Unraveling Mysteries: A Comprehensive Study of the Second Alternative Oxidase Gene in *Aspergillus niger*

Bence Veres

ikrek8@gmail.com

György Gimnázium Secondary Grammar School, Kisvárda, Hungary

1. INTRODUCTION

Terminal oxidation, the final step in aerobic respiration, occurs in the mitochondria or cell membrane, involving the electron transport chain (ETC) and resulting in ATP synthesis. The alternative oxidase (AOX) bypasses the ETC's proton gradient, accepting electrons without contributing to ATP production. AOX plays diverse roles, including stress management, cellular regulation, and redox balance, particularly in organisms like plants. In *Aspergillus niger*, AOX is crucial for citric acid production, and while literature mainly focuses on one *aox* gene, a less-discussed second copy (*aox2* gene) prompts research on its unclear biochemical function and necessity.

2. AIMS

This study seeks to address the limited information on the *aox2* gene in *A. niger*. We were curious whether through bioinformatics and molecular biological methods, we could better understand the gene, and provide an answer to what causes the potential loss of function of the gene. This knowledge could serve as a basis for the treatment of certain human disorders and diseases

3. METHODS AND MATERIALS

In our study, we investigated alternative oxidases in various *A. niger* strains. To analyze these genes, we utilized the NCBI database, employing the *A. niger* ATCC 1015 strain's protein sequence as a reference in a Blast search. After identifying 70 genome sequences, we conducted multiple alignments, followed by phylogenetic analysis using the BMGE program. Mutations were validated in our laboratory, with each mutation corresponding to a specific strain. Verification involved DNA isolation, PCR amplification, and gel electrophoresis. For sequencing, we ligated the gene into a plasmid and sent it to the Eurofins Scientific laboratory. Additionally, we assessed gene expression in the ATCC 1015 strain using RT-PCR, providing insights into the expression levels of the genes.

4. RESULTS AND DISCUSSION

In my investigation about alleles of the second alternative oxidase (*aox2*) in *A. niger* strains, the first crucial step to construct a gene-tree. This tree served to categorize strains into a wild-type group (I), and five mutated groups exhibiting various mutations affecting protein formation and function. The identified mutations included deletions (II), where specific gene sections were missing, transposon insertions (III), missense mutations (IV) altering start codons, and frameshift mutations (V) leading to reading-frame shifts. As I mentioned earlier we found a transposon which is a mobile genetic element that can change its position within a genome and altering it, which we found we named Anita2. The study concludes the examination of ATCC 1015 strain *aox2* gene expression wild type among citric acid overproducing strains. Through RT-PCR, we confirmed the expression of *aox* genes in this strain

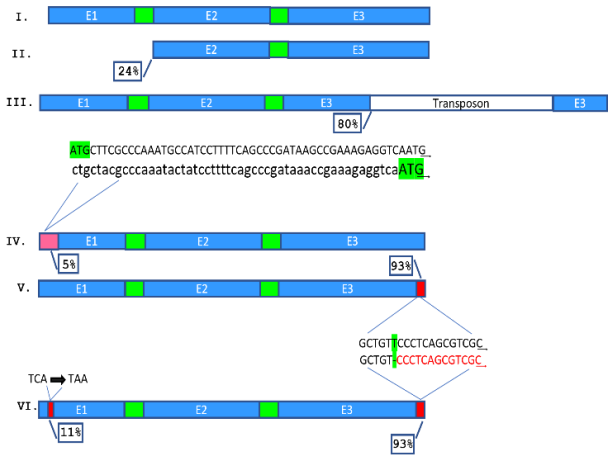

Figure 1 Schematic representation of detected aox2 mutations

5. CONCLUSIONS

AOX is vital for citric acid production in *Aspergillus niger*. While literature concentrates on one *aox* gene, a neglected second copy (*aox2*) was investigated bioinformatically in the current study. Six *aox2* alleles were found, with mutations in 70% of strains. Confirming *aox2* expression in citric acid-producing strains underscores its role in fermentation and strain development.

6. ACKNOWLEDGEMENT

I would like to express my gratitude to my teachers, Katalin Konczné, PhD and Gábor Koncz, PhD as well as to my mentors, Alexandra Márton and Vivien Bíró, and finally, to the Department of Bioengineering at the University of Debrecen for providing me with the opportunity. The research was supported by the István Gróf Tisza Foundation for the University of Debrecen.

7. REFERENCES

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