

Abstract

Title: The use of the filamentous fungus, *Aspergillus nidulans*, in undergraduate research and teaching.

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I have had success involving students in independent research projects with *Aspergillus nidulans* and have also used this system for research-based exercises in the sophomore level Genetics course at Mississippi College. In addition, I plan to use *A. nidulans* for several exercises in our new Cell Biology course. *A. nidulans* is well-suited for use in undergraduate research and teaching for several reasons. It is easily and inexpensively grown on simple microbiological medium. Stocks are easily made for long term storage and do not require frequent subculturing. A variety of long and short term projects can be designed for students. Numerous mutant strains are available for use in research and teaching. Many topics relating to genetics, cell biology and biochemistry can be introduced with *Aspergillus* in laboratory exercises. Auxotrophic mutants are available as well as mutants defective in some aspect of cell cycle progression, signal transduction or development.

In our genetics class, students analyze a cross between two mutant strains of *Aspergillus* that carry mutations in linked genes. By analyzing the combination of markers in the progeny of this haploid organism, students are able to easily see gene linkage and to construct a linkage map of three closely linked genes. I plan to use *Aspergillus* in an exercise to demonstrate the importance of biochemical controls in cell cycle progression and to provide examples of signal transduction pathways. No specialized equipment is required for these analyses.

In addition to its use in the classroom, I have involved students in independent research projects on the genetics and molecular biology of *Aspergillus*. Under my direction, students have isolated suppressor mutations of signal transduction mutants, made progress in sequencing a signal transduction gene and characterized cDNA clones of this gene. Students working with me have also carried out immunofluorescence microscopy with *Aspergillus* and performed 5' RACE to characterize a gene.

Thus, *A. nidulans* is a suitable system for research-based experiments in laboratory courses as well as for extended independent undergraduate research projects.

Manuscript

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I and colleagues at other undergraduate institutions, have had success in involving undergraduate students in independent research projects with *Aspergillus nidulans*. In addition, I have found *Aspergillus* to be very useful for laboratory teaching exercises. Here I will describe techniques for growing *Aspergillus* and exercises demonstrating important concepts of genetics and cell biology. I will describe in detail the procedure for crossing *Aspergillus* and analysis of progeny and other laboratory experiments in less detail. In addition, I will describe student research projects that I have directed involving *Aspergillus*.

Aspergillus nidulans is a filamentous ascomycete fungus having both an asexual and a sexual life cycle. Conidia (spores) will germinate on solid medium and grow into a colony covered with conidia in only two days. The short life cycle is an attractive and useful feature of this organism. Obtaining progeny from sexual crosses takes about two weeks and will be described below. *A. nidulans* is usually worked with as a haploid organism, but stable diploids can be formed which can be used in mapping studies - this is the parasexual cycle. In addition to its short life cycle, *Aspergillus* is grown on simple microbiological medium and long term stocks are easily made. Complete medium (see recipes below) is useful for propagation of strains and minimal medium for testing of many genetic markers and crossing. Genetic markers are tested in several different ways, including 1) on minimal medium lacking a particular nutrient, 2) at a restrictive temperature, and 3) on toxic substances.

Many mutant strains of *A. nidulans* are available from the Fungal Genetics Stock Center (FGSC, Department of Microbiology, University of Kansas Medical Center, Kansas City, KS 66160-7420. fgsc@ukanvm.cc.ukans.edu). Different mutant strains are useful for demonstrating concepts of biochemistry, cell biology or genetics. An extensive linkage map of *A. nidulans* has been established and is also available from the FGSC. This map can be used by students to compare results from crosses to establish linkage as described below.

The use of *Aspergillus nidulans* to demonstrate gene linkage

Crosses between strains differing in linked genetic markers can demonstrate linkage, crossing over and how linkage maps are constructed. Strains carrying complementary

genetic markers are easily crossed. *Aspergillus nidulans* is homothallic, so any two strains can be crossed. In our class, students enter the crossing process at the point of analysis of progeny which have already been transferred to appropriate media for scoring. Students could also set the cross and isolate the progeny, in which case several weeks would be required for the experiment.

In our sophomore level genetics class, students analyze a cross between two strains of *A. nidulans* that carry mutations in three linked genes. By analyzing the combination of markers in the progeny, students are able to easily see gene linkage and to construct a linkage map of the linked genes, *byA*, *yA* and *pabaA*. We have found this experiment to be a useful addition to our classical *Drosophila melanogaster* crosses. Mutations in the *yA* gene result in an easily scored (on complete or minimal medium) phenotype of yellow, rather than wild type green, conidia. Mutations in the *biA* or *pabaA* genes are easily scored on minimal medium lacking biotin or PABA (p-Aminobenzoic acid), respectively. Students analyze progeny from the cross, *biA*⁺, *yA2*, *pabaA1* X *biA1*, *yA*⁺, *pabaA*⁺, on media which allows them to determine if each progeny carries the mutant or wild type allele for each of the three genes. For each pair, students determine the number of progeny with the parental genotypes (eg. *biA*⁺, *yA2* and *biA1*, *yA*⁺ for *biA* and *yA*) and the number with recombinant genotypes (*biA*⁺, *yA*⁺ and *biA1*, *yA2*). Using that information, they can determine if there is linkage between any pair of genes: *biA* and *yA*, *biA* and *pabaA* or *yA* and *pabaA*. Having determined that all three genes are closely linked, they can then determine the order of the linked genes and compare their results to the published linkage map.

This analysis could be simplified for an introductory course by examining linkage between just two linked genes, *yA* and *biA*, for example. Strains used in the cross could be chosen to demonstrate additional important genetic concepts such as epistasis. For example, mutations in the *wA* gene result in white, rather than wild type green conidia. Mutations in the *wA* gene are epistatic to mutations in *yA* (a double mutant is white). A cross of a double mutant to a green strain would produce strains of all three colors. Students would need to understand epistasis to explain where the yellow progeny came from.

Several methods for crossing *A. nidulans* are used. The simplest is to make point inocula of the two strains to be crossed near each other on a plate of complete medium (see recipe below). Strains will grow together after two days at 37° C. Small blocks of agar from areas where the strains have grown together are now transferred to a plate of minimal medium. After two days at 37° C, the plate is sealed with tape, creating a low oxygen environment which facilitates crossing. After two weeks at 37° C, the cross can now be analyzed. The products of meiosis, ascospores, are present within dark, spherical cleistothecia. Several large cleistothecia are removed from the plate and cleaned of smaller cleistothecia by rolling on a 3% water agar plate. Each is then burst in 1 ml of sterile water and the tube is vortexed to release the spores. 5 - 20 µl of the suspension is spread onto medium which will support the growth of all possible progeny (usually complete medium). Individual colonies on these isolation plates are then transferred in an ordered fashion to another complete medium plate. After these have grown, they are transferred (using a

replicator, if available) to the test plates which includes a plate of synthetic complete medium (minimal medium with supplements to allow growth of all progeny - biotin and PABA in the above example) and a plate which lacks each of these supplements (a minus biotin plate and a minus PABA plate in the example).

Results obtained in a recent genetics laboratory are shown below. The number of parental and recombinant types for each gene pair was determined and the map distance, which is the percent recombinant progeny.

	<u><i>yA</i></u>	<u><i>biA</i></u>	<u>#s each type</u>	
parentals	+	-	44	
	-	+	40	
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recombinants	-	-	3	7% recombinants
	+	+	3	

	<u><i>yA</i></u>	<u><i>pabaA</i></u>	<u>#s each type</u>	
recombinants	+	-	7	
	-	+	6	
<hr/>				
parentals	-	-	41	14% recombinants
	+	+	36	

	<u><i>biA</i></u>	<u><i>pabaA</i></u>	<u>#s each type</u>	
parentals	+	-	35	
	-	+	39	
<hr/>				
recombinants	-	-	9	18% recombinants
	+	+	7	

The use of *Aspergillus nidulans* to demonstrate signal transduction

My area of research is the ambient pH signal transduction pathway of *A. nidulans*. This pathway ensures the production of pH-appropriate extracellular enzymes; for example, acid phosphatase in acid environments and alkaline phosphatase in alkaline environments. The products of seven *pal* genes, *palA*, *B*, *C*, *F*, *H* and *I* constitute this signalling pathway. Mutations in these *pal* genes result in decreased staining for extracellular alkaline phosphatase and increased staining for extracellular acid phosphatase. Activation of the *pal* signalling pathway in response to alkaline ambient pH results in the proteolytic activation of the *pacC*-encoded transcriptional activator, PacC. *pacC*^c truncation mutations result in an active form of PacC regardless of the ambient pH, causing increased staining for alkaline phosphatase but decreased staining for acid phosphatase. Students can easily perform the alkaline and acid phosphatase staining procedure, and thereby determine which mutation or mutations strains carry. An understanding of the signalling pathway will be required to understand why a strain carrying both a *pacC*^c mutation and a mutation in one of the *pal* genes has the same staining pattern as a strain carrying only the *pacC*^c mutation. This is also a demonstration of epistasis.

The use of *Aspergillus nidulans* to study the control of cell cycle progression

The Chromosome Mitotic Index (CMI) can easily be determined for *Aspergillus* by staining cells with a nuclear stain such as orcein and visualization under the light microscope. If a fluorescence microscope is available, the Chromosomes of mitotic nuclei appear very condensed and have no visible nucleolar region. A number of interesting temperature sensitive mutants are known that carry mutations in genes important for cell cycle progression. The *bimC* gene, for example, encodes a kinesin-like protein and *bimG* encodes a phosphoprotein phosphatase. At restrictive temperature (usually 42° C), these strains enter, but cannot exit mitosis and therefore become blocked in mitosis, demonstrating a dramatic increase in CMI. Strains carrying mutations in other genes are unable to enter mitosis at restrictive temperature. Strains carrying mutations in the *nimX* gene, which encodes a homolog of the *S. pombe cdc2* gene, transferred to restrictive temperature are never in mitosis, and demonstrate a decrease in the CMI. In a laboratory exercise, students can fix and stain cells growing at permissive temperature and cells which have been transferred to restrictive temperature and determine the CMI for both.

The use of *Aspergillus nidulans* in molecular biology exercises

A. nidulans can be used in a number of exercises of important molecular biology techniques. Genomic DNA and RNA can be easily isolated from *Aspergillus* for use in Southern or Northern blotting. Plasmid transformations can rescue auxotrophic mutants. Isolation of genomic DNA from transformants followed by restriction enzyme digestion and Southern blotting can demonstrate homologous integration, as this occurs with a high frequency in *A. nidulans*.

The use of *Aspergillus nidulans* in undergraduate student research projects

A. nidulans allows for long term research projects in areas of molecular genetics, classical genetics and cell biology. Short-term projects are also easily designed, depending on research interests, for students. Features of the system that make it attractive for student research are mentioned above. Some of the research projects, including classical genetics projects do not require constant attention by the student, but can be worked on periodically to fit the students' schedules. In my lab students have been involved in the following projects:

An undergraduate student working on an Honors research project has been determining the genomic sequence of the *pall* gene of *A. nidulans*. She first made deletion clones of the genomic clone and then sequenced the clones using our department's automated DNA sequencer. She has completed the sequence of our genomic clone. Comparison to the cDNA sequence has allowed her to identify introns in the genomic sequence. She is now performing 5' RACE to identify the 5' end of the gene.

Another student working on an honors research project has purified and characterized several new cDNA clones of the *pall* gene. The experience she gained was primarily in molecular biology, working with lambda clones and plasmid clones.

A student just beginning work in my lab will be using immunofluorescence microscopy to determine the cellular location of *pal* gene products. As a preliminary step to this project he has been carrying out tubulin immunofluorescence.

As part of an HHMI grant at Mississippi College, a high school teacher and his student performed a genetic screen for suppressor mutants during the summer months of 1997. Following 4-NQO mutagenesis of a *pall30* mutant strain, 500,000 viable spores were plated to identify suppressed strains. Of 400 strains initially identified as interesting, 34 have been chosen for further study.

Undergraduate research with *A. nidulans* at other colleges

Faculty at other undergraduate institutions also involve students in research with *A. nidulans*.

Dr. Sarah McGuire of Millsaps College has been directing students in the isolation of extragenic suppressors of strains carrying mutations in the *nimX^{CDC2}*, cell cycle regulatory gene.

Dr. Steven James of Gettysburg College has directed undergraduate students in research using a combination of cellular, genetic, and molecular approaches to isolate and study two genes important for cell cycle progression. These genes control the initiation of DNA replication, and include *nimO*, which encodes a protein containing a novel zinc finger motif, and *nimQ*, which is a member of the MCM family of DNA licensing factors.

***A. nidulans* media**

Complete medium

Amount per liter:

10 gm Dextrose
1 gm Yeast Extract
2 gm Peptone
10 ml *A. nidulans* Vitamin Solution (see below)
1 gm Casamino Acids
0.075 g Adenine
20 ml *A. nidulans* salt solution (see below)

pH to 6.5 with NaOH and add agar (10 gm/L) for solid medium

Mininal medium

Amount per liter:

10 gm Dextrose
20 ml *A. nidulans* salt solution (see below)

pH to 6.5 with NaOH and add agar (10 gm/L) for solid medium

Salt solution

Amount per liter:

26 gm KCl
26 gm MgSO₄ 7 H₂O
76 gm KH₂PO₄
10 ml *A. nidulans* Trace Elements Solution (see below)

Vitamin solution

Amount per liter:

400 mg p-Aminobenzoic acid (PABA)
50 mg Thiamine HCl
2 mg d-Biotin
100 mg Nicotinic Acid
250 mg Pyridoxine HCl
1.4 gm Choine HCl

100 mg Riboflavin

Trace elements solution

Amount per liter:

40 mg Sodium tetraborate 10 H₂O
400 mg Cupric sulfate 5 H₂O
800 mg Ferric orthophosphate H₂O
800 mg Manganese sulfate 4 H₂O
800 mg Sodium molybdate 2 H₂O
8 gm Zinc sulfate 7 H₂O

Supplements

Supplements are made as stocks and added to minimal medium:

d-Biotin stock is 0.1 mg/ml and added to 0.01 mg/L final concentration

Ammonium tartrate stock is 500 mM and is added to 5 mM final concentration

PABA stock is 0.4 mg/ml and added to 4 μl/ml final concentration