INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X
Impact Factor: 6.078
(Volume 7, Issue 4 - V7I4-1532)
Available online at: https://www.ijariit.com

Computational Identification of Promoter Regions in Fungal Genomes

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ABSTRACT

Transcription is the mechanism through which proteins are formed and it is done at the promoter regions. The structural property and the stability of DNA (Deoxyribonucleic Acid) are due to the promoters and these promoters are used to distinguish them from other genomic sequences. Genomic expression patterns were determined in the yeast S.cerevisiae in response to the environmental fluctuations. To measure these changes, DNA Microarrays were used. It is revealed that the yeast genome contains a TATA box. Those genes associated with the TATA box show response mainly to stress conditions.

Keywords: *Promoter Regions, Tata Box, Fungal Genome, Promoters Analysis, PPTASE, Saccharomyces Cerevisiae*

1. INTRODUCTION

For the investigation of transcriptional regulatory regions, reporter genes are widely used in eukaryotes like fungi(Ghim CM et.al., 2010). Promoters and other regulatory regions are fused with the reporter genes. Green fluorescent proteins (GFP), luciferase enzyme, β-Galactosidase (lacZ), etc are used as reporter genes (Nordeen SK et.al., 1991-2003).To study the structure and functionality of genes in the fungal genome green fluorescent protein (GFP), luciferase enzymes, etc are used (Fakhoury AM et.al., 2004). Aspergillus nidulans have Phosphopantetheinyl transferases (PPtase) which help in phosphopantetheinylation (covalent attachment of phosphopantetheine to a specific serine residue in specific protein) (Marquez Fernandez et.al., 2007).

At the promoters region, the TATA box (a DNA sequence that shows the genetic sequence, transcription starts from the TATA box) plays a significant role in transcription assemblance(Benoist et.al., 1981). by using TATA box function information, the following goals were to be achieved.

1- Identification of a TATA box sequence for promoters
2- Identification of TATA box containing or TATA-less genes in S.cereviciae
3- Identification of physiological mode of regulation in TATA containing or TATA-less genes

The description of promoters is necessary for the complete explanation of genomes in fungus and their regulatory networks.5' tag-based techniques, small RNA sequencing methods are used to locate and identify the promoter regions (Sandelin A et.al., 2007).

2. TATA BOX SEQUENCE

Approximately 13% of S.cereviciae genes are known as TATA-containing and less than 2% of genes are known as TATA-less. It was examined that the genes known as TATA-containing were sensitive to mutations with TATA-binding proteins (TBP).it was also concluded that both TATA-containing and TATA-less promoters bind with TATA-binding proteins and require it for their expression.

With the help of the TATA-binding protein method, 318 additional genes in the fungal genome were considered as TATA-containing and totally become 1071 approximately 19% of the genome. Other 80% were considered as TATA-less. Chromatin Immunoprecipitation (ChIP) analysis showed that both TATA-containing and TATA-less promoters have TATA-binding proteins (TBP) (Kuras et.al., 1999).

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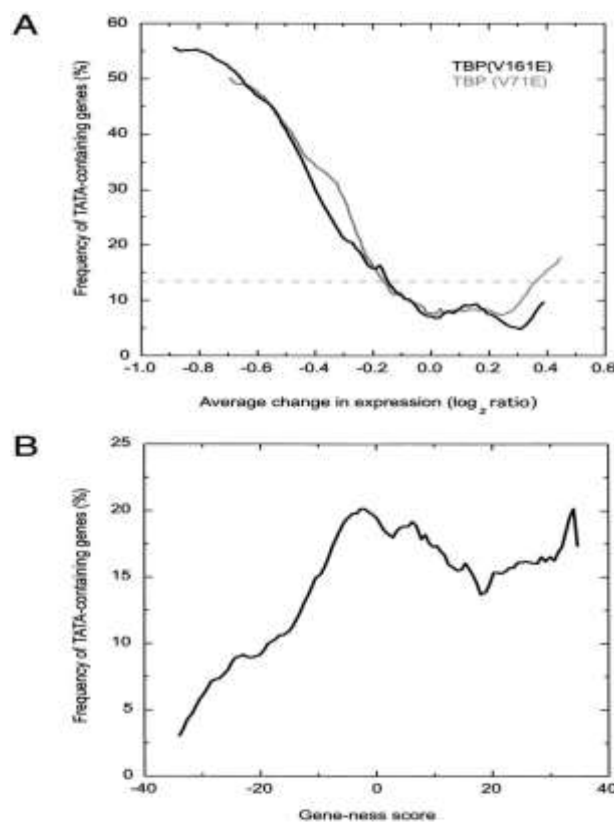

FIG 1. A) Frequency of TATA-containing genes
B) Presence of TATAbox sequence in -180 to -70 region of S.cerevisiae
C)

Whole Genome promoter in Sacchromyces Cerevisiae

The important model organism in the kingdom fungi is Saccharomyces cerevisiae and contains 72% of the entire sequence genome. S.cerevisiae has a 12Mb genome and contains 16 chromosomes. For both forward and reverse strands identification of promoters region has been done for complete genome I.e, for 16 chromosomes.

Relative to transcription start sites (TSS), -500 to +100 region promoters identifications were mapped. If the identified promoter region lies within the region of 500 upstream or region of 100 downstream of a given transcription start site (TSS), then it is considered a true positive.

Table 1. whole genonme promoter identification in S.cerevisiae

	Number of sequences	Transcript median length	TP	FP	TP promoters	Precision	Recall	F-score
ORF	4912	1548	5317	9072	3934	37.0	80.1	50.6
Cuts	501	428	540	242	404	69.1	80.6	74.4
Suts	729	964	727	704	552	50.8	75.7	60.8
Other	300	1272	296	447	223	39.8	74.3	51.9
All	6442	1436	6880	10465	5113	39.7	79.4	52.9
TATA and TATA-less promoters								
TATA	842	1384	978	1298	701	43.0	83.3	56.7
TATA-less	4070	1544	4206	7529	3139	35.8	77.1	48.9

Table 2. identification of promoters in 14 species of yeast using PromPredict algorithm

	Genome size in Mb	Genome GC %	Promoter sequences	Total predictions	TP predictions	TP genes	Recall
Yeast							
S. cerevisiae	12.5	38.4	6642	8113	6636	5386	81.1
S. bayanus	11.9	40.3	7216	8161	6415	5346	74.1
S. castellii	11.4	37	4655	5745	4528	3703	79.5
S. kluyveri	11	41.7	2932	2991	2433	2059	70.2
S. kudriazvevii	11.2	39.9	3736	4157	3431	2863	76.6
S. mikatae	10.8	38.2	3064	3773	3054	2465	80.5
S. paradoxus	11.9	38.6	7373	8578	6807	5608	76.1
C. albicans	14.5	33.7	5852	8628	6764	5063	86.5
C. dubliniensis	14.6	33.2	5933	8988	7114	5255	88.6
C. glabrata	12.1	38.5	5149	6913	5913	4575	88.9
C. lusitaniae	12.1	44.5	5797	5621	4867	4051	69.9
C. tropicalis	15.3	33.5	6119	8855	6714	5112	83.5
D. hansenii	11.5	35.4	6102	9100	7459	5592	91.6
L. elongisporus	15.5	37	5657	8337	6933	5076	89.7

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PromPredict Algoritm

To differentiate the promoter and non-promoter regions, the PromPredict algorithm is used along with DNA duplex stability (ability of DNA strands to unzip, depending upon the hydrogen bonding and base pairs).PromPredict algorithm uses the Gibbs free energy of DNA.

$$Dn=E1(n) - E2(n)$$

In this equation, Dn is the difference between E1 and E2 and E1 and E2 are Gibbs free energy.

Analysing promoter deletion constructs

For analyzing the promoter deletion constructs, the strain of Aspergillus nidulans was used as a wild type. Also, a mutant strain WX17 was used (Chung YS et.al., 2003). Aspergillus nidulans were fed on both complete and minimal media. For mycelial cultures, a complete liquid medium was used.

EP3 Program

The Easy Promoter Prediction Program (EP3) contains a base-pairing property to define the promoter regions from the other regions. For a specific sequence of DNA, the Easy promoter Prediction Program counts the inverted base-pairing energy values (Yella et.al., 2015).

Easy Promoter Prediction Program depends upon two parameters;
1- Length of the window
2- Deviation of value from the average genome window size

Easy Promoter Prediction Program is available at the given website; http://bioinformatics.psb.ugent.be/webtools/ep3.

Use of PPTase Gene

Promoter regions analysis is used not only to find the regulatory regions in the fungal genome but also to produce a promoter analysis system by using modern reporters (Mander GJ et.al., 2006, Elliott CE et.al., 2004). For the determination of fungal promoters, a new system is required. So, the PPTase gene as a reporter was used for system analysis by using different promoters such as trpC and cryparin (crp). The trpC gene takes part in the synthesis of tryptophan, glutamine amidotransferase phosphoribosylanthranilate isomerase, and indole glycerol phosphate synthase enzymes in Aspergillus nidulans (Mullaney EJ et.al., 1985). Crp gene is involved in the coding of the cell-surface hydrophobin protein (Zhang L et.al., 1994).

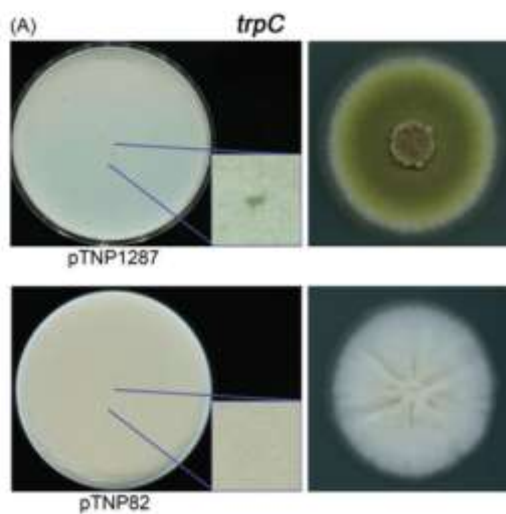

Fig 2. Colony morpholigical transformants of Aspergillus nidulans containing trpC gene by sing PPTase promoter assay

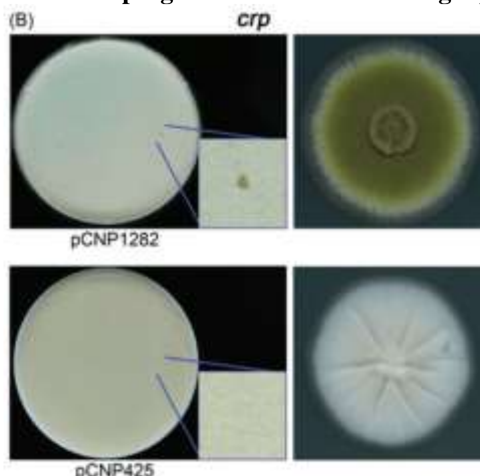

Fig 3. Colony morpholigical transformants of Aspergillus nidulans containing crp gene by sing PPTase promoter assay

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Promoter activity using PPTase

After the expression of the PPTase gene with trpC and crp promoter activity was checked. The number of restored green color mutants was counted. With the confirmation of PPTase activity in trpC and crp promoter-deleted constructs, regulatory regions were determined.

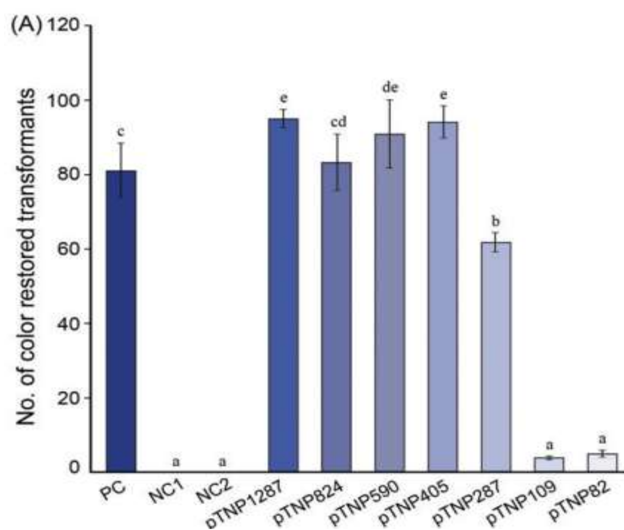

Fig 4. Promoter activity in trpC gene for PPTase

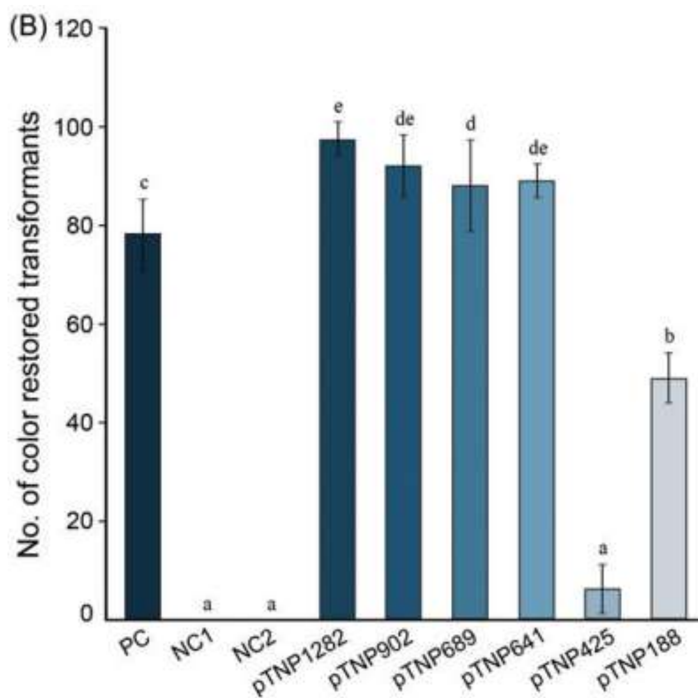

Fig 5. Promoter activity in crp genes for PPTase

3. DISCUSSION

For survival in the natural environment, organisms must be respond to changes. The study of genome analysis and identification of promoters revealed that fungi show response to the environmental changes by changing the genes expressions.Aspergillus species contains NRPSs/PKSs, so the mutant strain can be produced on PPTase in Aspergillus nidulans as reporter systems. For the prediction of whole genome in fungi, only coding regions were chosen.

4. CONCLUSIONS

It is concluded from the whole study that trancription is done at the promoter regions. There are some regions in the fungal genome that are TATA-containing regions and some are TATA-less.About 19-20% are TATA-containing and remaining are TATA-less. For the identification of promoter regions in the fungal genome several methods were adopted such as PromPrediction analysis, DNA Microarrays and Easy Promoter Prediction Program.

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