The Effect of an Acute Nicotine Exposure on *ACR-16* Gene of *C. elegans.*

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Introduction

Tobacco use is regarded as a worldwide cause of preventable death. Nicotine is one of the psychoactive components found in tobacco and its long-term exposure induces brain changes resulting in addiction. Nicotine stimulates the release of neurotransmitters endorphin and dopamine in reward circuits in human brain. Increased dopamine release is responsible for compulsive drug taking and seeking behaviors found in addiction (Picciotto and Mineur, 2014). Nicotine's action in human body is also due to its binding of receptors called nicotinic acetylcholine receptors (nAChRs) which are required for dependence behavior in humans (Liu and Su, 2018). These receptors, when bound by ligand transduce signals to the central and peripheral nervous systems, as they are expressed in many tissues as muscles, nerves, and sensory cells among others (Rose, 2007). Every nAChR is formed of five subunits: alpha, beta, gamma, delta, and epsilon. The alpha subunit has ten subtypes (alpha 1: alpha 10), and beta subunit has four types (beta 1: beta 4). Every subtype is encoded by a distinct gene (Benowitz, 2009). Due to the addictive nature of nicotine studies on other organisms can aid in ethically investigating nicotine's effects. One model organism that is used extensively in neural studies is the soil nematode, *C. elegans*. The *ACR-16* subunit group genes in *C. elegans* is similar to the mammalian alpha 7 subunit group of nAChR genes. This makes it an exceptional model to study the effect of nicotine on gene expression. Additionally, exposure effect of nicotine on such nematodes mirrors the exposure effect on vertebrates (Feng et al., 2006).

Hypothesis

This study aims to investigate changes in expression of the subunit *ACR- 16* gene in response to nicotine exposure. It is expected that nematodes exposed to nicotine will have an increase in alternative splicing of the RNAs encoded by the *ACR-16* gene to produce increased numbers of functional nicotine receptors compared to the control.

Purpose

Exposing the *C. elegans* nematode to nicotine and detect the genome changes.

Methods

Inoculate *C. elegans* nematodes onto growth agar plates (NGM plates) previously treated by OP50 *E. coli*, to increase nematode population.

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One population of nematodes is treated with nicotine (62.3 mM) while the other population is considered control and is not exposed to nicotine.

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Isolation of *C. elegans* RNA (Ly protocol) for both experiment & control nematodes, then use reverse transcription to produce cDNA by Maxima H Minus RT'ase.

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Use the primer that was designed earlier to define the gene of interest (*ACR-16*) which is amplified using PCR technique

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Prepare gradient PCR on cDNA using Fusion Master Mix (hot to cold). Run agarose gel electrophoresis to get the DNA fragment sizes.

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Determined occurrence of alternative splicing events and search the domain to estimate the changes of the protein function.

Results

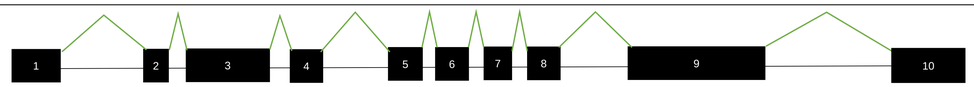

Figure 1: *ACR-16* Gene Structure. The black boxes represent the exons and they are classified according to their sizes, which is exon. The black horizontal lines represent introns. Sizes are relative. The green angled lines that join the edges of the exons represent the constitutive splicing pattern. These features are adapted from information acquired from Ensembl Metazoa (*Kinsella et al., 2018).*

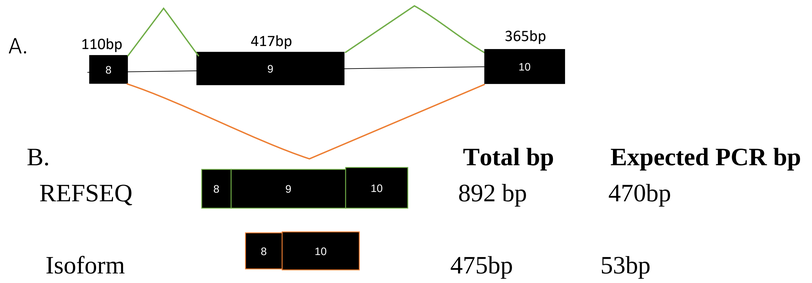

Figure 2: Transcript comparison of Expected RT-PCR sizes of *ACR-16*. Figure A. The region of interest of *ACR-16* gene; which are exons 8,9, and 10. The green angled lines show the constitutive splicing pattern and the orange angled line shows the alternative splicing pattern. Figure B. Transcript REFSEQ results from the constitutive splicing of the exons with the total length of the exons and the expected RT-PCR sizes for the RNA shown in base pairs (bp) at right. Transcript Isoform 1 results from the alternative splicing in which exon 9 is skipped. Total bp and bp expected by RT-PCR are shown at right. Primers to detect both transcripts were designed using primer 3 plus (Untergasser et al., 2007).

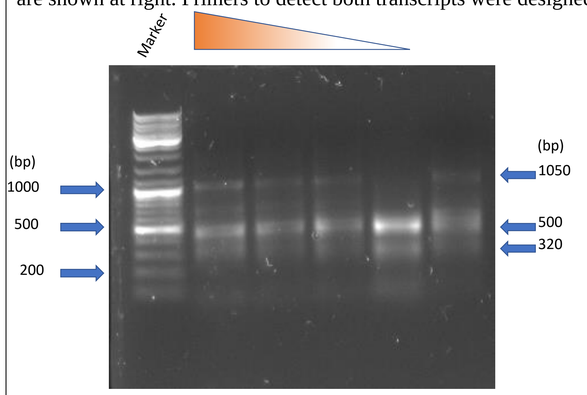

Figure 3: Agarose Gel Electrophoresis Results of Gradient RT-PCR of *ACR-16*. Products of reverse transcription polymerase chain reaction for ACR-16 at different annealing temperatures were separated using agarose gel electrophoresis. The triangle shape above the picture represents the annealing temperature (orange-hot to white-cold). The numbers on the left are the marker sizes in bp. (OGene Ruler; ThermoFisher) The estimated DNA fragment sizes are shown as horizontal bands (Kinsella et al., 2018).

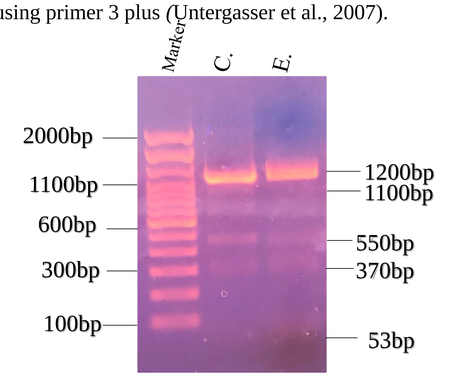

Figure 4: Control vs Experimental Alternative Splicing Gel. RT-PCR products were separated using agarose gel electrophoresis after annealing at 57 °C. Marker Sizes (Tracklt 100 bp DNA Lader; ThermoFisher) are noted at left while estimates of observed sizes are noted at right.

Figure 6: Protein Domain of REFSEQ. The protein family domain are showing as a rectangular colored shapes. The main domain is showing in orange and it is the neurotransmitter-gated-ion channel that is has the nicotinic acetylcholine receptors. Information used to generate this image was obtained using InterPro (Mitchell et al.,2019).

Figure 5: Multiple Alignment Translation Comparison. Translated Isoform RNA and REFSEQ RNA in silico to protein sequences, then aligned and compare REFSEQ protein product to Isoform protein product. Information used to generate this image was from ExPASy (Gesteiger et al., 2004).

Conclusions

- The preferred annealing temperature for the primer set is 57 degree Celsius.
- There is an alternative splicing of 2 fragments of both experimental and control that are the same with length of 550 bp and 1200 bp which is something new and 370 bp which is the expected PCR of REFSEQ.
- There is a difference in splicing between control and experiment which is control fragment around 53bp which is the isoform expected PCR size.
- Greater number of alternative spliced RNA were found in the experimental treatment than the control treatment
- The nicotinic acetylcholine receptors proteins increased by exposing the nematodes to nicotine which is the functionally will increase, as they are responsible for the dependence behavior.

Future Direction

Further research is needed to determine:

- What will happen if the dosage of the nicotine increased.
- What are the new unexpected alternative splicing in in my results.
- what other proteins are effected by nicotine.
- What will happen if treatment time changed.

Literature Cited

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