Characterizing Genetic Elements on Conidiation in *Neurospora crassa*

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Introduction

Fungal diseases have adverse effects on immunocompromised individuals, and the ability to treat them effectively is becoming difficult due to the efficacy of antifungal drugs available. It is known that conidia, or asexual spores, is the primary means of spreading plant/human diseases. Conidiation is a multi-gene trait. Although many genes are known to be involved in the quantitative production of conidia, it is not well understood on the mechanism of quantitative production of conidia. For identifying and characterizing genes playing a major role in conidiation, we have performed a QTL analysis on a mapping population (N6) produced by crossing two ecotypes of the model fungal species *Neurospora crassa*, FGSC2223 and FGSC4825. We identified three major QTLs and several minor QTLs for conidiation trait. To clone a major QTL gene in Chromosome I, we have performed two backcrosses and narrow down the QTL region into 322,000 base pairs. We are in the process of characterizing the list of candidate genes in the region. The successful outcome of the project will provide an understanding of the genetic mechanisms of the quantitative variation of the number of conidia, and also provide useful insight into better targeted therapies for controlling the spread of fungal disease.

Methodology

Parent strain 2223 was crossed with Parent strain 4825 in order to generate F1 population N6.

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QTL Analysis was performed. QTL Analysis identifies specific regions on chromosomes that correspond with number of conidia.

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The QTL region was narrowed down by backcrosses to 322,000 base pairs on chromosome I.

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Several candidate genes where narrowed down using FungiDB. FGSC knock-out strains were found for genes within the QTL region.

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Conidia counting assay was carried out in the knock-out strains.

Results

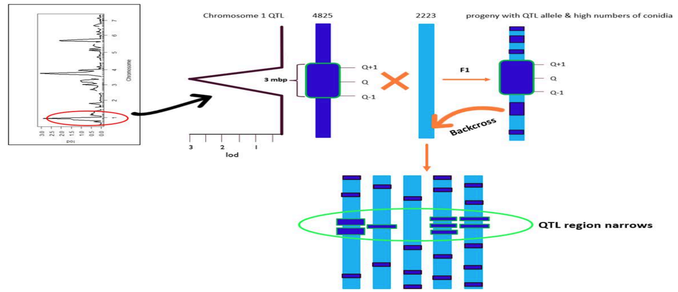

Figure 1: QTL analysis and cloning QTL genes for conidiation

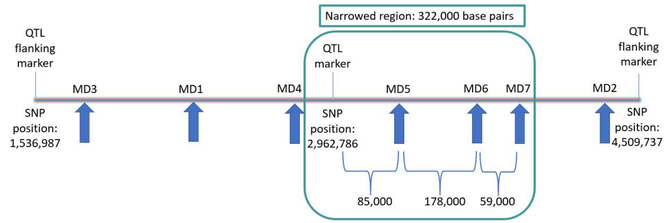

Figure 2: Region narrowed down on chromosome I

Chromosome I

Gene ID	Transcript ID	Genomic Location (Gene)	Product Description
NCU02630	NCU02630-t26_1	CM002236:2,990,148..2,993,018(-)	heat shock protein 78
NCU02636	NCU02636-t26_1	CM002236:3,008,027..3,009,803(-)	ubiquitin conjugating enzyme
NCU02666	NCU02666-t26_1	CM002236:3,125,823..3,129,123(-)	zinc finger transcription factor-58
NCU02713	NCU02713-t26_1	CM002236:3,284,468..3,286,740(-)	conidial separation-1

Chromosome IV

Gene ID	Transcript ID	Genomic Location (Gene)	Product Description
NCU04866	NCU04866-t26_1	CM002239:536,988..539,552(-)	All development-altered regulator ADA-6

Figure 3: Potential candidate genes

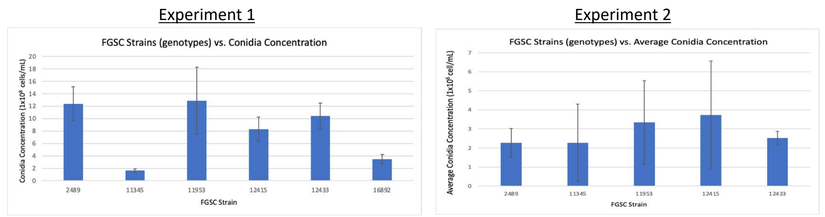

Figure 4: Preliminary data on candidate genes.

Conclusion

- As hypothesized, conidiation is a multigene trait because various major and minor QTLs were identified.
- As predicted, the number of conidia vary compared to the wildtype strain (2489) if the gene is a causative gene. This is seen in experiment 1 where strain 11345 shows a significant variation from the wildtype.
- The conidia counting assay will be repeated because there is variation and inconsistencies in the data.
- The variation and inconsistencies seen could be due to inaccurate conidia count of the original suspension, which was done manually using a hemocytometer. There could have been partial evaporation of this suspension due to it being left out overnight or improper mixing could also have contributed to the variation after the suspension was placed into the chamber.

Discussion and on-going experiment

- The conidia counting assay will be improved and repeated in order to obtain reliable phenotypic data.
- The knockout strains of the few candidate genes shows the 'necessity' of the genes in the quantitative expression of the conidia.
- In the future, a transgenic strain test will be carried out to determine the 'sufficiency' of the potential candidate gene. To determine the sufficiency, the gene is put back into the organism to see if it directly affects conidia production.

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References

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