6308	38	<i>Bgh</i> inoc. leaf, seed, callus
Hv5295	29	<i>Bgh</i> inoc. leaf, callus, flower
Hv5097	8	Seed, leaf, root
Hv5147	10**	<i>Bgh</i> inoc. leaf, callus, seed
Hv877	10	Seed, leaf
Hv13165	6**	<i>Bgh</i> inoc. leaf, callus
Hv2292	62	<i>Bgh</i> inoc. leaf, root, seed, flower
Hv17199	3	<i>Bgh</i> inoc. leaf, seed
Hv2154	6	Seed
Hv4823	26	<i>Bgh</i> inoc. leaf, stem, callus
Hv1254	30	<i>Bgh</i> inoc. leaf, callus, root
Hv15785	70**	<i>Bgh</i> inoc. leaf, root, shoot, seed
Hv19392	14	Seed
Hv19815	9	Seed, flower, <i>Fusarium</i> inoc. leaf
Hv6910	10	Seed
Hv18811	4	Seed, <i>Fusarium</i> inoc. leaf
Hv18323	18	<i>Bgh</i> inoc. leaf, root, callus
Hv17607	10	Seed, <i>Fusarium</i> inoc. leaf
Hv5292	47	<i>Bgh</i> inoc. leaf, seed, flower
Hv21351	7	<i>Bgh</i> inoc. leaf, callus, seed
Hv2626	23	<i>Bgh</i> inoc. leaf, seed
Hv21779	3	<i>Bgh</i> inoc. leaf, stem, root
Hv19852	26	<i>Bgh</i> inoc. leaf, root, stem
Hv19885	14	Stem, leaf

HvNAC4
 Blast of EMBL gives 400 sequences
 Sequences aligned using UniGene Build #48
<http://www.ncbi.nlm.nih.gov/sites/entrez>

HvNAC6
 Collinge et al. *Eur. J. Plant Pathol.* 121:257-266, 2008

HvNAC1
 ** Full-length NAC cDNA clones recovered in our lab (Per L. Gregersen)

© David B. Collinge 2009 Slide 10



UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

Are they relevant?

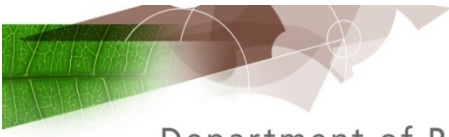
RNAi - targeted gene silencing

- Inoculation
 - Inoculated with *Bgh* 4 hours post-bombardment
- GUS staining
 - GUS stained 40 hours post-inoculation, followed by staining of epicuticular fungal structures by Evan's Blue

Stable slides for months

Michael Krogh Jensen

© David B. Collinge 2009 Slide 12



Department of Plant Biology and Biotechnology

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

Are they relevant? Manipulation of transcript levels

RNA Interference – to disrupt gene expression

Over expression

■ = statistical significant (P < 0.05)
 ■* = statistical significant (P < 0.01)

Frequencies of haustorium formation by *Bgh* calculated relative to pUbiGUS cells

© David B. Collinge 2009 Slide 13 Jensen, et al. Plant.Mol.Biol. 65: 137-150, 2007.

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

HvNAC6 belongs to the *ATAF* subfamily

The move to Arabidopsis...

© David B. Collinge 2009 Slide 14 Jensen, et al. Plant.Mol.Biol. 65: 137-150, 2007.

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

ATAF1 in the *Arabidopsis-Bgh* interaction same phenotype as for *HvNAC6* in barley

■ Bgh penetration
 ■ Invasive growth

Relative *ATAF1* expression

Hours after inoculation

Arabidopsis mutated in the *ATAF1* gene show enhanced susceptibility to *Bgh*

The expression of the *ATAF1* gene is induced by *Bgh*

© David B. Collinge 2009 Slide 15 Jensen, et al. Plant.Mol.Biol. 65: 137-150, 2007.

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

The 10K dollar question (70,000 DKKr)

What are the regulatory targets of *HvNAC6* and *ATAF1*?

or

What genes kinds of genes are being differentially regulated upon *Bgh* inoculation when knocking out *HvNAC6* and/or *ATAF1*

© David B. Collinge 2009 Slide 16 Michael Krogh Jensen

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

Experimental setup: It's all about design

Bgh effect

X 3

Control *Bgh*

Wild-type
 8 x Col-0

Mutant
 8 x *ataf1-1*

WT *Bgh* *ataf1-1* *Bgh* WT *Bgh* *ataf1-1* *Bgh*

© David B. Collinge 2009 Slide 17 Michael Krogh Jensen

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

ATAF1 and *Bgh* –dependent regulation in Arabidopsis

A

Treatment: ctrl, *Bgh*

Genotype: *ataf1-1*, Col-0

1st principal comp. (85%)
 2nd principal comp. (5%)

B

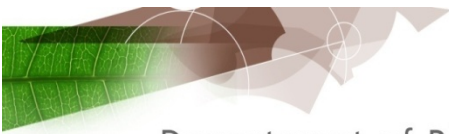
I *ataf1-1* ctrl vs Col-0 ctrl
 22 up, 10 down

II *ataf1-1* *Bgh* vs Col-0 *Bgh*
 341 up, 679 down

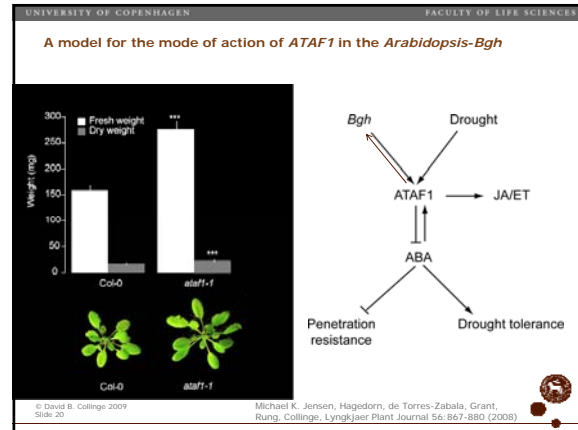
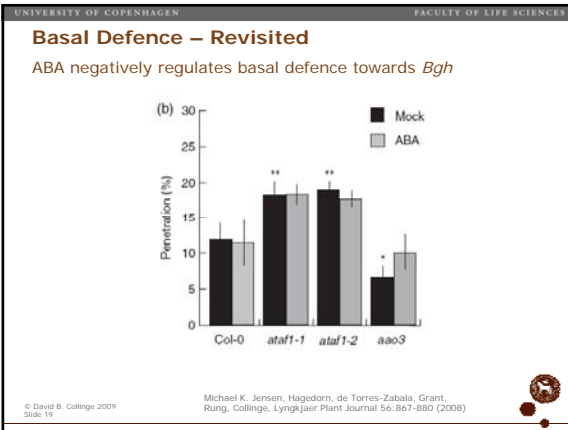
III Col-0 *Bgh* vs Col-0 ctrl
 3987 up, 4832 down

IV *ataf1-1* *Bgh* vs *ataf1-1* ctrl
 4097 up, 5433 down

© David B. Collinge 2009 Slide 18 Michael K. Jensen, Hagedorn, de Torres-Zabala, Grant, Rung, Collinge, Lyngkjaer Plant Journal 56:867-880 (2008)

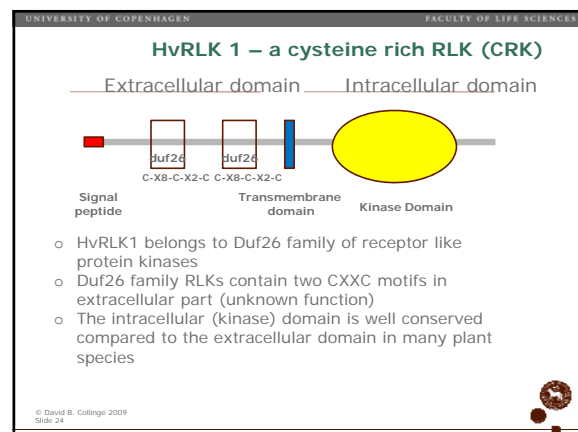
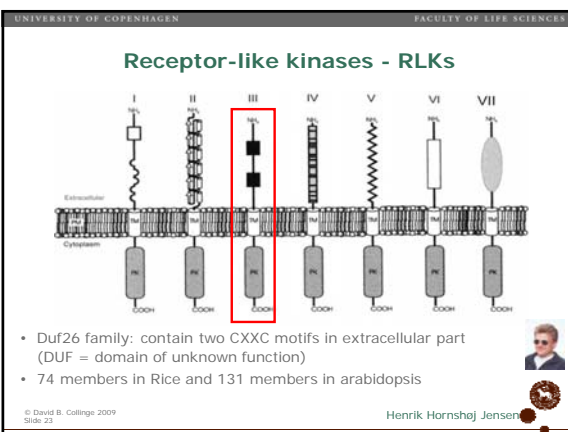


Department of Plant Biology and Biotechnology



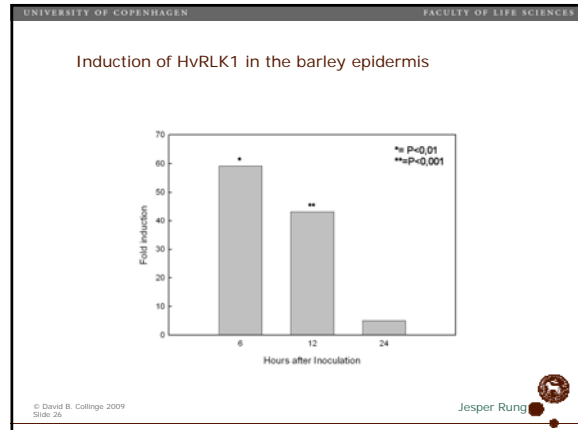
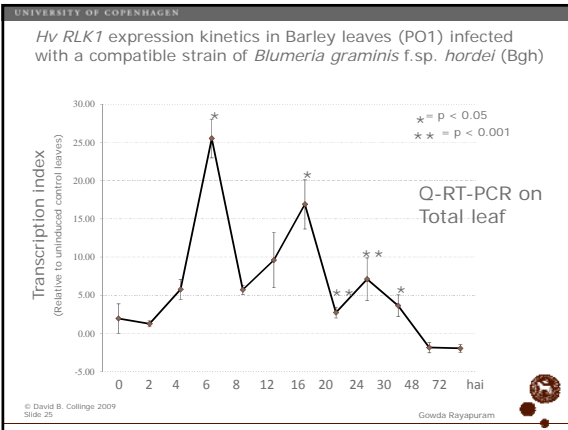
- Current studies on barley (which will also use the weed)**
- We are studying to determine the expression of the barley NAC'ome to determine when the plant uses the different NACs
 - PhD student Michael Wagner with Per Gregersen at Flakkebjerg
 - Michael Krogh Jensen with Karen Skriver at NAT
 - Angie Chen at LIFE with Michael Lyngkjær & DBC
 - is making transgenic overexpressing and antisense barley plants
 - Will look for interaction partners of HvNAC6
 - We also have interesting data from the *Arabidopsis* microarray in relation to carbohydrate metabolism.
 - Danish Research Council, FTP: "Unravelling plant regulatory networks: NAC transcription factors in senescence and disease resistance"
- © David B. Collinge 2009
Slide 21

- Receptor-like kinases – RLKs**
- Important roles in development, plant growth and defence responses
 - Involved in signal transduction
 - Converting binding of a ligand to the receptor part to an intracellular signal (phosphorylation cascade the kinase part of the protein phosphorylates a target)
 - The large RLK family:
 - More than 600 members in *Arabidopsis*
 - More than 1100 members in Rice
- Jesper Rung
Johanna Vikgren
Gowda Rayapuram
- © David B. Collinge 2009
Slide 22





Department of Plant Biology and Biotechnology



UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

Duf26-RLK family members

Gene	Organism	Function	Expression				Promotor
			SA	Oxidative	Pathogen	W-Box	
At4g23130	Arabidopsis	Cell death	x	x	x	x	
At4g23270	Arabidopsis	Cell death	x		x	x	
At3g45860	Arabidopsis	Cell death	x		x	x	
At4g23280	Arabidopsis	Cell death	x		x	x	
At4g23190	Arabidopsis	No	x	x	x	x	
PvRK20	Common Bean				x		
Q8VX53	Potato				x		
Q8VX50	Potato				x		
AK110461	Rice					x	
AK064115	Rice					x	
AK120072	Rice					x	
HvRLK1	Barley	maybe		x	x		
Hze60	Barley				x		

Chen, et al *Plant.Mol.Biol.* 56:271 (2004) Jesper Rung

© David B. Collinge 2009 Slide 27

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

Summary RLK:

- Characterized a novel *duf26*-related (*HvRLK1*) Bgh responsive gene in barley
- Pictures showing localisation of the protein in barley were shown.
- Silencing *HvRLK1* increased penetration resistance in barley against virulent Bgh
 - [Data not shown in the web version]

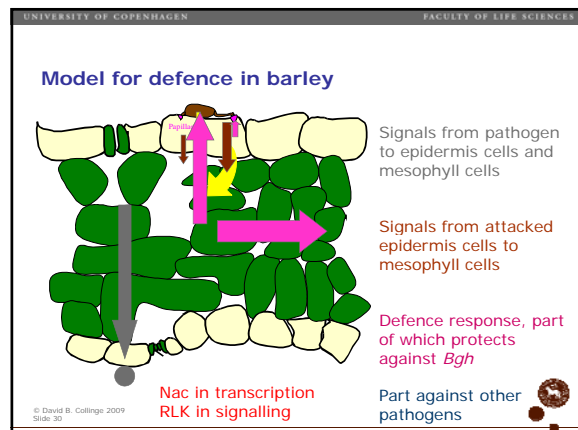
© David B. Collinge 2009 Slide 28

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

Conclusions:

- The range of pathogen induced plant genes identified is far from exhausted:
 - Five novel pathogen induced genes in barley were identified.
 - None of the 36 sequenced genes represented sequences previously known.
 - Involvement of a putative transcription factor
 - NAC domain protein
 - Receptor-like protein kinase Duf26 family
- Differential gene transcript accumulation in the epidermis was substantiated
- Though microarray is clearly the way forward, Differential display has a role for difficult tissues like epidermis
- A microarray is only as good as the genes you put on it!

© David B. Collinge 2009 Slide 29





Department of Plant Biology and Biotechnology



UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

The future

- More transient expression assays for looking at important components by RNAi and by over-expression transiently and stably.
- Proteomic approaches based on epidermal tissues to look for interacting proteins.
- Use of Arabidopsis microarray data for hypothesis design
- Use of Arabidopsis T-DNA lines where appropriate
- Why? To understand the nature of disease resistance for sustainable use!

© David B. Collinge 2009
Slide 31

UNIVERSITY OF COPENHAGEN FACULTY OF LIFE SCIENCES

People involved NAC/RLK:

*Angie Chen
Michael Krogh Jensen (now NAT)
Michael Lyngkjær
Gowda Rayapuram
Jesper Rung
Johanna Vikgren*

The Riso Lab:
*Michael Lyngkjær
Torben Gjetting
Peter Hagedorn*

Flakkebjerg:
*Per Gregersen (ex LIFE)
Preben Bach Holm
Michael Wagner*

Gatersleben:
Patrick Schweizer

© David B. Collinge 2009
Slide 32

People involved Septoria & Fusarium:

*Jens Due Jensen
Nandini Shetty
Hans Jørgensen
Michael Lyngkjær*

Funding:
FTP: Climate, NAC & Septoria
DFFE/PBD: *Fusarium*
Danida: Rice-*Rhizoctonia*
Banana - *Mycosphaerella*

Opportunities for PhD studentships at LIFE
Mail to dbc@life.ku.dk