Middle-East Journal of Scientific Research 4 (3): 133-136, 2009
ISSN 1990-9233
© IDOSI Publications, 2009

Expression of Eukaryotic Gene in *E. coli*

Yesubabu and T. Elavazhagan

Department of Biotechnology,
Thanthai Hans Roever College, Perambalur-621 212, Tamilnadu, India

Abstract: Gene expression is the process by which inheritable information from a gene, such as the DNA sequence, is made into a functional gene product, such as protein or RNA. Several steps in the gene expression process may be modulated, including the transcription step and translation step and the post-translational modification of a protein. A wide range of microorganisms produce invertase and can, thus, utilize sucrose as a nutrient. Commercially, invertase is biosynthesized chiefly by yeast strains of *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis.* The bacteria can also be grown easily and its genetics are comparatively simple and easily-manipulated, making it one of the best-studied prokaryotic model organisms and an important species in biotechnology. *E. coli.* The objectives of the present study is Isolation and identification of yeast *(Saccharomyces cerevisiae)* from bakers Yeast. Separation of protein by SDS PAGE,Gene Cloning and Expression of Cloned Gene.

Key words: Gene expression · Manipulation · Isolation · Identification · Gene cloning

INTRODUCTION

Gene expression is the process by which inheritable information from a gene, such as the DNA sequence, is made into a functional gene product, such as protein or RNA. Several steps in the gene expression process may be modulated, including the transcription step and translation step and the post-translational modification of a protein [1]. In addition to these biological tools, certain naturally observed configurations of DNA (genes, promoters, enhancers, repressors) and the associated machinery itself are referred to as an expression system [2].

Yeasts are eukaryotic microorganisms classified in the kingdom Fungi, with about 1,500 species currently described they dominate fungal diversity in the oceans [3]. Most reproduce asexually by budding, although a few do so by binary fission. Yeasts are unicellular, although some species with yeast forms may become multicellular through the formation of a string of connected budding cells known as *pseudohyphae*, or *false hyphae* as seen in most molds Yeast size can vary greatly depending on the species, typically measuring 3-4µm in diameter, although some yeasts can reach over 40µm [4].

Fusions of bacterial genes to the *lacZ* gene of *Escherichia coli* have been extremely useful for determining the level of expression of gene products that otherwise [5]. would be difficult to detect because there are convenient methods for monitoring the activity of 3galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) [6,7]. In *Escherichia coli*, this property results in certain characteristic phenotypes that have been used to select mutations that have permitted the genetic dissection of the process of prokaryotic protein export [8,9]. In yeast, this feature of 83-galactosidase might limit its utility in the analysis of eukaryotic secretory transport (which includes protein translocation into the endoplasmic reticulum, delivery to the golgi complex, protein sorting, packaging into vesicles and final targeting to unique cellular destinations [10].

The present objectives of the study was to isolate and identify the yeast (*Saccharomyces cerevisiae*) from bakers Yeast [11]. Plasmid DNA isolation by Yeast and to separate protein by SDS PAGE. Plasmid DNA undergo Amplification, cloning and expression of *E.coli* [12]. The objectives of the present study is Isolation and identification of yeast (Saccharomyces

Corresponding Author: T. Elavazhagan, Department of Biotechnology,
Thanthai Hans Roever College, Perambalur-621 212, Tamilnadu, India

cerevisiae) from bakers Yeast.Separation of protein by SDS PAGE,Gene Cloning and Expression of Cloned Gene.

MATERIALS AND METHODS

Sample Collection: Isolated the *Escherichia coli* from soil samples of Perambalur. Bakers yeast (*Saccharomyces cerevisiae*) were obtained from Bakery and the cultures were maintained at low temperature (i.e., nearly 4°C) by periodic transfer on YEPD agar plate.

Media Preparation: 100 ml of YEPD (Yeast Extract Peptone Dextrose) medium was prepared for the growth of yeast. 100 ml of Nutrient Agar Medium was prepared for the growth of *E. coli.* All the media were sterilized in an autoclave at 15lb pressure for 20 minutes.

Simple Staining: A heat fixed smear of the desired culture was prepared. Then the smear was flooded with Methylene blue. The dye was allowed to remain for approximately 1 min. Excess stain was washed off. The slide was observed under microscope. The shape, arrangement and size were examined.

Optimization of Physical Parameters for the Enhanced Cell Growth: The various parameters selected for this study are temperature, pH and the incubation time. All experiments were conducted in duplicates and repeated twice. The results presented in the results section are mean of the obtained values.

Isolation and Separation: Isolation of protein from yeast, separation of protein by SDS-PAGE was done.

Preparation of 10% APS: This is the initiator and should be added immediately before pouring-old APS may appear to work but the quality of the cross-linking was reported to be compromised. TEMED speeds up the rate of polymerization but cannot start the polymerization. The rate of polymerization is also affected by temperature. Extraction of Plasmid DNA from yeast and to amplify DNA by RAPD was carried out in the experiment.

Transformation of Vector DNA to Competent *E. coli* Cells: Vector (0.1µg) was added to 20µl of competent cells in a sterile eppendorff tubes. The tubes were incubated on ice for 30 min. These cells were given heat shock by transferring tubes to a 42°C water bath and treated for 40 seconds. LB medium (1 ml) was added to the tubes and incubated at 37°C in an incubator shaker at 200 rpm.

RESULTS

SDS-PAGE: Separation of enzyme invertase by SDS PAGE. Bands is appeared (Fig. 1).

Plasmid DNA Isolation: After run the agarose gel electrophoresis this type of band observed (Fig. 2).

PCR: After run the agarose gel electrophoresis this type of band observed (Fig. 3).

5' GGG GAA TTC AAG CTT GAA AGA TGC CG 3'
3' CGA CCA GCT TTA AGC ATT CAG CTG GGG 5’

Fig. 1: SDS-PAGE

Fig. 2: Plasmid DNA

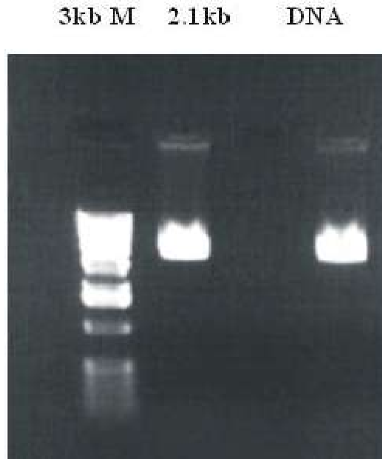

Fig. 3: PCR product

Fig. 4: Streak plate

Fig. 5: Gene expression
A: Normal *E.coli* negative to Benedict's test.
B: After gene cloning *E.coli* shows positive to Benedict's test

Characterization of the Isolated *E. coli:* In the streak plate techniques are carried out in the experiment and colour fewer colonies were observed over the medium (Fig. 4).

Gene Expression: The transformed cells were cultured for 2-3 generations in order to check the stability of the transformation. And the cells were found to be stable (Fig. 5).

DISCUSSION

Post-transcriptional mechanisms contribute in many important ways to the overall control and regulation of gene expression and in doing so employ a veritable army of proteins that bind a wide range of targets in messenger RNA (mRNA). The full range of these RNA-protein interactions is only just beginning to emerge and much remains to be learned about the mechanisms underlying the rapidly increasing number of regulatory systems now being described [13]. Genes that are part of the same operon in prokaryotes, or have the same expression pattern in eukaryotes, are transcriptionally co-regulated. If genes are consistently co-regulated across distantly related organisms, the genes have closely associated functions [14]. It has been shown previously that such genes have a strong tendency to belong to the same protein complex in prokaryotes and we show by an analysis of the sequences and their expression in the yeast *Saccharomyces cerevisiae* and the worm *Caenorhabditis elegans* that this is also true for eukaryotes [15]. Our analysis reveals that the number of conserved co-regulated genes is small in eukaryotes, as has been shown previously in prokaryotes, indicating that there are extensive variations in the gene regulatory network across organisms [16].

Comparisons of the gene order in closely related genomes reveal a major role for inversions in the genome shuffling process. In contrast to prokaryotes, where the inversions are predominantly large, half of the inversions between *Saccharomyces cerevisiae* and *Candida albicans* appear to be small, often encompassing only a single gene. Overall the genome rearrangement rate appears higher in eukaryotes than in prokaryotes and the current genome data do not indicate that functional constraints on the co-expression of neighboring genes have a large role in conserving eukaryotic gene order. Nevertheless, qualitatively interesting examples

of conservation of gene order in eukaryotes can be observed [17]. *E. coli* is frequently used as a model organism in microbiology studies. Its generation time is very less compare to all microorganisms. Production of enzyme invertase from various parameters like temperature and pH. Effect of different carbon source and different nitrogen source. Production of the high yield by using cheap substrate potato peel. Then amplified the DNA by using PCR. Cloned the invertase producing genes and expression in *E.coli* cells.

ACKNOWLEDGEMENT

The authors are gratefully acknowledged to The Principal, Thanthai Hans Roever College, Perambalur, India and The Head, Department of Biotechnology, Thantahi Hans Roever College, Peramablur, Tamilnadu, India for their constant support during the study period

REFERENCES

1. Sarah, A., Teichmann and M. Madan Babu, 1 October 2002. MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK. Trends in Biotechnol., 20(10): 407-410.
2. Arya, R., A. Bhattacharya and K.S. Saini, 2008. Dictyostelium discoideum a promising expression system for the production of eukaryotic proteins. FASEB J., 22: 4055-4066.
3. Attila Remenyi, R. Hans Scholer andMatthias Wilmanns, 2004. Nature Structural and Molecular Biology, 11: 812-815.
4. Ballou, C.E., 1982. In Molecular Biology of the Yeast Saccharomyces: *Metabolism and Gene Expression*, eds. J.N. Strathern, E.W. Jones and J.R. Broach (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp: 335-360.
5. Doolittle, RF., D.F. Feng, K.L. Anderson and M.R. Alberro, 1990. Center for Molecular Genetics M-034, University of California, 92093 San Diego, La Jolla, California, USA. J. Molecular Evolution., pp: 553-565.
6. Bassford, P., T.J. Silhavy and J. Beckwith, 1979. Studies in *Escherichia coli* and more recently in yeast demonstrate that, 3-galactosidase is unable to be translocated through a membrane. Bacteriol., 139: 19-31.
7. Beckwith, J., 1981. Fusions of bacterial genes to the lacZ gene of *Escherichia coli* have been extremely useful for determining the level of expression of gene products. Cell*,* 23: 307-308.
8. Emr, SD., M. Hall and T.J. Silhavy, 1981. J. Cell Biolo., 86: 701-711.
9. Bierman, M., R. Logan, K.O. Brien, E.T. Seno, RN. Rao and B.E. Schoner, 2000. Lilly Research Laboratories. A Division of Eli Lilly and Company. Indianapolis. IN 46285-0424.
10. Silhavy, T.J., S.A. Benson and S.D. Emr, 1983. Isolation of yeast from the fertile soil Microbiol. Rev., 47: 313-344.
11. Thorner, J., 1981. In Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance, eds. J.N. Strathern., E.W. Jones and J.R. Broach (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp: 143-180.
12. Seleem, M.N., M. Ali, S.M. Boyle, B. Mukhopadhyay, S.G. Witonsky, G.G. Schurig and N. Sriranganathan, 2006. Establishment of a Gene Expression System in Ochrobactrum anthropi. Appl. Environ. Microbiol., 72: 6833-6836.
11. McCarthy, E.G. and Heike Kollmus, 1995. Department of Gene Expression, National Biotechnology Research Center (GBF), Mascheroder Weg 1, D-38124, Braunschweig, Germany. Trends in Biochemical Sci., 20(5): 191-197.
12. Gilson, P.R. and M.C. Fadden, 2002. Gene expression in eukaryotes Genetica, 115: 13-28.
13. Douglas, S., S. Zauner, M. Fraunholz, M. Beaton, S. Penny, L.T. Deng, X. Wu, M. Reith, T. Cavalier-Smith and U.G. Maier, 2001. Nature, 410: 1091-1096.
14. Sarah, A., Teichmann and M. Madan Babu. 1 October 2002. MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK. Trends in Biotechnol., 20(10): 407-410.
15. Martijn, A., Huynen, Berend Snel and Peer Bork. 2001. EMBL, Biocomputing, Meyerhofstrasse 1, 69117 Heidelberg, Germany. Trends in Genetics, 17(6): 304-306.
16. Peterson, J.A. and A.M. Myers, 1993. Nucleic Acids Res., 21: 5500-5508.
17. Silverman, S.J., M. Rose, D. Botstein and G.R. Fink, 1982. Molecular Cell Biolo., 2: 1212-1219.