

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

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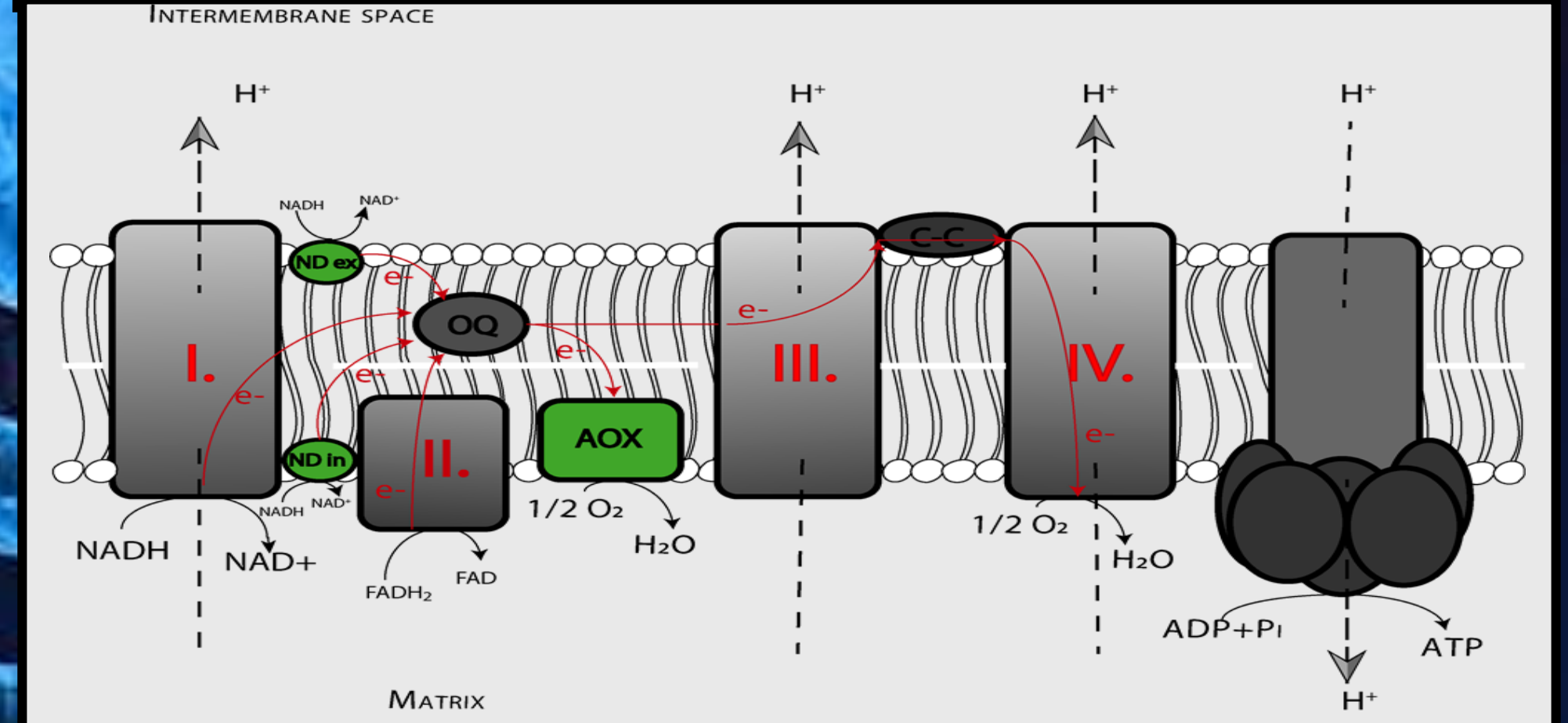


A The alternative oxidase (AOX) is an enzyme in eukaryotic cells that catalyzes an alternative respiratory pathway in the mitochondria. AOX is located on the inner membrane's matrix side. Unlike other respiratory complexes, it does not pump protons, leading to no ATP synthesis. AOX reduces oxygen to water, releasing energy as heat. AOX is absent in mammals and yeast but found in filamentous fungi.

O

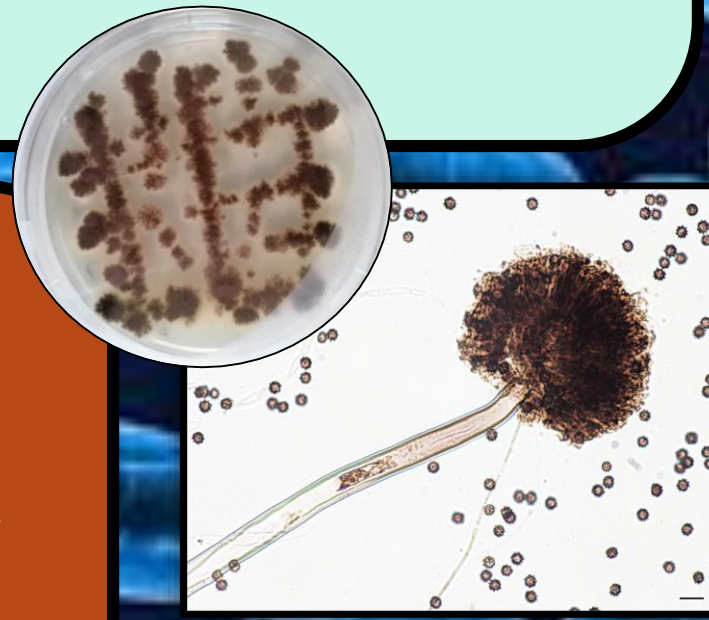
X

The electron transport chain and the AOX on the mitochondrial membrane



Aspergillus niger

Filamentous fungus known for its industrial uses, easily grows in diverse environments. It produces organic acids and enzymes. In citric acid fermentation, the fungus utilizes AOX to regenerate NADH without producing ATP, supporting glycolysis for increased organic acid production, and a defense mechanism against oxygen radicals (aeration).

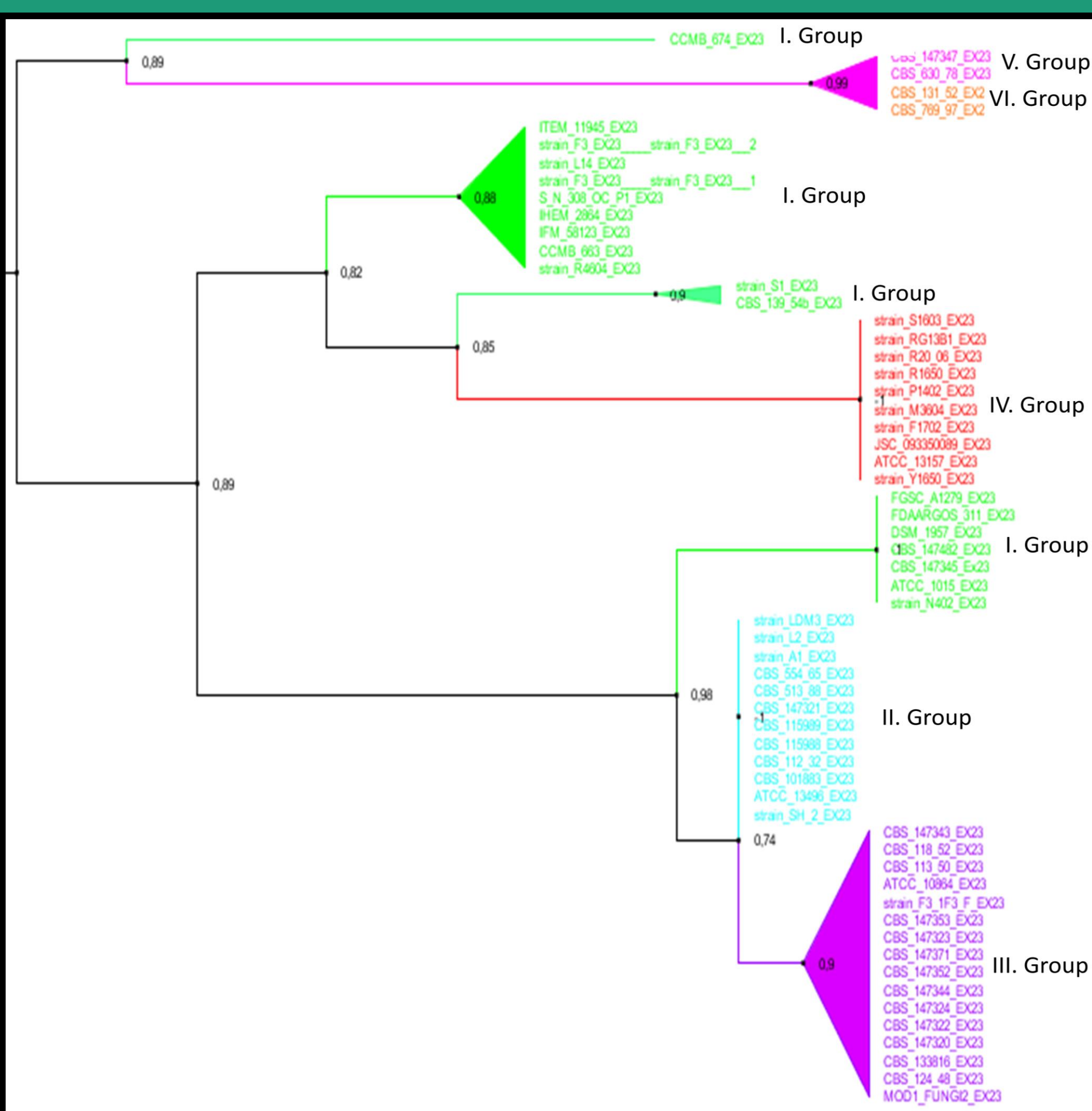


The importance of AOX research in human diseases

- Mitochondrial-origin myopathies (Cardiomyopathia)
- Oxidative stress (ageing, cancer)
- Antifungal resistance (AOX specific inhibitor) Aspergillosis
- Alzheimer's disease (reducing the production of β -amyloid)

- A** Understanding genes is crucial for technology development.
- I** Examination of aox gene alleles in different *A. niger* strains.
- M** Explanation for the lack of literature (only addressing *aox1*).
- S** Basis of treatment for human mitochondrial-origin diseases.

FILOGENETIC TREE



MUTATIONS

- I. Group - Wild-type
- II. Group - Deletion
- III. Group - Transposon insertion
- IV. Group - Missense
- V. Group - Frameshift
- VI. Group - Frameshift and nonsense

Materials and methods

Molecular biological methods

Gene expression in ATCC 1015

RNA isolation, cDNA synthesis
RT-PCR, gel electrophoresis
Sequencing

Mutation analysis

DNA isolation
PCR, gel electrophoresis
Blue-white screening

Bioinformatics

NCBI, MAFFT,
BMGE, PhyML,
FigTree

Gene expression in ATCC 1015

The gel image indicates two bands for cDNA. One aligns with gDNA, suggesting intron splicing hasn't occurred. The lower band represents cDNA, cut, re-amplified, and sequenced. Data were compared with the database sequence. The sequences match, indicating that the *aox2* gene is expressed.

