Analysis of Morphology and Secretion Mutants in *Aspergillus nidulans*

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Filamentous fungi are important economically and medically due to their capacity to produce secondary metabolites or as human and animal pathogens. The genes and molecular mechanism responsible for secretion is poorly understood. Using classical genetics, we derived temperature-sensitive (Ts) fungal mutants in *Aspergillus nidulans*. These mutants were then analyzed for their secondary metabolite secretion capacity.

In this study, we used the Ts strains of *A. nidulans* to determine how knockouts affect morphological and protein secretion by examining the phenotypes under the microscope and also by staining the mutants with Congo red staining. Mutants were stained with Congo red to determine enzyme activity, which was then used to select mutants with the highest enzymatic activity to be used in the study.

Our objective was to identify the mutants responsible for the morphological and protein secretion defects by identifying numerous mutants with previously unknown roles in hyphal morphological and protein secretion factors by examining the phenotypes under the microscope and also by staining the mutants with Congo red staining. Mutants were stained with Congo red to determine enzyme activity, which was then used to select mutants with the highest enzymatic activity to be used in the study.

Our objective was to identify the mutants responsible for the morphological and protein secretion defects by identifying numerous mutants with previously unknown roles in hyphal morphogenesis and secretion and performing a screen to determine hyper-secretors.

### Materials and Methods

- Ts stock mutants were made in our laboratory and were generated by UV radiation or through chemical mutagenesis.
- Mutants were grown in Yeast Extract and Glucose liquid media on cover slips and then placed on slides for 12-16 hours at 42°C and were then analyzed under a microscope to determine the phenotypes. After incubation, they were stained with calcofluor stain.
- The mutants were stained with Congo red solution for 30 minutes, and then washed with sodium chloride solution. White light was used to determine the halo index.

### Results

<table>
<thead>
<tr>
<th>A. nidulans mutant</th>
<th>Halo Index</th>
<th>Halo Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>A4 (WT)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>C13</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>G7</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>I12</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>M7</td>
<td>1.8</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Figure 1: For the *A. nidulans* mutant strains, A4 is known as wild-type (WT). All mutants were grown out on MN+CMC media for 48 hours at 42°C. They were then stained with Congo red and washed with sodium chloride solution to display the halos. These halos indicated the amount of enzymatic activity secreted by each individual mutant. Compared to A4, C13 (not shown), G7, I12, and M7 displayed higher amounts of enzymatic activity compared to the amount of mutant plated, resulting in a ratio known as the halo index. Overall, these four mutants were used to further our research regarding enzyme activity for *A. nidulans*.

### Conclusion

The various morphological defects can explain a lot about the fungus *A. nidulans*. The phenotype that shows the tip of the hypha splitting in two is known as dichotomous branching. This phenotype does not occur in wild-type. Another morphological defect is delayed spore polarization, in which a spore cannot readily establish a hypha and therefore undergoes considerable swelling before polarizing.

Lastly, another morphological defect is reduced extension rate of growing hyphae. In this case, hyphae are often wider than normal or possess uneven width.

The Congo red staining process is useful because it explains how much enzymatic activity is occurring in an organism. The higher halo index around the mutant the more enzymatic activity that occurred. When screening these mutants, four mutants had a large diameter, some even bigger than wild-type A4. These mutants were C13, G7, I12 and M7.

In conclusion, by screening the *A. nidulans* mutants, four different mutants emerged that appeared as hyper-secretors, or mutants that secreted better than wild-type. This was determined by performing a Congo red staining process on these mutants to screen for mutants with the highest enzymatic activity. This process helped us pick out mutants that in the future can be verified and used in industries to improve the production of enzymes and proteins. It can also be used for industrial-relevant enzymes by filamentous fungi. Morphology and secretion factors are able to determine the improvement of greater production of enzymes and renewable chemicals within industries.

### References


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