Molecular Bio-imprinting of Biocatalysts

Israr Khan¹ and Muhammad Waheed Akhtar¹

Author 1:

Israr Khan

Affiliation:

School of Biological Sciences, University of the Punjab, 54590, Lahore, Pakistan

E-mail:

Khanisrar07@gmail.com. israrks@live.com

Phone number: 00923349301550.

Author 2: Muhammad Waheed Akhtar

Affiliation:

School of Biological Sciences, University of the Punjab, 54590, Lahore, Pakistan

E-mail: mwapu@brain.net.pk

Phone number :92-42-99230-960

Fax No : - 92-42-99230-980

Abstract

Energy conservation is the cry of the day. Attempts are made all over the world to occupy and use energy reserves. Increased industrialization and machanisation has led to the depletion of natural energy reserves. Its un avoidable to search for renewable sources of energy, may be not used now but can be used by the future generations. we are using the expertise of our ancestors. Thus exploiting the nature and newer techniques in this area would yield the best results. Bio-imprinting is one of those techniques whereby chemical modification of the E.coli culture medium is done in order to achieve highly expressed protein which can be stored in its highly active form in specific solvent.

Key Words: Biocatalyst, Bioimprinting, E. coli

Introduction

C.thermocellum bglA gene for beta-D-glucosidase.

LOCUS X60268 2021 bp
SOURCE Clostridium thermocellum ATCC 27405
CDS 361..1707

ORIGIN

```
gtcatcatatataaaaagcgccagattagctccattggttgcttagctggaacctcgccctt
ctctttaacctgcctaattccaccaaacattacccccatattatattaatattatatttg
atatataattctttaatttatataattctttaattatctcaatctattttgctattttatc
acattattcaatttaatacaaaaagggtcataagctcaccataatataccataatc
tttatgctttttcagggtattcattttttcgattaaaaataaatagtattttatggttcct
ctaggttataattatattattacactgtttgcaaataatatcttaaagggtgtggtaaac
atgtcaaagataactttcccaaaagatttcatatggggttctgcaacagcagcatatcag
attgaagggtgcatacaacgaagacggcaaagggtgaatctatatgggaccgtttttccac
acgccaggaaatatagcagacggacataccggcgatgttgcatgcgaccactatcatcgt
tatgaagaagatatcaaaataatgaaagaaatcggtattaaatcatacagggttttccatc
tcatggcccagaatctttcctgaaggaacaggtaaatataaatcaaaagggactggatttt
tacaaaagggtcacaaatctgcttctggaaaacggaattatgcctgcaatcactctttat
cactgggaccttccccaaaagcttcaggataaaggcggtggaaaaaccgggacaccacc
gattattttacagaatactctgaagtaatatataaaaatctcggagatatcgttccaata
tgggtttactcacaatgaaccgggtgttggtttctttgcttggccacttttttaggaattcat
gcccctgggataaaaagacctccgcacttcattggaagtctcgcacaatcttcttttgcc
cacggcaaggccgtgaaactgttttagagaaatgaatattgacgccc aaattggaatagct
ctcaattttatcttaccattatcccgcatccgaaaaagctgaggatattgaagcagcggaa
ttgtcattttctctggcggaagggtggtatctggatcctgtgctaaaaggccggtatcct
gaaaacgcattgaaactttataaaaaagaagggtattgagctttctttccctgaagatgac
ctgaaacttatcagtcagccaatagacttcatagcattcaacaattattcttcggaattt
ataaaaatgatccgtccagtgagtcagggtttttcacctgcaaactccatattagaaaag
ttcgaaaaaacagatatgggctggatcatatcctgaaggcttgatgatctgcttatg
ctccttgacagggtatttgaaaagccaaacattgttatcagcgaacggagccgccttc
aaagatgaaataggtagcaacggaaagatagaagacacaaagagaatccaatatcttaaa
gattatctgacccagggtcacagggaattcaggacgggtgtaaacttaaaagcatactac
ttgtggtcgcttttggaacaactttgaatgggcttacgggtacaacaagagattcggaatc
gttcacgtaaattttgatacgttggaaagaaaaataaaggatagcggctactggtacaaa
gaagtaatcaaaaacaacgggttttttaatcatacacagcaaacacggatacacatcataaa
acataatgctaattgggcattcacgtccgaatgccatttagcattatattttcactctata
actctaaaacaatatattttaccaatgttaatatgtcaaccctgtttttctcttaagc
tcaatccagccatcccggaatcacttaccagtccagatacaccaacatgaaagagcata
gaccacagcgaaagatacacaaactattttctccccacatatgttttaataaacatcc
attaaaataaccacccttcacatttttctggatagttcacat
```

Materials and Methods

The bglA gene was PCR amplified from Clostridium thermocellum ATCC 27405 strain. TA cloned in pTZ57RT vector and transformed in DH5-alpha. Blue/White screening done to know the positive colonies. White colonies picked up and colony PCR done to amplify the bglA gene. Plasmid isolated and amplified

for the presence of the gene. Positive colonies were replica spotted on L.B ampicillin.

The purified gene was cloned in pET28a vector and transformation done in BL21C(+). Positive colonies were replica spotted on L.B kanamycine. The spotted colonies picked up and cultured on L.B and M9NG medium and induced with IPTG and Lactose. Expression of the protein checked on 12% SDS-PAGE.

The protein was purified, lyophilized and stored in different Inorganic and organic solutions like Na-P buffer, PBS, isopropanol and Glycerol.

The theoretical PCR product with forward and reverse primers:

ctcc atgggtgtcaaagataaaccttcccaaagatttcatatggggttctgcaacagcagcatat IF 1-60
 cagattgaaggtgcatacaacgaagacggcgaaggtgaatctatatgggaccgtttttcc
 cacacgccaggaaatatagcagacggacataccggcgatgttgcacgaccactatcat
 cgttatgaagaagatatcaaaataatgaaagaaatcggtattaaatcatacagggttttcc
 atctcatggcccagaatcttttctgaaggaacaggtaaatataatcaaaagggaactggat
 ttttcaaaaagggtcacaaatctgcttctggaaaacggaattatgcctgcaatcactctt
 tatcactgggaccttccccaaaagcttcaggataaaggcggatggaaaaaccgggacacc
 accgattattttacagaatactctgaagtaatatataaaaatctcggagatatcggtcca
 atatgggtttactcacaatgaacccgggtgttgtttctttgcttggccacttttttaggaatt
 catgccccctgggataaaaagacctccgcacttcattggaagtctcgcacaatcttcttttg
 tcccacggcaaggccgtgaaactgttttagagaaatgaatattgacgcccaaattggaata
 gctctcaatttatcttaccattatcccgcacccgaaagctgaggatattgaagcagcg
 gaattgtcattttctctggcgggaaggtggtatctggatcctgtgctaaaaggccggat
 cctgaaaacgcattgaaactttataaaaagaagggtattgagctttctttccctgaagat
 gacctgaaacttatcagtcagccaatagacttcatagcattcaacaattattcttcggaa
 ttataaaaatgatccgtccagtgagtcagggtttttcacctgcaaactccatattagaa
 aagttcgaaaaaacagatatgggctggatcatatatcctgaaggcttgatgatctgctt
 atgctccttgacagggattatggaaagccaaacattgttatcagcgaaaacggagccgcc
 ttcaaagatgaaataggttagcaacggaaagatagaagacacaaagagaatccaatatctt
 aaagattatctgaccagggtcacagggcaattcaggacgggtgtaaacttaaaagcatac
 tacttgtggtcgcttttggacaactttgaatgggcttacgggtacaacaagagattcgga
 atcggttcacgtaaattttgatacggttgaaagaaaaataaaggatagcggctactggtac 1340
aaagaagtaatcaaaaacaacggttttttaatacacacagcaaacacggatacacatcat 1400
gagctcag

Results and discussions

Recent efforts have been directed towards molecular cloning, sequencing, mutagenesis and crystallography of the enzyme. The sources and properties of recombinant beta glucosidase, the classification schemes based on similarity at the structural and molecular level, elucidation of structure function relationship, directed evolution of existing enzyme toward enhanced thermostability, substrate range,

biosynthetic properties and application. (Khan and Akhtar., 2010). Protein engineering is the novel field which has wide applications from pharmaceuticals, industry, commercial, laundry and research. It may apply rational design or non rational design or both. Site directed mutagenesis is a classical approach involving the protein folding principles and as such different techniques involving multidisciplinary research and broad knowledge is required involving biocomputing of complex data obtained from various sequencing projects and prediction of the future protein structure either chemically or genetically modified. Non rational mutagenesis or directed evolution involves random mutations in the gene encoding protein or shuffling the genes encoding different domains producing a random set of numerous large libraries of mutant proteins, using advanced technology the desired protein can be selected but the exact structure or changes may remain unnoticed (Khan and Akhtar., 2010). Sodium dodecyl sulfate (SDS) is an anionic detergent which denatures proteins by wrapping around the polypeptide backbone and SDS binds to proteins fairly specifically in a mass ratio of 1.4:1. In so doing SDS confers a negative charge to the polypeptide in proportion to its length i.e the denatured polypeptides become rods of negative charge with equal charge or charge densities per unit length. In denaturing SDS-PAGE separations therefore, migration is determined not by intrinsic electrical charge of the polypeptide, but by molecular weight. 12% SDS-PAGE of both Lactose induced (fig. 3) and IPTG induced (fig. 4) showed an estimated protein of 51 Kda. Besides developments in molecular biology and biochemistry, biocomputing and methods for the determination of 3D structures, are indispensable tools in this field. Since the three-dimensional structure of the

protein determines its biological function, it becomes important to have available a high resolution 3D structure, especially when the protein engineer aims to redesigning the protein function. Often enzymes exposed to particular pH or ligands before liophylization and subsequent dissolution in organic solvents, prove to be able to retain specific properties, i.e affinity for the ligand, a property referred to as “memory”. Based on this observation, a strategy for the activation of proteins has been developed called “**Molecular Bio-imprinting**” and can be adapted to particular conditions where the reaction of interest has to take place. Thus Bio-imprinting of is demonstrated to be an easy, feasible and effective method for obtaining overexpressed and highly active enzymes in organic solvents.

Acknowledgement:

We are Very Much thankful to Prof. Dr Muhammad Akhtar, Director General, school of Biological sciences, University of the Punjab, Pakistan and Prof. Dr. Atta Ul rehman, ex chairman Higher Education Commission Pakistan (HEC) for their valuable contribution.

Literature cited:

1. Jarvis M. 2003. Cellulose stacks up. *Science*. 426:611 – 612.
2. Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS. 2002. Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol Mol Biol Rev*. 66:506–577.
3. Gardner KH, Blackwell J. 1974b. The hydrogen bonding in cellulose. *Biochim Biophys Acta* 343:232–237.
4. Spezio, M., D.B Wilson, and P.A Karplus. 1993. "Crystal structure of the catalytic domain of thermophilic endocellulase" *Biochem. J.*, 222:729-736.
5. Pommier J. C.; Goma G.; Fuentes J. L.; Rousset C.; Jokinen O. *Tappi* **1990**, 73, 197–202.
6. Wildman, K.H. and D. Kern., Dynamic personalities of Proteins. 2007. *Nature*/vol450/doi:10.1038/nature06522
7. Khan I, Shuaib M, Sikandar, Zia M A, and Akbar H. 2010. A Micropropagation, Molecular and Physiochemical Analysis of Canola Brassica napus L. From Seeds. Available from Nature Precedings <http://hdl.handle.net/10101/npre.2010.4781.2>
8. Khan I and Akhtar M W. 2011. Different Approaches For Protein Engineering In Industrial Biotechnology. *Nature Precedings* <http://hdl.handle.net/10101/npre.2011.5601.1>

Figures and Tables legends

Figure 1: Colonies on X-Gal plate screened by Colony PCR for *bglA*/pTZR and loaded on 0.8% Agarose gel. showing. M; VC 1Kb protein marker. Purified gene cleaned *bglA* 1.4 Kb (Lane 1). Positive colonies/white (Lane 4 and Lane 5). Negative colonies/blue (Lane 2 and Lane 3)

Figure 2: 12% SDS-gel of total cell protein of *E. coli* BL21(DE3) cells expressing β -glucosidase (pET28a-BGIA-1) after induction Lactose concentrations. M; vivantis protein marker. Induced samples and Uninduced samples.

Figure 1.

Figure 2.