Characterization of alpha-(1-3)- Glucan Synthases function in cell wall biosynthesis in *Neurospora crassa*

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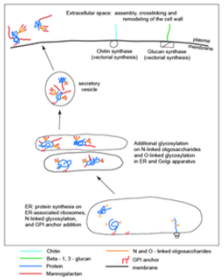
Cell Wall Biosynthesis



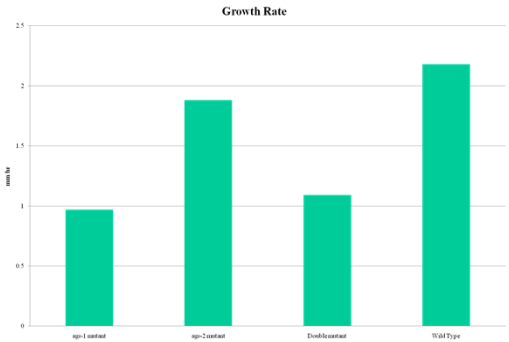
Abstract

Two genes are identified that encode alpha-(1-3)-glucan synthases, *ags-1* (NCU08132) and *ags-2*(NCU02478), in *Neurospora crassa*. We were able to isolate those knock out mutants (deletion mutants) from the heterokaryon strains available in the knockout library. The *ags-2* mutant had mild mutant phenotype whereas the *ags-1* mutant showed a severe mutant phenotype. The presence of the knock out mutation (deletion) was confirmed by polymerase chain reaction (PCR). We analyzed knock out mutants in terms of morphology, cell wall integrity, and growth rate. We also created double mutant by mating *ags-1* mutant and *ags-2* mutant. The double mutant showed a similar mutant phenotype to *ags-1* mutant. We concluded that the *ags-1* encoded alpha-(1-3)-glucan synthase plays an important role in normal cell growth, however it is not required for *Neurspora crassa* to survive.

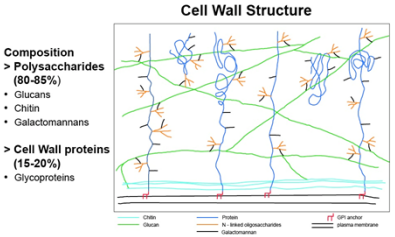
Introduction

The fungal cell wall performs a number of critical biological functions, such as defining cell morphology, and protecting the cell from osmotic lysis,dessication, and pathogens. The fungal cell wall is mainly composed of glucan (polymers of glucose), chitin(polymers of beta 1-4-N-acetylglucosamine), and mannoproteins. Alpha-(1-3)-glucan is the basic components of the fungal cell wall. Alpha glucan synthases are the glucosyltransferase enzymes involved in alpha-glucan synthesis.

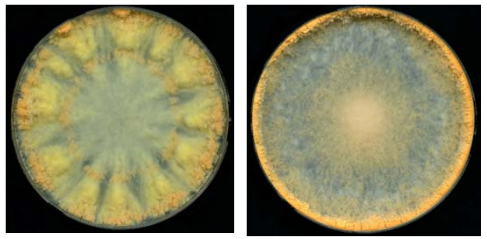
Growth rate



Cell Wall Structure

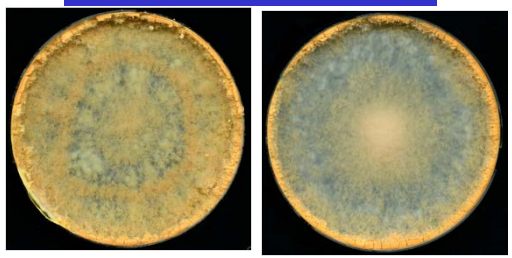


ags -1 Gene(NCU08132) knock out mutant



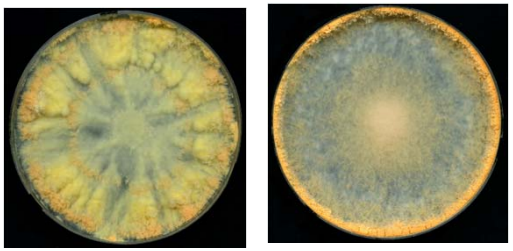
***ags-1* mutant (7days)** **Wild type (7days)**

ags-2 Gene (NCU02478) knock out mutant



***ags-2* mutant (7days)** **Wild type (7days)**

Double knock out mutant



Double mutant (7days) **Wild type (7days)**

Wild type vs Double mutant

Wild type (7days) **Double mutant (7days)**

Cell wall integrity test

1. Salt test(growth in 10% NaCl)

2. Cell wall stress test (growth in 0.01% SDS)

3.Hyper-osmotic stress test (growth in 2M glycerol)

4. Chitin inhibitor test (10mg/ml Calcoflour white)

5. Glucan inhibitor test (10µg/ml Caspofungin)

Mutants were grown in 2% Vogel's sucrose containing the above concentrations of cell wall stress reagents and their growth was observed over 72 hours and compared to wild type.

Result: Both *ags-1* and *ags-2* mutants did not show cell wall sensitivity to any of those cell wall stress reagents.

Discussion and Conclusions

The *ags-1* mutant had a more severe phenotype than *ags-2*, which had a mild mutant phenotype. Double knock out mutants show a similar mutant phenotype to the *ags-1* mutant in terms of morphology and growth rate. We concluded that *ags-1* mutant plays more critical role in determining cell wall morphology. Although the *ags-1* mutant shows slower growth rate and different morphology compared to wild type, deletion of these genes ate not lethal in *Neurospora crassa*.

Future Directions

1. co-segregation experiment; We will mate *ags-1* mutant with a wild type and collect ascospores. These will be inoculated onto 2% Vogel's sucrose slants and transferred to hygromycin tubes to verify that the phenotype co-segregates with hygromycin resistance.

2. Isolation of beta-(1-3)- glucan synthase; We found out (using PCR) that the beta-(1-3)-glucan synthase was not knocked out in the Neurospora knock out library. We will try to isolate beta-(1-3)-glucan synthase mutants.

3.Triple mutant; Once we are able to isolate beta-(1-3)-glucan synthase knock out mutant, we will mate it with an *ags-1*/*ags-2* double mutant.