

Genetic Diversity in Hyper Glucose Oxidase Producing *Aspergillus niger* UAF Mutants by RAPD and SSR

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FAISALABAD



Contents

Genus *Aspergillus*

Aspergillus niger

Glucose oxidase (Gox)

Molecular markers

Objectives of the study

Progress

Planning ahead

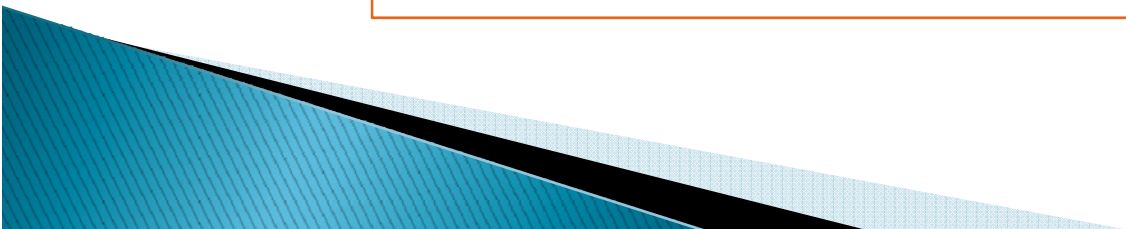
Aspergillus

Group of filamentous fungi

180 species

Causes diseases

Economically important



Aspergillus niger

1

- Haploid
- Filamentous fungi

2

- Carry out efficient post translational modification
- Produces enzymes, aflatoxins, mycotoxins, PGRs

Glucose Oxidase

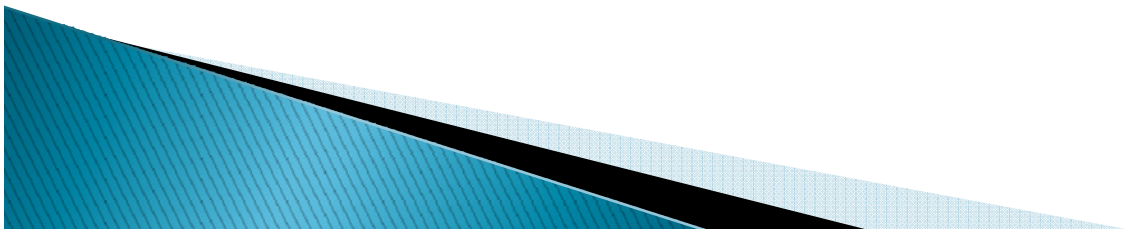
β -D Glucose : oxygen-oxidoreductase

Production of gluconic acid in food and beverage industries

Maintains food flavor and color stability

Quantification of glucose in biological fluids

Removal of glucose and oxygen.



Utility of DNA based markers

Brilliant tool

RAPD, SSR, RFLP &
transposons are mostly
used

DNA markers

Helps to understand
polymorphism in
mutants

Measures genetic
distances

objectives

Evaluation of genetic distance among 20 mutants and wild type strain of *A.niger* UAF

Estimating the level of *transcription* of GOx gene.

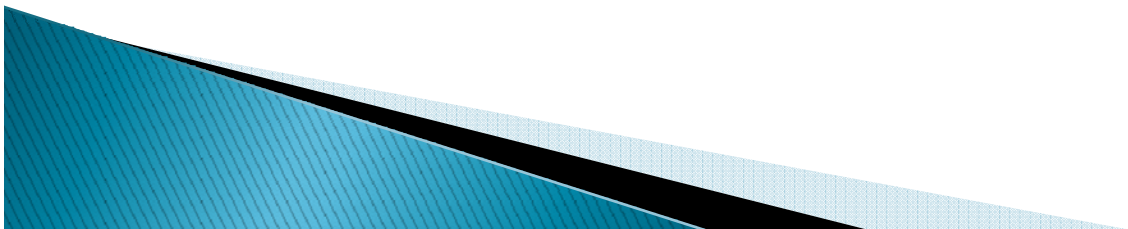
Exploitation of information generated in the studies for the stable and enhanced GOx production

Exploitation of results to prepare Glucose diagnostic kit.

Isolation and cloning of GOx gene with high transcription level in microbial species.

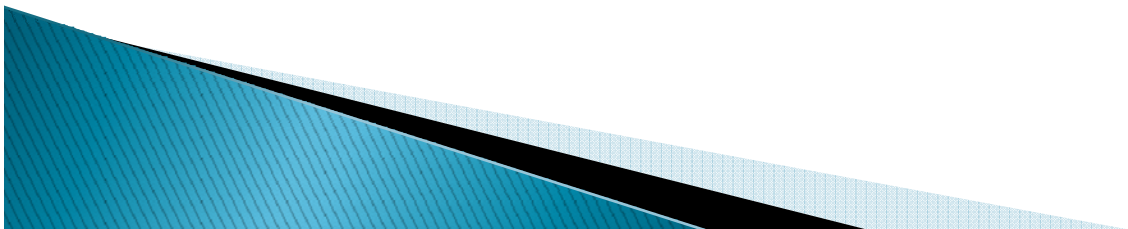
Progress

- ▶ 20 mutants and parent strain of *A. niger* have been obtained from Enzyme Biotechnology lab. Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad.



Methodology used

- ④ Growth of Mutants on Potato Dextrose Agar (PDA)
 - ④ a- Medium preparation and composition
 - ④ b- Streaking and culture conditions:
- ④ Production of Mutants on Vogel's Medium
 - ④ a- Medium preparation and composition
 - ④ b- Inoculation and culture conditions



Media composition

Potato Dextrose Agar

Chemical	Quantity (g/100ml)
Glucose	2g
Agar	2g
Starch	2g
Urea	3g
KH_2PO_4	0.008g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.05g
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.001g
KCl	0.015g

Vogel's medium

Chemical	Quantity (g/100ml)
Glucose	2g
Peptone	0.1g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.02g
NH_4NO_3	0.2g
$(\text{NH}_4)_2\text{SO}_4$	0.4g
Tri-sodium citrate	0.5g
Yeast extract	0.2g

Planning ahead

Optimization of protocol for extraction of DNA of *A. niger*

Molecular characterization of *A. niger* UAF mutants by RAPD and SSR

Optimization of protocol for extraction of total RNA and mRNA (Gox gene)

Development of cDNA from mRNA of Gox gene

Determining level of transcription of Gox gene



THANKS

