Phenotypic Characterization of Neurospora crassa fsd-1 overexpression strains

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Introduction

Neurospora crassa is a filamentous fungus that is often used as a model organism, as it can reproduce either asexually or sexually.

• Little is known about the mechanisms that control signaling during sexual development.

• The transcription factor FSD-1 regulates female sexual development, and fsd-1 deletion strains do not develop mature female sexual development structures (Hutchison and Glass, 2010).

Methodology

RNA extraction:

• Samples of both wild type and fsd-1 overexpression strains will be lysed and mixed with TRIzol reagent to promote the extraction of RNA.

• After washing with ethanol and centrifugation according to the (Hutchison and Glass, 2010) protocol, we will have a mix of nucleic acids (mostly RNA) attached to the column which we can then elute.

• The sample will then be treated with DNase, to ensure we have a pure RNA sample.

• RNA quality and concentration will be assessed using the Nanodrop and gel electrophoresis.

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

• Using 2 µg of the RNA that was extracted, we converted the mRNA into cDNA with reverse transcriptase. A Stratagene QPCR mix, which fluoresces when bound to DNA, was added to the cDNA. This allows us to quantify the amount of amplification for each PCR cycle.

• The amplification data was analyzed using the 2−ΔΔCT method for calculating gene expression changes (Livak, 2001).

Crossing fsd-1 mutants

• Female strains are plated on minimal media + histidine and allowed to develop for 7-10 days. After protoperithecia have formed, male mycelia are inoculated onto the plates and grown 7-10 days to cross.

• If the two strains crossed, then dark spores will form

• These spores can be germinated to analyze the fecundity of the mutant offspring

Analyzing the fecundity of mutant offspring

• Spores picked from crosses were heat shocked at 60°C water bath and plated on minimal media + histidine, with hygromycin added as a selective agent in some cases.

• Next, spores were picked from these plates and allowed to germinate on BDES and histidine. Hygromycin was added as a selective agent to some of the crosses. The number of germinated and ungerminated spores were counted and recorded.

• Overexpression strains have a hygromycin resistance marker, so any spores with an fsd-1 overexpression parent will be able to grow on a medium with hygromycin.

Conclusions and next steps

Conclusions:

• Crosses with a deletion of fsd-1 in the female strain do not produce spores; however, if the fsd-1 deletion is in the male strain, spores are produced.

• FSD-1 is confirmed to be overexpressed in the overexpression strains.

• The overexpression strains are able to cross with other strains, but their offspring is infertile, meaning that their spores do not germinate.

Future Steps:

• The next step in this experiment is to use RNA sequencing to find genes that are being abnormally expressed in the fsd-1 overexpression strains, and use it to find possible downstream targets of FSD-1.

• We also want to understand why the overexpression spores are not germinating after being heat shocked. Are they spontaneously germinating and dying, or not germinating at all? We can also assess fsd-1 expression in germinating ascospores (via microscopy and/or qRT-PCR)

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