Production of L-Ribose from L-arabinose by Co-expression of L-arabinose isomerase and D-lyxose isomerase in E.Coli.

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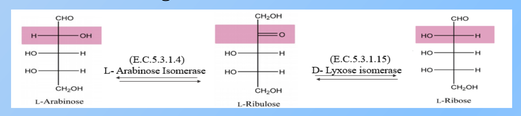
Introduction

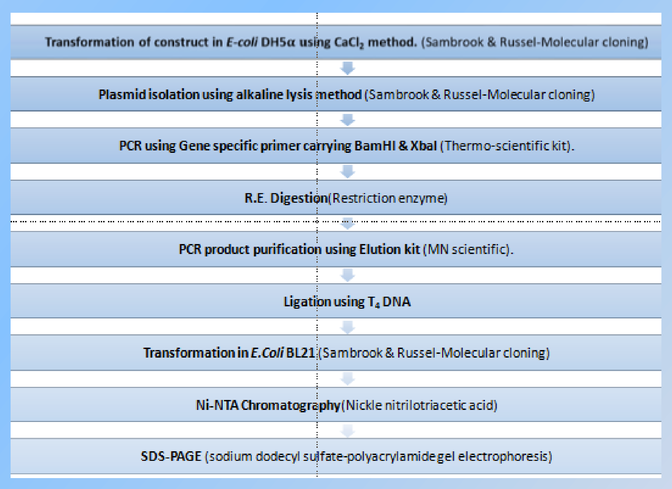
- L-Ribose is a rare and unnatural aldo pentose which is an important precursor for the synthesis of various L-nucleoside derivatives for anticancer and antiviral drugs (Gumina et al, 2001; Mathe and Gosselin, 2006).
- L-Ribulose is a precursor of L- ribose, to synthesize L- nucleoside to produce antiviral drugs. L-ribulose is rare pentose sugar that can be synthesized from abundantly available L-arabinose and found to be served as a variable starting molecule for conversion into L- ribose.
- L- Arabinose isomerase is a key enzyme in microbial pentose phosphate pathway. It is also regarded as potential biocatalyst for the production of rare sugars



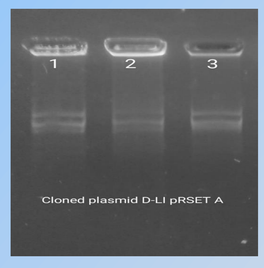
Material and Method

➢pRSET A+ D-LI and pET + L-AI both the Construct were obtained from Dr. Darshan Patel lab.

➢Mini BEST Agarose Gel DNA Extraction Kit Ver.4.0.by TaKaRa (Cat log no.:9762) -50rxn

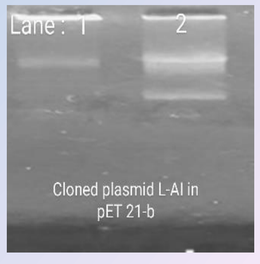


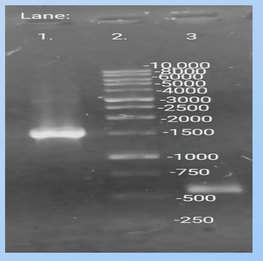
Results



1.Isolation of plasmid pRSET A containing
D-LI gene from E-coli DH5-α by Alkaline lysis:
➢plasmid bands were obtained by plasmid isolation method on 0.8% agarose gel

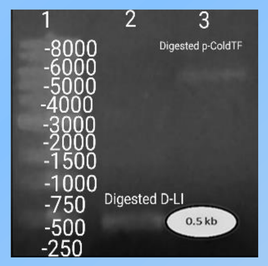
2.Isolation of plasmid pET-21b containing L-AI gene from the E-coli DH5-α
➢plasmid bands were obtained by plasmid isolation method on 0.8% agarose gel.

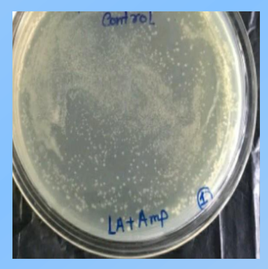




3. The gene specific primers for D-LI gene as well as L-AI gene used. PCR reaction was carried out with both the primer pair separately and the product was electrophoresed on 1% agarose gel.
➢The expected size amplicon, 568bp gene fragment of D-LI(lane:1) and 1519bp gene fragment of L-AI(lane:2) was amplified. Which further conform by comparing the size with 1Kb DNA ladder. Amplified product of D-LI and L-AI were purified from agarose gel by gel extraction Kit (TaKaRa) and used further for cloning in p-Cold vector.

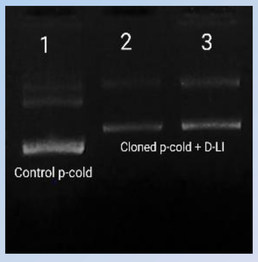
➢ 4.Restriction digestion of D-LI gene and p-Cold TF. Lane 1: 1 kb DNA ladder, lane 2: digested D-LI gene and 3: digested p-Cold TF These both the RE sites were present in out interested vector (p-Cold) multiple cloning cite. Hence, vector p-Cold was also digested enzymes (BamH1 and Xho1). After the restriction digestion both PCR product (D-LI) and vector (P-Cold) were purified and used for ligation



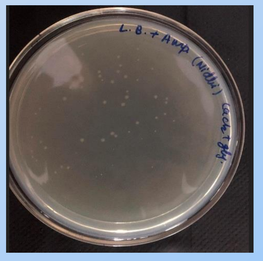


➢5.Transformation of ligated product in E.coli DH5-α
➢After transformation of ligated product in *E.coli* DH5-α isolated colony on the LB agar plate having Ampicillin was observed which confirms the successful transformation.

➢6.1:Plasmid isolation from transformed cells by Alkaline lysis:
➢For the further conformation plasmid was isolated from transformed colonies used for the confirmation. Isolated plasmids were loaded on agarose gel with non-recombinant p-Cold, which shows shifting of bands in plasmid isolated transformed colonies compared to non-recombinant p-Cold. This result gives the preliminary confirmation for cloning of D-LI in P-Cold.



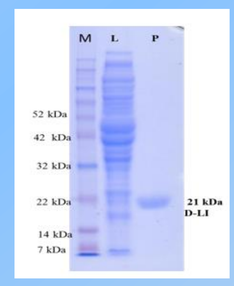
➢ 6.2.Clone conformation by PCR For the further conformation D-LI was amplified from the same plasmid .Through the gene specific primers which were earlier used for the amplification of the gene of interest.
➢ To Confirm the clone by comparing the size of the gene with DNA ladder.



7.Expression of p-Cold D-LI in *E.coli* BL-21: the presence of transformed cells as an isolated colony on the LB agar plate having Ampicillin. The plate shown here the insertion of the D-LI plasmid contained pCold TF vector was transformed in E.coli BL21
E.coli BL-21 strain is widely used non-T7 expression and is suitable for protein expression. It does not express the T7 RNA polymerase. It is deficient in some proteases

➢ Hence the plasmid p-Cold containing D-LI was further transformed in *E.coli* BL 21. After conformed transformed colonies ware preserved and used for the protein expression studies.
➢ Through sonication the crude of protein was prepared in the lysis buffer which is further use to purified the protein using Nickle NTA chromatography.

8.SDS–PAGE analysis D-LI. The recombinant D-LI was expressed in *E. coli* strain BL21 (DE3) and subjected to SDS–PAGE (10%). The gel was stained with Coomassie Brilliant Blue



Conclusion

➢D-LI gene containing pRSET vector was isolated and gene fragment was successfully amplified by polymerase chain reaction. After amplification, D-LI gene was ligated in pCold TF.
➢D-LI containing pCold TF ligated product were transformed into E.Coli DH5α. After, successful transformation, plasmid was isolated from transformed colony and clone was confirmed through Insert release by restriction digestion as well PCR amplification (568 bp).
➢Positive clone was transformed in E.coli BL21 for protein expression. Recombinant Protein was purified by nickel affinity chromatography and purity was checked by 10% SDS- PAGE gel electrophoresis. On SDS page single band of recombinant protein (21.0 kDa) was observed that confirmes expression and purity of D-LI.
➢L-arabinose isomerase gene of about 1519 bp was successfully amplified from pET21b-L-AI by gene specific PCR amplification. Which need to be further cloned p-Cold D-LI and needs to study the expression and stability of fusion protein (D-LI + L-AI) .

Future aspects

- PCR product of L-AI gene is already available which need to further ligation for the construct of fusion gene.
- These construct further need to analyze for fusion gene expression.

Reference

➢Wu, H., Huang, J., Deng, Y., Zhang, W. and Mu, W., 2019. Production of l-ribose from l-arabinose by co-expression of l-arabinose isomerase and d-lyxose isomerase in Escherichia coli. Enzyme and microbial technology, 132, p.109443
➢Patel M J ,Akhani R C, Patel AT, Dedania S R ,Patel DH(2017)A single and two step isomerization process for D-tagatose and L-ribose bio-production using L-arabinose isomerase and D-lyxoseisomer- ase. Enzyme Microbe Techno 97:27–33.