# A Genetic Linkage Map of *Phycomyces blakesleeanus*

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## INTRODUCTION

*Phycomyces blakesleeanus* is a filamentous fungus of research interest because of its ability to sense and respond to its environment. The unicellular sporangiophores show growth responses to light, gravity, wind, chemicals and presence of objects near the growing zone. The mycelium also shows responses by induction of β-carotene synthesis and initiation of sporangiophores. The responses to light have been most carefully analyzed, in part driven by the efforts of Nobel laureate Max Delbrück who tried to develop *Phycomyces* into the "phage of vision". Strains with impaired phototropism were isolated in the 1960s-1980s, as well as mutants affected in other sensory responses or phenotypes. These properties have made *Phycomyces* a model of intracellular sensory transduction processes. However, the inability to transform DNA into *Phycomyces* has blocked the identification of genes in this fungus. To achieve a better understanding in all these studies, it is important to know more about features of this fungus. One of these features is a genetic map, which is fundamental to the understanding of the various mechanisms and processes of interest. A genetic linkage map is an important component of *Phycomyces* genome project, which is currently comprised of 481 distinct DNA fragments. The genome map of *Phycomyces* is constructed largely from amplified fragment length polymorphism of progeny derived from a cross between two wild type strains. The goal of the current study is to prepare reliable and unambiguous single-locus genetic markers and create a dense linkage map.

## MATERIALS AND METHODS

***Mapping population:*** We used a mapping population of 121 progeny, generated by a cross between a pair of wild-type strains, i.e UBC21 (+) and NRRL1555 (-). The crosses were performed as follows: mycelia of two strains of opposite sex were inoculated at opposite margins of 10 cm diameter petri dish containing potato-dextrose agar supplemented with 100 mg/liter thiamine. The dishes were incubated in darkness at room temperature until a line of zygospores appeared. The zygospores are harvested individually with tweezers and transferred to petri dishes containing filter paper moistened with water. Approximately two months later, the zygospores begin to germinate, each giving rise to a germsporangiophore whose germsporangium contains the germspores. The germspores are collected in 10 µl of sterile $H_2O$ and heat-shocked at 45°C for 15 min to break dormancy before being plated onto PDA medium. Pieces of mycelium, from each colony were subcultured to investigate their genotype.

***Molecular marker (PCR-RFLP) development:*** Polymorphic regions were identified by comparison of Solexa sequencing data generated from strain UBC21 with the genome sequence of strain NRRL1555 (Fig 2). From the Solexa sequencing, which provides short DNA reads, an estimated 1 polymorphism (SNP) per 850 bp are present between the two strains used for mapping. At least two markers on the 20 largest contigs, or near regions of specific interest (*sex* locus, *furA madD*, and *lysA madE*) were designed to amplify ~1 kb fragments including the polymorphic region, with each polymorphism selected to include a change in restriction enzyme site. Oligonucleotide primers were designed manually to avoid repetitive regions or those with multiple restriction enzyme cut sites. These regions were amplified from 121 progeny of the UBC21 x NRRL1555 cross, the PCR products were digested with restriction enzymes, and DNA resolved on agarose gels to assign alleles.

***Data Analysis and map construction:*** Strains NRRL1555 (-) has been crossed to UBC21 (+) and 121 progeny have been isolated and DNA prepared for analysis. Oligonucleotide primers are designed to amplify polymorphic regions yielding restriction enzyme site changes from parents and 121 progeny, the PCR products digested and products resolved by gel electrophoresis. This has been performed for 78 markers. To provide a comprehensive framework, markers are placed every 500-1000 kb across the genome, thus requiring approximately another 110 markers to be generated and incorporated into the map.

When all the marker information is assembled, JoinMap 4.0 software will be used to analyze the allele data to create the molecular map. The data will be analyzed with an algorithm based on anonymous markers, that is creating the map from the raw data, and with an algorithm that considers used-defined physical distances. It is anticipated that both algorithms will lead to congruent genetic maps, and those regions that are not consistent between both will be subject to a further round of marker developed and analysis.

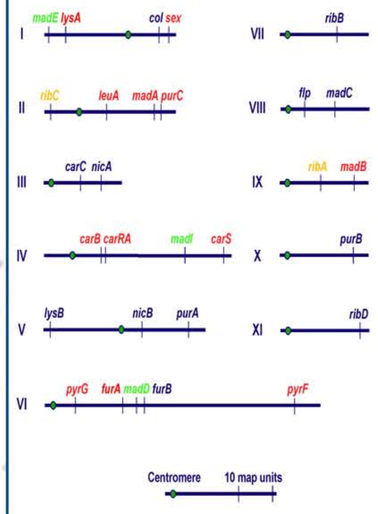



**Fig. 1.** Genetic map of *Phycomyces blakesleeanus,* Red loci represent genes that have been published and orange markers represent loci whose identity can be inferred with high certainty. The centromeres were mapped using tetrad analysis. Green markers are *mad* phototropism genes close to being identified.

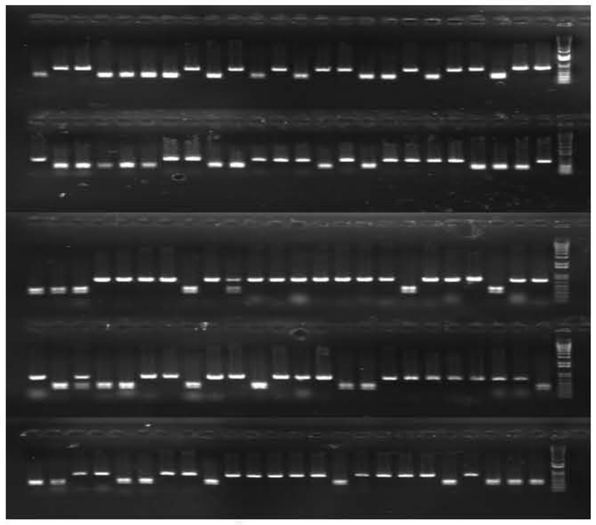


**Fig 3.** Agarose gel of PCR-RFLPs of 120 progeny of a cross between *Phycomyces* strains NRRL1555 x UBC21.

## RESULTS AND DISCUSSION

At present, 78 markers have been developed and used in PCR analysis to assign alleles from the mapping population (Fig 3). These were incorporated into the mapping population database (Fig 4). Preliminary analysis provides an estimate of the frequency of recombination in the genome and an estimate of how many markers are needed to complete the genetic map. Markers spacing a collective total of 7.1 Mb exhibited a total of 247 map units of recombination (centiMorgans, cM). The average is 1 cM = 29 kb, with a range of 1 cM = 8 kb (adjacent to the *sex* locus) to 1 cM = 106 kb (on contig 7). With the *Phycomyces* genome comprising an estimated 55 Mb, we need 190 markers for complete coverage with 10 cM resolution.

The rationale behind the map-based cloning approach is due to the current inability to perform stable genetic transformation of *Phycomyces*. Indeed, this is the major hurdle for the species hindering advances in understanding gene function. Mapping approach is reliable method to identify the *mad* genes, and has additional advantages in yielding information about the meiotic process in a basal fungal lineage from which no resolution genetic map has been made. While map-based cloning is a traditional approach to identify mutated genes, particularly in plant species, the ease of cloning by complementation in fungi has meant that this is a preferred approach.

A genetic map of *Phycomyces* based on mutant phenotypes and tetrad analysis was generated over a 20 year time frame (Fig 1). This map has already been used to narrow the search for *mad* genes, with the identification of point mutations in *fur*A and *lys*A genes that flank *madD* and *madE*, respectively. Additionally, using the *carRA* and *carB* genes as a starting point, the DNA regions near the *madI* gene have been identified, and the adjacent *carS* gene also found.

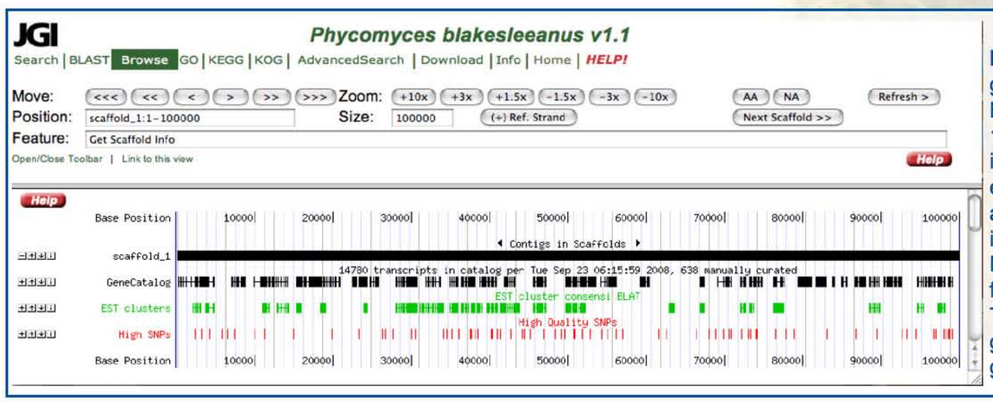
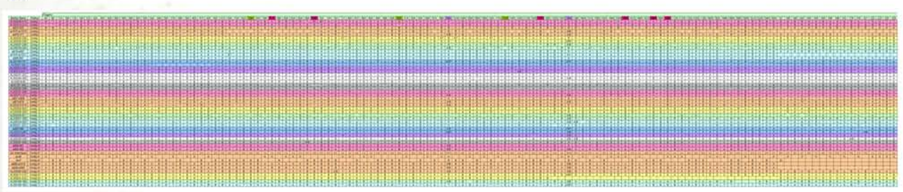



**Fig. 2.** Example of the *Phycomyces* genome browser from the Department of Energy. The bottom window shows the first 100 kb on scaffold 1 of strain NRRL1555, including the positions of the 27 genes and expressed sequence tags (EST clusters) for a subset. The red "High SNPs" section indicates polymorphisms between strains NRRL1555 and UBC21 that were identified from Solexa sequencing data of UBC21. The frequency of these polymorphisms per gene illustrates the potential resolution for genetic mapping down to a single gene.

**A.**



**B.**

| Marker Name | Position | Progeny 4A | 5A | 11A | 15A | 17A | 18A | 21A | 22A | 24A | 25A | 26A | 28A | 29A | 30A | 32A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ALID0209-210 | Contig 1 | B | ? | A | B | B | B | A | B | B | ? | A | B | A | | |
| ALID0312-313 | Contig 1 | ? | ? | B | B | A | A | A | B | A | A | A | B | B | B | A |
| ai904-905 | Contig 2 | B | B | A | A | A | B | A | A | B | A | A | A | B | ai904 | |
| ai1072-ai1073 | Contig 2 | ? | ? | A | A | B | A | A | A | B | A | ? | B | B | A | A |
| ai916-917 | Contig 2 | A | A | A | B | A | B | B | B | B | B | B | A | A | A | A |
| ALID0233-234 | Contig 3 | B | A | A | B | A | A | B | B | B | A | B | A | A | A | B |
| ALID0235-236 | Contig 3 | B | ? | A | B | B | A | B | B | B | B | B | A | A | B | A |
| ALID0237-238 | Contig 4 | A | ? | A | B | A | B | B | B | B | B | B | A | A | B | A |
| ALID0239-240 | Contig 4 | A | A | A | ? | A | A | B | B | B | B | | A | A | A? | B |
| ai814-ai861 | Contig 5 | A | B | A | B | B | B | B | A | A | A | A | B | A | B | A |
| ai1070-ai1071 | Contig 5 | A | ? | A | B | B | B | A | A | A | A | B | A | A | B | A |
| ai886-887 | Contig 5 | A | B | A | A | B | B | B | A | A | A | A | B | A | B | A |
| ALID0190-191 | Contig 6 | A | A | A | A | A | A | B | A | A | A | B | A | A | B | B |
| ALID331-332 | Contig 6 | ? | ? | A | A | A | A | B | A | A | A | ? | A | B | B | B |
| ALID0243-244 | Contig 7 | A | A | A | B | A | B | A | B | B | B | B | A | A | B | A |
| ALID314-315 | Contig 7 | A | ? | A | B | A | B | A | ? | B | B | B | A | A | B | A |
| ALID0251-252 | Contig 8 | B | A | A | B | B | A | B | B | B | A | B | B | A | B | B |
| ALID344-345 | Contig 8 | B | B | B | B | A | A | B | B | B | A | A | A | A | B | B |
| ALID0035-69 | Contig 8 | B | ? | A | B | B | B | B | B | A | B | B | A | A | B | B |
| ALID 351-352 | Contig 9 | B | A | A | ? | ? | B | A | B | B | B | ? | B | B | A | A |
| ALID0255-256 | Contig 9 | B | ? | A | B | D | B | A | B | B | B | B | B | B | B | A |
| ALID0257-258 | Contig 10 | A | ? | B | B | B | B | B | A | A | B | B | A | A | B | A |
| ALID0310-311 | Contig 10 | A | ? | B | B | A | B | B | A | ? | B | B | A | A | A | A |
| ai857-ai858 | Contig 11 | B | A | A | A | B | B | B | A | B | A | B | B | A | B | A |
| ai878-879 | Contig 11 | B | A | A | A | B | B | B | A | B | A | B | B | A | B | A |
| ALID0273-274 | Contig 11 | ? | B | A | B | B | B | B | A | B | A | ? | B | A | B | A |
| ALID0302-303 | Contig 12 | A | A | A | B | B | A | B | A | B | B | B | A | B | B | A |
| ALID0304-305 | Contig 12 | A | A | A | B | B | A | B | A | B | B | B | A | B | B | A |

**Fig. 4.** Data matrix for the *Phycomyces* genetic map. **A.** Global overview of >5000 data points. **B.** A subset of the data. For most markers, the regions of the genome polymorphic between NRRL1555 and UBC21 were amplified by PCR and cut with restriction enzymes. Colors indicate unique contigs for the markers.

## ACKNOWLEDGEMENTS

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