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Isolation and Characterization of Aconitate Hydratase from *Lipomyces starkeyi*

Malaya Misra

Abstract

The characterization of oleaginous yeast such as *Lipomyces starkeyi* was done by high through-put technology to produce novel and potent genes and to identify the homology percentage of one of the genes among different species. Aconitate hydratase of *L.starkeyi* is one of the genes that convert citrate to isocitrate in Tricarboxylic acid cycle and plays an important role in the iron regulatory protein (IRP) which supervises the uptake, transport and storage of cellular iron in *L.starkeyi*. cDNA library of Lipomyces starkeyi was constructed. The isolation and characterization of Lipomyces starkeyi was performed and successfully confirmed on agarose gel electrophoresis and the amplification of the desired gene was done by PCR. Bioinformatics tool such as BLAST and Chromas Lite were used in order to determine the presence of aconitase and in different species. Restriction mapping was done to find out the restriction enzymes that were not involved in cutting the sequence, were later used in cutting the DNA fragments of desired sample in different fragments. Inverse PCR was successfully used by designing the forward and reverse primers through computer software. Insights were provided in terms of screening and characterization of aconitase through robust techniques and software tools. This research contemplated potentially valuable information regarding the role and the steps of Inverse PCR techniques.

Key-Words: *Isolation, Lipomyces starkeyi,PCR, cDNA library, BLAST*

Introduction

Lipomyces starkeyi, an oleaginous micro-organism has a capability for the production of Aconitate hydratase and other novel and potent genes that are useful for the biotechnological purposes. Its usefulness helps the researchers to find out its significance in the field of Life Sciences. Genomic DNA library was constructed from *L. starkeyi* in Molecular Biology Laboratory at University of Greenwich, which already have a hold of cDNA library of *L. starkeyi*. Screening of *L. starkeyi* clone were done from cDNA library of *L. starkeyi* in which it contains many novel genes which are very much useful for biological purposes. Some of the important genes are Aconitate hydratase, Allantoin permease, Acetyl CoA synthase, Protein tyrosin phophatase, Kinesin, Ubiquitin specific protease2, etc. Aconitate hydratase, also known as Aconitase are normally classified in the form of two isomers Mitochondrial aconitase and Cytoplasmic aconitase, plays an important role in the metabolic pathway of oleaginous yeast in TCA cycle. Its function is to catalyses the isomerisation of stereo specific from citrate to isocitrate through cis-aconitic acid in a non – redox active process or TCA cycle.

*** Corresponding Author**
E.mail: malaya.misra@gmail.com

Aims and Objectives

Important aims and objectives of this experiment were:

- Screening and characterization of *L.starkeyi* through bioinformatics tool.
- To find out the homology percentage of aconitate hydratase among different species through BLAST and Chromas Lite software.
- Application of Inverse PCR to identify the flanking sequence of known fragment region of mitochondrial aconitate hydratase.

Development of filtration technique along with the centrifugation – sedimentation, followed by an iron exchange column is an important separation method to isolate M-aconitase and C-aconitase from different animal resources. Reinforcement of chromatographic techniques enhances the purification of two isoform of aconitase in-order to produce higher enzyme activity/quality. Purification of aconitase is done by over-expressing in *Escherichia coli* using pDNOR222 vector with **Hind III** and **Pst I** restriction sites, a promoter and finally ampicillin as the selection antibiotic.

Material and Methods

Isolation and characterization of *L.starkeyi* was performed and successfully confirmed on Agarose Gel Electrophoresis and the amplification of desired gene was done by PCR

Bioinformatics tool such as BLAST and Chromas Lite (Technelysium Pty) helped to characterize the desired sample to produce novel and potent genes. The sequence that was obtained from the DNA sample was found to be similar with the nucleotides of aconitate hydratase present in the database through BLAST (NCBI). Restriction mapping using Web-Cutter tool was used successfully in order to identify the restriction enzymes which are not responsible for cutting the sequences. These enzymes cut the DNA sequence into different fragments.

Inverse PCR plays an important role in this research in order to know the flanking region of known fragments of aconitase and also to know how big the gene is. Application of online software tool such as Primo Inverse 3.4 was implemented to do the primer design for Inverse PCR by selecting the forward and reverse primers with proper melting temperature (T_m).

Results and Discussion

Agarose gel Electrophoresis was used to estimate the size of DNA fragments with the help of an electric field which helps the negatively charge molecules (DNA molecules) to move towards the positive charge through an agarose matrix.

Sample purification has been done at both $O.D_{260nm}$ and $O.D_{280nm}$ in order to know the presence of DNA in the sample through UV spectrophotometer.

BLAST was done to find out the percentage of homologous of gene among different species. Through BLAST, Mitochondrial aconitase was easily identified from the sequence of desired sample and also identified the identical percentage within the database. At first the desired sequence was aligned with vector sequence (pDNOR222) with the help of **BLAST N**, followed by the identification of desired gene through BLAST X. Finally the desired gene (Mitochondrial aconitate hydratase) was identified through **BLAST X** which also helped to find out the gene of the remaining sequence highlighted in the bold letter with the help of BLAST X by comparing the plasmid base pairs with the PCR product base pairs.

Chromas Lite helped to rectify the presence of junk genes in the sequence by giving the correct nucleotides in the sample. Restriction mapping using Web-Cutter tool was done to identify the restriction enzymes which are not responsible for cutting the sequences.

Online software tool such as Primo Inverse 3.4 was implemented to do the primer design for Inverse PCR in order to know the flanking region of known fragments of aconitase

From the result, it was confirmed that DNA was found and the PCR products were checked for proper amplification by AGE.

Conclusion

Finally in the conclusion, utilization of robust techniques and computer software helps to characterize *Lipomyces starkeyi* successfully. Hopes this tool in the future would usher a new age for the biotechnological purposes.

Future Prospective:

This future direction of this research work is to:

- Find out the actual size and length of the gene encoding protein (aconitate hydratase) by using Inverse PCR techniques.
- Retrieve the exact location of Loci and the number of exons of aconitase present in *Lipomyces starkeyi*.
- Ascertain the role and the application of aconitase in *Lipomyces starkeyi*.

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