ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Study and Evaluation Of Pathway Process Of Polyol Producing Microorganisms And Their Enzymes through *In Silico* Approaches.

Romit Choudhary[1], Padmini Padmanabhan[1], and Koel Mukherjee[2]*.

[1]Bioprocess Engineering Lab, Bio-Engineering Department, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, 835215.
[2]Bioinformatics Lab, Bio-Engineering Department, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, 835215.

ABSTRACT

The constituent of agro-wastes such as starch and cellulose are a source of large number of polyols and other chemicals. Several polyols find application in the manufacture of personal care and cleaning products and hence are very economical. One such polyols is 1,3-Propanediol (1,3-PDO), is a valuable bifunctional molecule which is mainly produced from renewable resources using several bacteria, including *Citrobacter*, *Clostridium, Enterobacter, Klebsiella*and *Lactobacillus* species, via the fermentation of glycerol, however no natural microorganisms are capable of fermenting sugars directly to 1,3PDO. Now, in this currently planned work we intend to study on pathways involved in the desired production in above mentioned microorganisms. Modeling of their pathways of catalysis in 1,3-PDO production, characterization of enzymes involved in pathways and to integrate system biology approach to introduce some engineered enzymes whose enzyme activity will be more than the natural one. To achieve this idea computational platform will be applied. Engineering the available microbes can further aid in finding optimal and rational strategies in production part of 1,3-PDO. The results of this research will be used for fermentative production of 1, 3 PDO from agro waste for achieving higher yields than reported till date.

Keywords: Polyols, 1,3- PDO, KEGG pathway, protein modelling

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Millions of tons of agricultural wastes such as cornstalks, corncobs, straws and hulls which are made up largely of cellulose, lignin is produced annually. These are well segregated, readily collected, and suitable for many purposes as a raw material in case of wood [1]. Agro-wastes like wheat straw, rice straw, sugarcane bagasse can be used to produce valuable chemicals from this waste. Energy production by chemical process causes large environmental footprint such as depletion of fossil fuels, production of greenhouse gases, water consumption, toxic gas emissions etc. While biosynthesis is a greener and safer process where microbes digest their food (feedstock) and produce the desire products[2].

Several polyols find application in the manufacture of polytrimethylene terephthalate (PTT), polyurethane, cosmetics, personal care and cleaning products and hence are very economical and will help in generating large money. One such polyols is 1,3-Propanediol (1,3-PDO), is a valuable bifunctional molecule which is mainly produced from renewable resources using microorganisms. It has several promising properties for many synthetic reactions, particularly for the use in polymer and cosmetic industries. Microbial fermentation is a widely used technology for the bioconversion of renewable resources to chemicals. In nature1,3-PDO is produced by several bacteria, including *Citrobacter*, *Clostridium, Enterobacter, Klebsiella* and *Lactobacillus* species[3], via the fermentation of glycerol, however no natural microorganisms are capable of fermenting sugars directly to 1,3PDO. It involves a two-step process from glycerol to 1,3-PDO formation - the rearrangement of glycerol to 3-hydroxypropionaldehyde, followed by its NADH-dependent reduction to 1,3-propanediol, which is excreted from the cell[4]. Two important enzymes involved in the bioconversion of 1,3 PDO from Glycerol **Glycerol dehydratase (**GDHt)and **1, 3 Propanediol oxidoreductase (**PDOR)**.** GDHt, is a key and rate-limiting enzyme for the conversion of glycerol to 3-HPA. 3-HPA is further reduced to 1,3-PD by the NADH-linked PDOR [5]. PDOR, which directly hydrogenates 3-HPA to 1,3-PD.The PDOR is inhibited by 1,3-PDO in metabolic pathways, and hence it resulted in the accumulation of 3-HPA. The accumulation of 3-HPA can inhibit the activity of GDHt to prevent the growth of bacteria and result in reducing the production of 1,3-PDO, leading to a major influence in the production of 1,3-PDO [5].However, common problem associated with anaerobic growth is the generation of excess reducing equivalents in the form of NADH, whose reoxidation to NAD+ requires formation of a by-product that can serve as an electron sink [4].

Currently there are no microbes that can produce 1, 3-PDO in a massive scale. Therefore, industrial microbes are introduced with foreign genes that undergo the biosynthetic production pathways to achieve the desired phenotype. Engineering the available microbes can further aid in finding optimal and rational strategies in production part of 1,3-PDO[6]. Computational or *in silico* metabolic engineering [7], involves the modeling, optimization and simulation of related microorganisms to computationally obtain valuable knowledge on the metabolic system so that rational intervention strategies can be altered.

The present proposal aims at interfacing System Biology to Bioprocessing at laboratory scale and of **truly interdisciplinary nature**. It might lead to at a later date to industrial scale- up.

MATERIAL AND METHODS

Natural microorganisms slack the ability to directly use sugars to produce 1,3-propanediol (PDO). They always convertthe sugar to glycerol and then to 1,3 PDO in two steps.

Selection and Retrieval of target sequences:

The amino acid sequence of Glycerol dehydratase (ENZYME: 4.2.1.30) and 1,3 P.D Dehydrogenase from four different *Lactobacilli* species was retrieved in FASTA format. These two enzymes are the key component in conversion from Glycerol to 1, 3PDO so main focus was given on these enzymes.

Physiochemical and functional characterization:

Using the ProtParam tool of Expasy, molecular weight, theoretical isoelectric point (pI value), total number of negatively (Asp+Glu) and positively (Arg+Lys) charged residues, extinction coefficients [8], instability index [9] and aliphatic index [10] were computed. Secondary structural features were predicted with Self Optimized Prediction Method from Alignment (SOPMA) [11]. Multiple sequence alignment was also performed

by Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) for both the enzymes to check the matches and mismatches among the lactobacilli species with *Klebselia*.

Modeling of enzyme:

The structure of glycerol dehydratase and 1,3 PD Dehydrogenase of *lactobacillus reuteri* was unavailable in Protein Data Bank and thus it was modeled by Swiss Model (https://swissmodel.expasy.org/interactive). It is an online tool and follows the homology modeling algorithm and thus requires template to model the structure. The modeling tool gave a list of suitable templates [12] from which the best template was selected depending upon the percentage similarity and coverage area.

Network Modeling

String (https://string-db.org/) is a biological database and web resource to predict protein protein interaction. The resource also serves to highlight functional enrichments in user-provided lists of proteins, using many functional classification systems such as GO, Pfam and KEGG. Individual protein sequence from different organisms was given in the box from where direct and indirect interactions can be obtained [13].

Pathway study

KEGG PATHWAY (http://www.genome.jp/kegg/pathway.html) is a collection of manually drawn pathway maps representing our knowledge on the molecular interaction, reaction and relation networks [14]. The production of 1,3 PDO from Glycerol was studied through KEGG map and *Lactobacili* was the selected organism.

RESULTS AND DISCUSSION

Glycerol to 1,3 PDO production involved two enzymes namely glycerol dehydratase and 1,3 PD dehydogenase (oxidoreductase). Here, we plan to characterize the enzymes which are involved in two step procedures of conversion and then design a new route for the biosynthesis of PDO from glycerol in one step. This idea can overcome the limitations of the existing synthesis pathway related to time and cost.

From literature [4-5] it was clear that *Klebsiella pneumonia* is an established organism that can produce enough amount of 1,3 PDO from agrowastes through two step processes. So, here in this work *Klebsiella pneumonia*is our model organism, with emphasis on two enzymes glycerol dehydratase (BAO20817.1) and 1,3 PD dehydrogenase (PJH57787.1) to compare our result. Three species of Lactobacillus (Table 1) were identified and selected for the following work.

Table 1: List of organism name and concerned enzymes with their Accession Number

Sl.No.	Organism Name	Enzymes	Accession Number
1.	*Lactobacillus brevis*	Glycerol Dehydratase	KWT48525.1
		1,3-PD Dehydrogenase	WP_043022596.1
2.	*Lactobacillus plantrum*	Glycerol Dehydratase	WP_021338259.1
		1,3-PD Dehydrogenase	OUT05911.1
3.	*Lactobacillus reuteri*	Glycerol Dehydratase	KRK52068.1
		1,3-PD Dehydrogenase	OXE60023.1

Physiochemical properties of the target sequence were computed using ProtParam tool (Table 2). The computed isoelectric point (pI value), extinction coefficient, instability index, aliphatic index and Grand average of hydropathicity (GRAVY) value of the enzyme are shown below [15-16]. The extinction coefficient calculated gives us a measure of the amount of light absorbed by the protein at a particular wavelength (280nm). Instability index relies upon the occurrence of certain dipeptides along the length of the enzyme. Table 2a shows that sequence length and molecular weight value of *Lactobacillus reuteri* and *Klebsiella pneumoniae* were very much alike. Table 2b corresponds the result of 1,3-propanediol dehydogenase for all the four enzymes.

ISSN: 0975-8585

Table 2: (a) Details of Glycerol Dehydratase of selected Organisms by Protparam (b) Details of 1, 3-propanediol Dehydogenase of selected organisms by Protparam

Name of Organism	No. of Amino Acid	Mol. Wt.	Theoretical PI	-ve charged residue	+ve charged residue	Extinction Coefficient	Instability Index	Hydropathicity
Lactobacillus brevis	616	65950.21	4.86	80	55	16390	29.05	-0.017
Lactobacillus plantarum	378	40714.19	5.08	46	25	27390	28.37	0.006
Lactobacillus reuteri	558	62092.29	4.74	90	61	55280	35.46	0.393
Klebsiella pneumoniae	555	60659.78	4.68	77	51	39100	41.84	-0.124

Nameof Organism	No. of Amino Acid	Mol. Wt.	Theoretical PI	–ve charged residue	+ve charged residue	Extinction Coefficient	Instability Index	Hydropathicity
Lactobacillus brevis	390	42034.01	4.97	55	37	20650	27.70	-0.102
Lactobacillus plantarum	390	42159.16	5.38	47	33	19160	30.20	-0.083
Lactobacillus reuteri	390	42179.58	5.65	44	36	20900	29.28	-0.074
Klebsiella pneumoniae	387	41465.60	5.94	40	34	22140	26.36	0.051

Secondary structural features of the enzyme using SOPMA are as follows, where significant amount as, 42.47% was helix structure, 17.56%was beta strand and significant number of random coils are observed (Table 3) for glycerol dehydratase of *Lactobacillus reuteri*. Percentage value of helix can represent a compact 3D structure of both the enzymes [16].

ISSN: 0975-8585

To check the similarity in context of matches and mismatches, multiple sequence analysis (MSA) was performed using Clustal Omega tool (https://www.ebi.ac.uk/Tools/msa/clustalo/). MSA was individually performed for both the enzyme sequences (Fig 1a & b) where the reference sequence was of *Klebsiella*. The alignment shows a patch of matches for 1,3 PD dehydogenase sequences. For Fig 1b where alignment was performed on 1,3 PD dehydrogenase, identical amino acids were found in many sections, among those here two blocks were annotated with box. For both the cases, phylogenetic analysis shown that *lactobacillus reuteri* is a close neighbor to *Klebsiella* (Fig 1a & b). In the first tree diagram (Fig 1a), it can be noticed that accession number KRK52068.1 represents Glycerol dehydratase of *lactobacillus reuteri* which is the closest neighbor of accession number BAO20817.1 of *Klebsiella.*

Table 3: Secondary structure result of three selected organisms

Organism Name	Enzymes	Alpha Helix	Beta Strand	Random Coil
Lactobacillus brevis	Glycerol Dehydratase	35.55%	24.68%	31.17%
	1,3-PD Dehydrogenase	46.15%	14.62%	28.97%
Lactobacillus plantrum	Glycerol Dehydratase	52.91%	14.29%	24.87%
	1,3-PD Dehydrogenase	47.18%%	13.59%	30.00%
Lactobacillus reuteri	Glycerol Dehydratase	42.47%	17.56%	28.49%
	1,3-PD Dehydrogenase	41.03%	17.95%	29.49%

The sequences of *lactobacillus reuteri* were analyzed to find the conserved domain through NCBI Conserved Domain Database. It was found that glycerol dehydratase is having a propendiol dehydratase large subunit which starts from 2nd position and finish at very end of the sequence (Fig 2a & b). It reflects the concept of 1, 3PDO generation by this specific organism. Similar, study was conducted on 1,3 PD dehydrogenase and a long stretch of sequence (5-383 amino acid) is falling in oxidoreductase superfamily (Fig 2b) which catalyzes the concerned enzymatic reaction.

Table 4: Template information for 3D structure modeling through Swiss Model. (a) Glycerol dehydratase (b) 1,3 PD Dehydrogenase

(a)

Template	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Range	Coverage	Description
1dio.1.A	65.34	homo-dimer	HHblits	X-ray	2.20Å	0.50	2 - 554	0.99	Protein (diol dehydratase)

(b)

Template	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Range	Coverage	Description
4fr2.1.A	76.88	homo-dimer	BLAST	X-ray	3.20Å	0.53	4 - 386	0.99	1,3-propanediol dehydrogenase

ISSN: 0975-8585

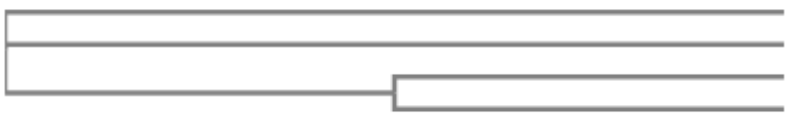
```
CLUSTAL O(1.2.4) multiple sequence alignment

KWT48525.1          ----------MQKVIGV-D-IGNSSTEVALADIS---DQGAVDFINSDIAETTGIKGTKQ
WP_021338259.1      MSEDLMFVSP-DKYIQGAGLLEHS--ASYINALG---SHP-LIMADALIWKIVGEDF---
BAO20817.1          MKRSKRFAVLAQRPVNQDGLIGEWPEEGLIAMDSPFDPVSSVKVDNGLIVELDGKRRDQF
KRK52068.1          MKRQKRFEELEKRPIHQDTFVKEWPEEGFVAMMGPNDPKPSVKVENGKIVEMDGKKLEDF
                              .: :     : .      :   .       : . :. * :  *

KWT48525.1          NLIGI-------------------KKAITQVLNKSHLALSDIDLIRINEAT-PVIGDVAM
WP_021338259.1      -----------------ATYLNQHDFDVTRVTFNGEASV--QEIARITQIA---------
BAO20817.1          DMIDRFIADYAINVERTEQAMRLEAVEIARMLVDIHVSR--EEIIAITTAITPAKAVEVM
KRK52068.1          DLIDLYIAKYGINIDNVEKVMNMDSTKIARMLVDPNVSR--DEIIEITSALTPAKAEEII
                                          ::::  . . :    ::  *.

KWT48525.1          E--TITETVITESTMIGHNPNTPGGVGIGSGYTVSLLQLLQETDKTRPYIVLVPAEVDFE
WP_021338259.1      -KDNHNDIIIGLG--GGKTLDTSKGIAEEVGA----------------KLVIAPTAASAD
BAO20817.1          AQMNVVEMMMALQ--KMRARRTPSN--QCHVT----------------NLKDNPVQIAAD
KRK52068.1          SKLDFGEMIMAVK--KMRPRRKPDN--QCHVT----------------NTVDNPVQIAAD
                          : ::       :   .  .                       *.    :

KWT48525.1          DA-AKLINLYQQSGYQ-IN--AAILQNDDGVLIDNRLEHKIPIVDEVARIDK-----VPM
WP_021338259.1      APTAGLSVLYTEEGSFD--QYKFYQRHSDLVLMDTQVIANAPVRTLISGIADAMATNIEA
BAO20817.1          AAEAGIRGFSEQETTVGIARYAPFNALA--LLVGSQCG-RPGVLTQ-CSVEEA--TELEL
KRK52068.1          AADAALRGFPEQETTTAVARYAPFNAIS--ILIGAQTG-RPGVLTQ-CSVEEA--TELQL
                       * :  :  :.                 :*:. :   .  :    . : .     :

KWT48525.1          G---MMAGVEVAGKGQVISQLSNPYGIATLFDLTADETKNIVPVSRALIGNRSAVVIKTP
WP_021338259.1      KATAQSHGT-TMG------NGAATFTGRAIAAECE-----RVIWKYAYEAV---------
BAO20817.1          G----MRGLTSYA------ETVSVYGTEAVFTDGD-----DTPWSKAFLASAYA-----S
KRK52068.1          G----MRGFTAYA------ETISVYGTDRVFTDGD-----DTPWSKGFLASCYA-----S
                           *    .      :    :    :           .  .  .  .

KWT48525.1          KGDVKARVIPAGSIQIEGDRDSDKVNVAAGAEAIMKKVNQ----FDRIQDITGEAGTNVG
WP_021338259.1      ------------------------------------------------------ESNVQ
BAO20817.1          R-GLKMR-----------------YTSGTGSEALMGYSESKSMLYLESRCIFITKGAGVQ
KRK52068.1          R-GLKMR-----------------FTSGAGSEVLMGYPEGKSMLYLEARCILLTKASGVQ
                                                                        :.*
```

Phylogenetic Tree

This is a Neighbour-joining tree without distance corrections.

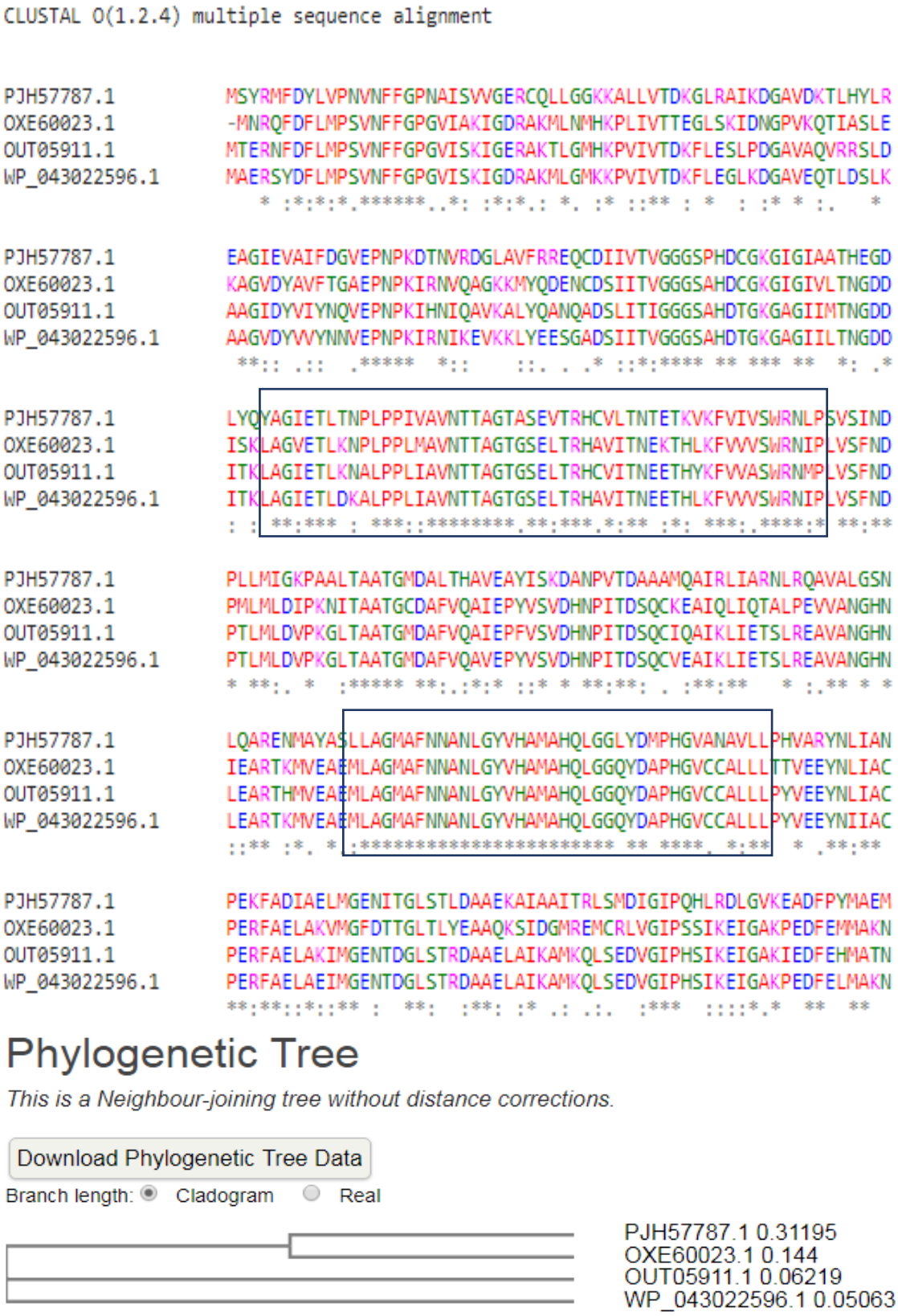
Download Phylogenetic Tree Data

Branch length: Cladogram Real



KWT48525.1 0.41111
WP_021338259.1 0.42952
BAO20817.1 0.18267
KRK52068.1 0.1903

ISSN: 0975-8585



Phylogenetic Tree

This is a Neighbour-joining tree without distance corrections.

Fig 1: Multiple sequence alignment and phylogenetic analysis of selected enzymes (a) Glycerol Dehydratase (b) 1,3 PD dehydrogenase

ISSN: 0975-8585

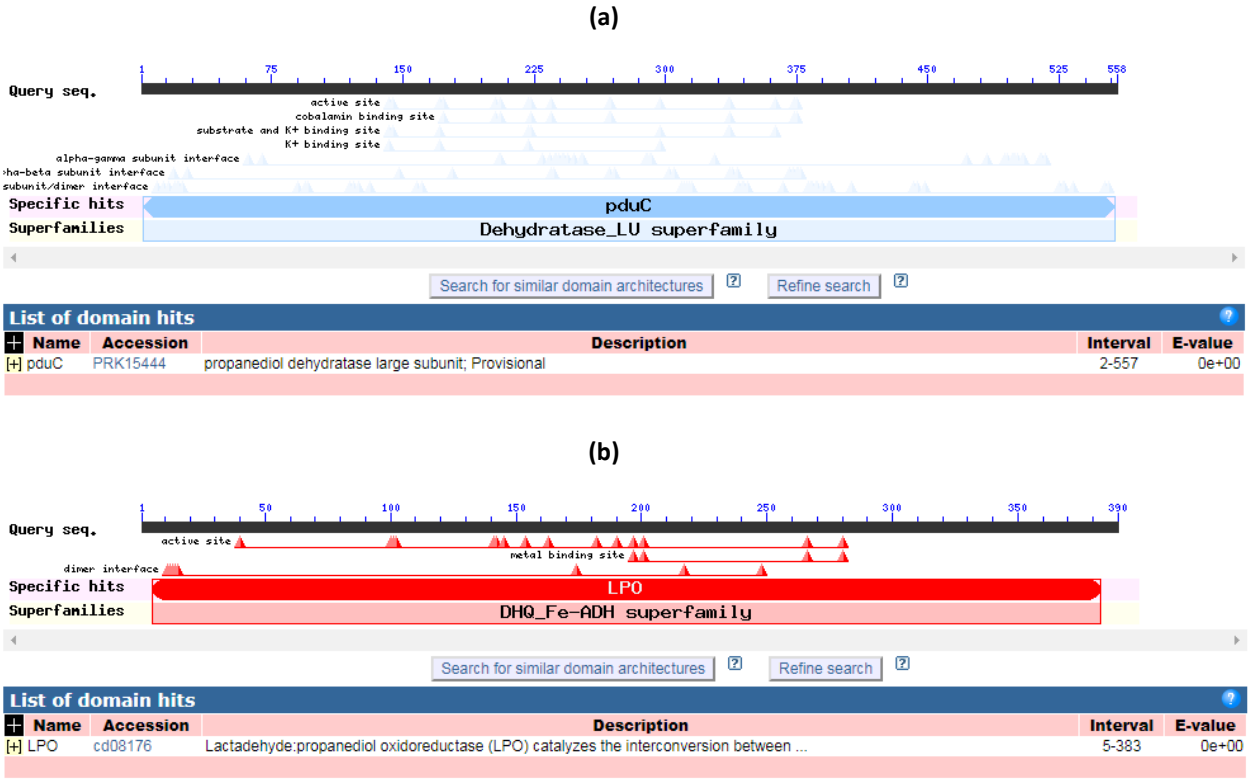


Fig 2: Conserved Domain analysis of selected enzymes (a) Glycerol Dehydratase (b) 1,3 PD dehydrogenase

As MSA result suggests the maximum similarity between *lactobacillus reuteri*and *Klebsiella pneumonia,* so the structure modeling was performed only on *lactobacillus reuteri* enzymes. For the first enzyme Glycerol dehydratase, 21 templates were predicted by SwissModel with different sequence identity, percentage coverage area and range. In this method, template search has been performed with Blast and HHBlits against the SWISS-MODEL template library. As the tool follows the homology modeling so the maximum sequence identity with 65.34% and coverage area of 0.99 template (1DIO.pdb) was selected for model build (Table 4a). The modeled structure (Fig 3a) was having QMean value of -0.82 which represents a good structure (Fig 3b) validation result [12]. The second enzyme (1,3 PD dehydrogenase), was also modeled with similar approach but the template was different. 4FR2.pdb file was the best template with sequence identity of 76.88% and coverage area was 0.99 (Table 4b). model was generated with QMean value of -1.63 (Fig 4a&b).Both the structures were validated with Ramachandran plot and statistics were generated and it was evident from the following table that both the structures having more than 95% residues in the favored region (Table 5a).It is previously informed in Rungta and Mukherjee (2014) that validation of modelled protein structure can be predicted by Ramachandran plot [15-16]. More than 90% value for favored region residues depicts less steric clashes and thus allow the structure to be in stable mode [17].

Table 5: Ramachandran plot statistics for selected enzymes

Name	Number of residues in favored region	Number of residues in allowed region	Number of residues in outlier region
Glycerol Dehydratase	94.6%	4.9%	0.5%
1,3 PD dehydrogenase	91.1%	8.9%	1.0%

This database, String (https://string-db.org/) provides information on both experimental and predicted interactions from varied sources based on their neighborhood, gene fusions, co-occurrence, co-expression, experiments and literature mining [12]. Lreu23DRAFT_4458 represents our selected 1,3 P.D Dehydrogenase in the following figure (Fig 5a) where it forms networking with similar type of enzymes

ISSN: 0975-8585

(structural or functional) from *Lactobacillus reuteri.* The network view summarizes the network of predicted association for the selected group of protein manly glycerol dehydratase and 1,3 P.D Dehydrogenase. In the figures proteins are represented by nodes and functional associations by edges. The first figure (Figure 5a) represents the enzyme (1,3 P.D Dehydrogenase) for *Lactobacillus reuteri* along with its network statistics. Total 11 nodes and 42 edges are counted that represents the correlation among different similar type of proteins in selected organism. In string edges can be drawn with seven different color (Fig 5b) scheme which depicts the existence of seven types of association among the nodes.

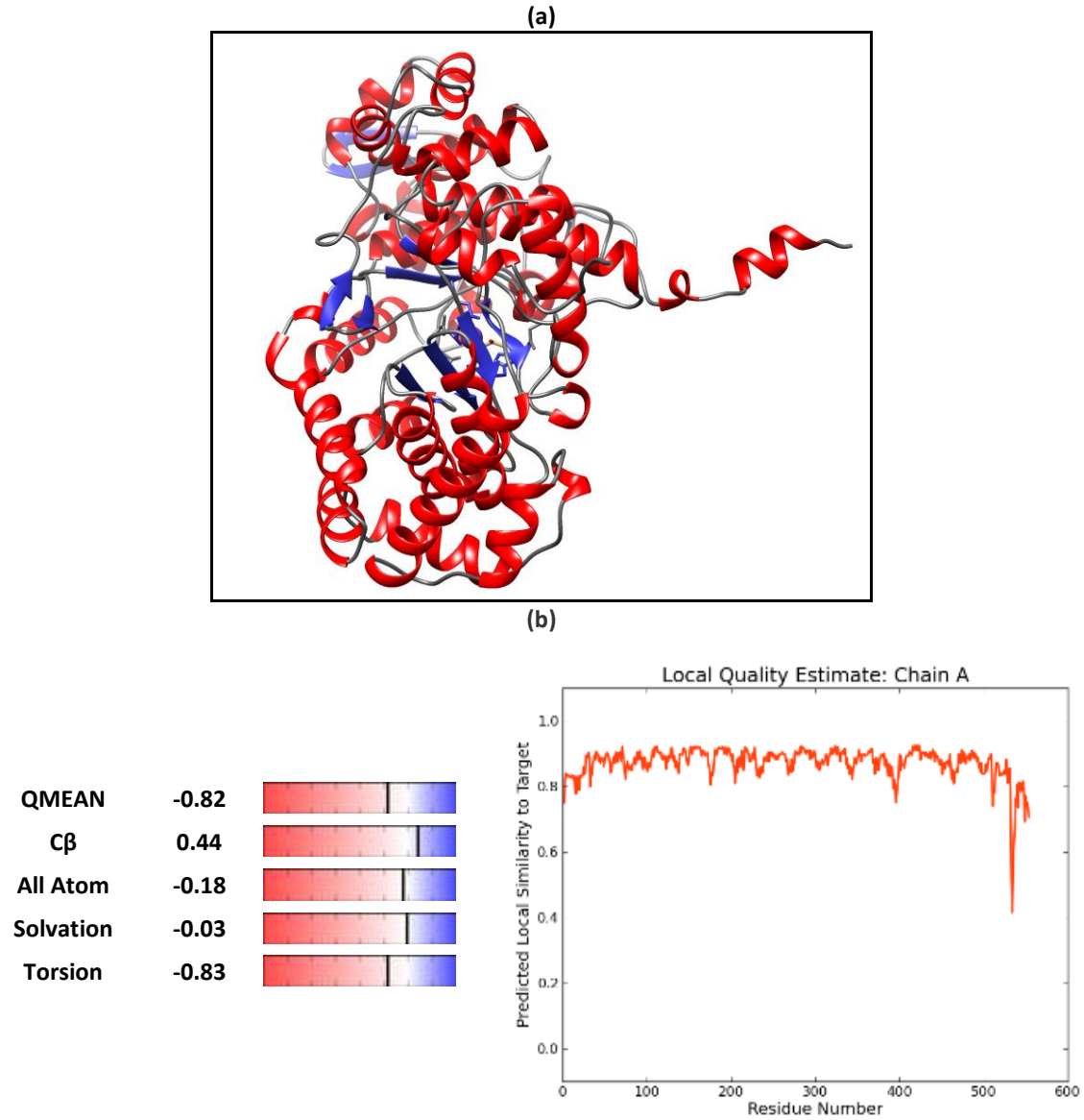
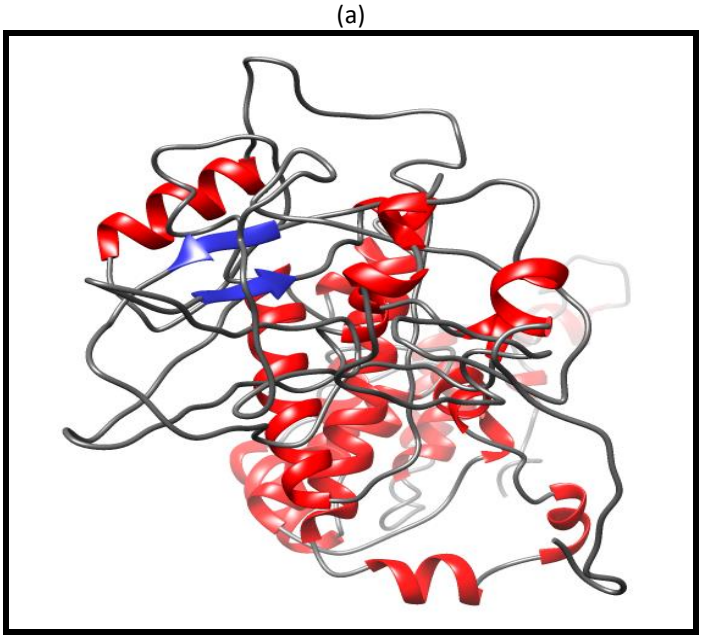


Fig 3: Schematic of Glycerol Dehydrataseenzymes of *Lactobacillus reuteri* by Swiss Model. Red, navy blue and gray color represents helix, strand and coil structure of the protein (a)3D structure model of Glycerol Dehydratase enzyme (b) Model building result

In the last decade, pathway analysis has become the first choice for explaining biological interpretation for statistically significant pathways or to validate the computationally derived results. Today, almost every bioinformatics study looks for pathways studies and network analysis [18]. The term "pathway analysis" has been used as physical interaction networks (e.g., protein–protein interactions), kinetic simulation of pathways, steady-state pathway analysis (e.g., flux-balance analysis), and in the inference of pathways from expression and sequence data [19].

ISSN: 0975-8585

(a)



(b)

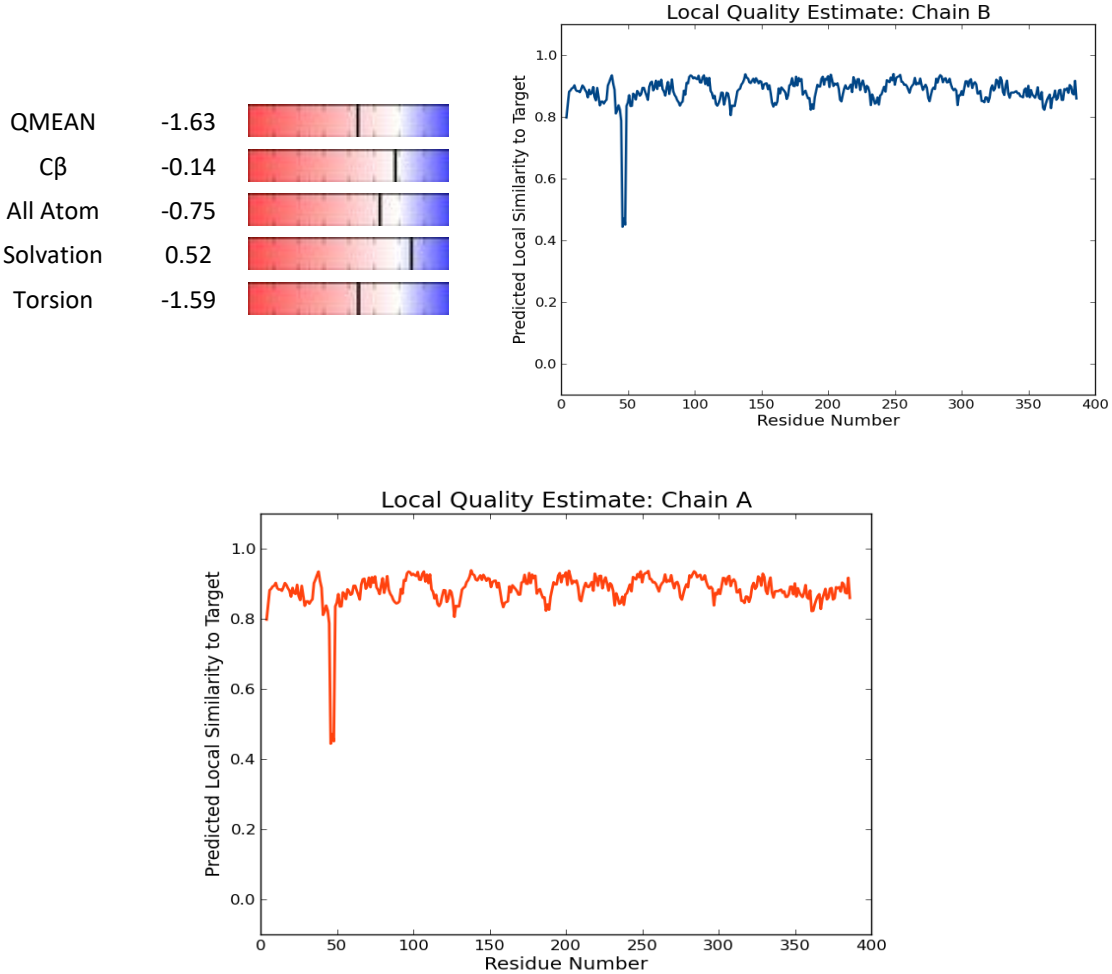
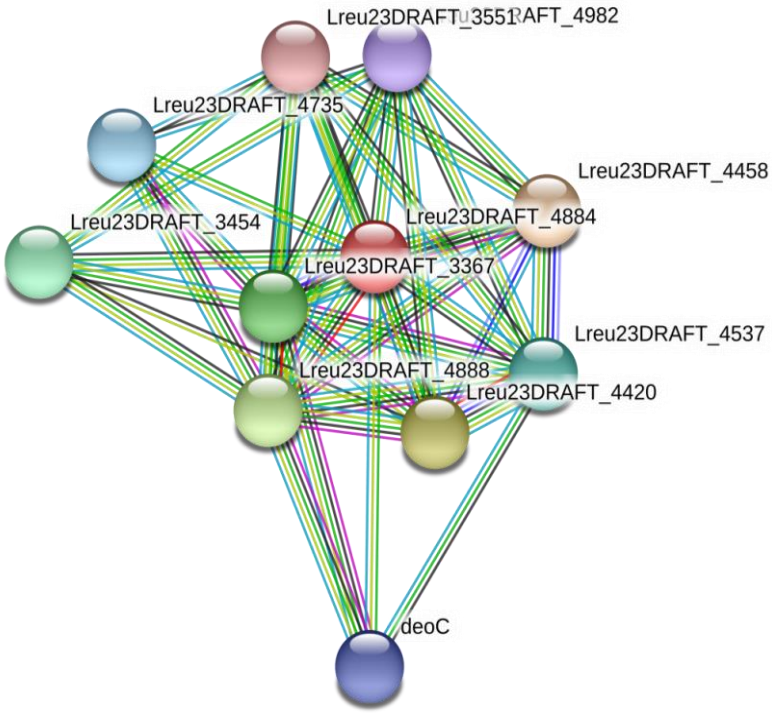


Fig 4: Schematic of 1,3 PD dehydrogenaseenzymes of *Lactobacillus reuteri* by Swiss Model. Red, navy blue and gray color represents helix, strand and coil structure of the protein (a) 3D structure model of selected enzyme (b) Model building result

ISSN: 0975-8585

(a)



Network Stats

number of nodes:	11	expected number of edges:	17
number of edges:	42	PPI enrichment p-value:	1.5e-07
average node degree:	7.64	*your network has significantly more interactions than expected (what does that mean?)*	
avg. local clustering coefficient:	0.837		

Functional enrichments in your network

Note: some enrichments may be expected here (why?)

PFAM Protein Domains			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
PF00465	Iron-containing alcohol dehydrogenase	3	0.000117
PF08240	Alcohol dehydrogenase GroES-like domain	3	0.00116
PF00205	Thiamine pyrophosphate enzyme, central domain	2	0.00569
PF02775	Thiamine pyrophosphate enzyme, C-terminal TPP binding domain	2	0.00569
PF02776	Thiamine pyrophosphate enzyme, N-terminal TPP binding domain	2	0.00569

(b)

ISSN: 0975-8585

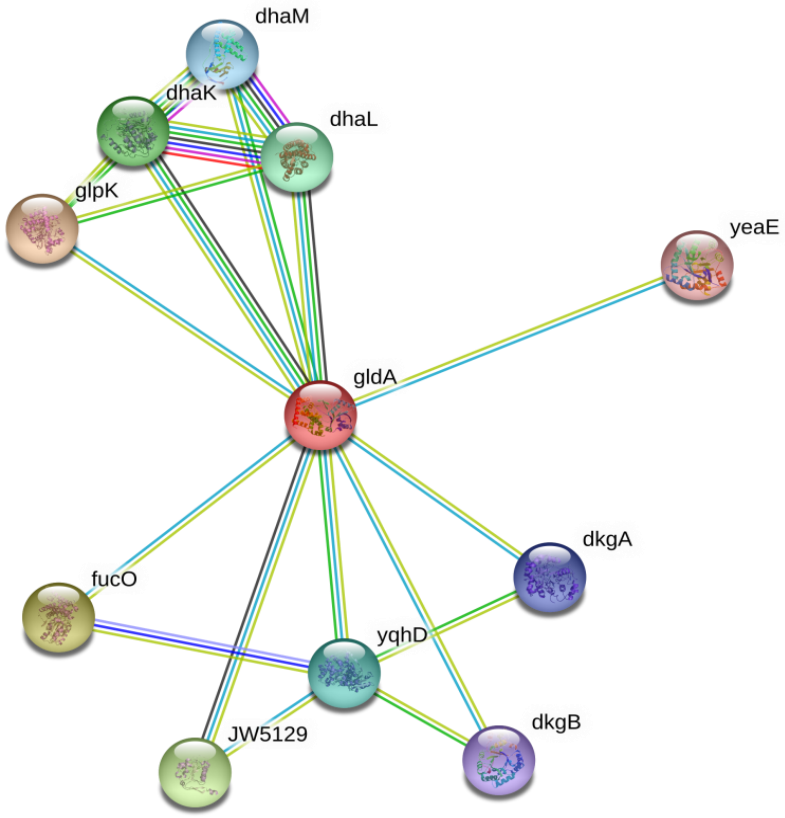
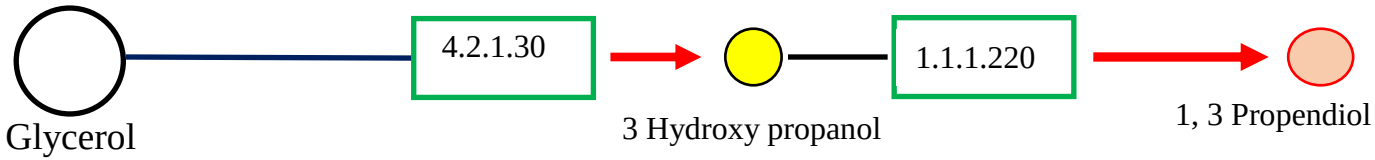


Fig 5: Network model and analysis of *Lactobacillus reuteri*enzymes (a) 1,3 P.D Dehydrogenase (b) Glycerol dehydratase. Different color scheme are:

Red line - presence of fusion evidence; Green line - neighborhood evidence; Blue line - Co-occurrence evidence; Purple line - experimental evidence; Yellow line - text mining evidence; Light blue line - database evidence; Black line - co expression evidence

Biebl et al., 1999 represent a flow diagram of microbial 1,3 PDO generation [20]. The main focus was given on the fate of Glycerolwhere three subsequent pathways can be processed; first is 1,3 PDO production, second is pyruvate generation and third is Biomass production. As, our work focuses on 1,3 PDO so we selected the first section.In the figure 6a the substrate and product are shown in circle and enzymes (glycerol dehydratase and 1,3 PD dehydrogenase) are in the rectangular box. One sided arrow means the reaction head for unidirectional. So, system biology approach can be performed to stop the other reaction pathways and enhance the concerned one by channelizing the above unidirectional reaction with help of network analysis and characterization of enzymes. So, first we tried to find the pathway from KEGG database for *Lactobacillus reuteri*. Figure 6b represents the full pathways of glycerol metabolism for *Lactobacillus reuteri,* from where we focus on the section which involved in the 1,3 PDO production (Fig 6a).

ISSN: 0975-8585



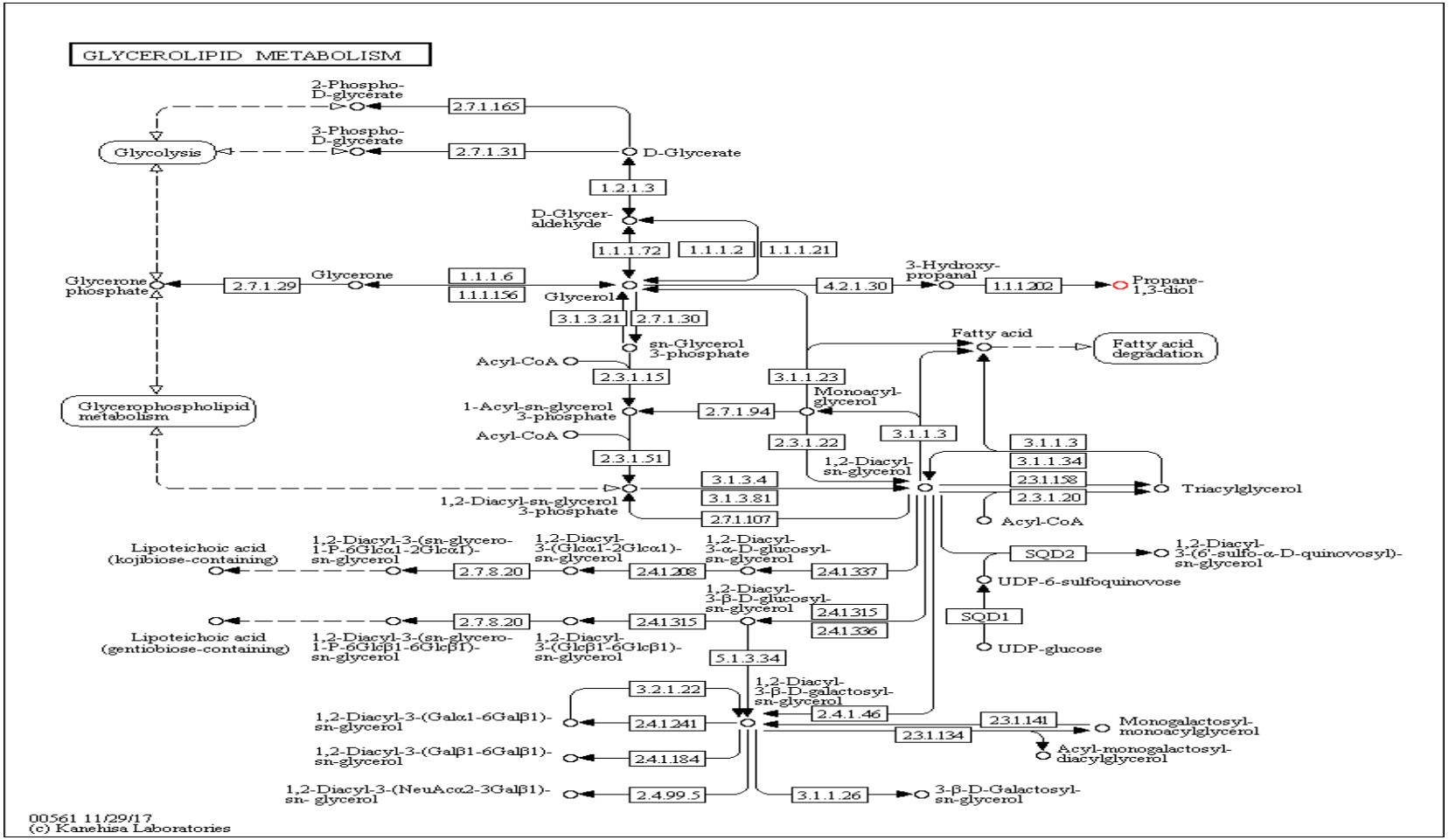


Fig 6: Pathway of *Lactobacillus reuteri*involved in1,3 PDO production (a) Section wise only 1, 3 PDO conversion (b) Full pathway

CONCLUSION

The project aimed to model the pathway for *Lactobacillus reuteri* where focus is to shut down all the other alternative pathways of glycerol utilization. Theoretically, it can be proved that all the glycerol can be converted to 1,3 PDO production if the above condition can be applied. To execute the idea the first stage was to select three species of *Lactobacillus* and identified which can produce 1,3 PDO from glycerol. *Lactobacillus reuteri* was found to be best alternative of *Klebsiella pneumonia.* Different omics tool was performed to characterize the two enzymes (Glycerol dehydratase and 1,3 P.D Dehydrogenase) which are involved in the bioconversion of 1,3 PDO from glycerol. Further, a network model of *Lactobacillus reuteri* and its enzymes were developed with different nodes and edges. KEGG pathway showed stepwise conversion from glycerol to 1,3 PDO production.

ACKNOWLEDGEMENT

We gratefully acknowledge TEQIP-III, Birla Institute of Technology, Mesra, Ranchi, Jharkhand for providing all the facilities to carry out this work.

REFERENCES

[1] Arnold KL. J. Chem. Educ. 1931; 8 (12): 2310
[2] Willke T, Vorlop KD. Appl. MicrobiolBiotechnol. 2004;66(2):131–42.
[3] Jian H, Rihui L, Zongming Z, Hongjuan L, Dehua L. World J MicrobiolBiotechnol. 2008; 24:1731–1740
[4] Saxena RK, Anand P, Saran S, Isar J. Biotechnology Advances. 2009; 27: 895–913
[5] Wie J, Shizhen W, Yuanpeng W, Baishan F. Biotechnol Biofuels.*2016; 9:57*
[6] Tang X, Tan Y, Zhu H, Zhao K, Shen W. Appl. Environ Microbiol. 2009;75(6):1628–34.
[7] Wilbert BC, Bryan AB, ChandranD, GaldzickiM, Kyung HK, Sean CS, Costas DM, Herbert MS. Metab. Eng. 2012; 14(3): 270–280
[8] Guruprasad K, Reddy BB, Pandit MW. Protein Eng Des Sel. 1990;4(2):155-61
[9] Ikai AJ. Biochem. 1980;88(6):1895-8
[10] Kyte J, Doolittle RF.JMolBio. 1982;157(1):105-32
[11] Geourjon C, DeleageG. Bioinformatics. 1995;11(6):681-4
[12] Biasini M, Bienert S, Waterhouse A, Arnold K, Studer G, Schmidt T, Kiefer F, Cassarino TG, Bertoni M, Bordoli L, Schwede T.Nucleic Acids Research. 2014; 42 (W1): W252-W258
[13] Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ, von Mering C.Nucleic Acids Res. 2015;43 (Database issue): D447–52
[14] Kanehisa M, Goto S. Nucleic Acids Res. 2000; 28:27–30.
[15] RoyD and MukherjeeK.Journal of Proteins and proteomics. 2015; 6(2); 183-196
[16] Pramanik K, Ghosh PK, Ray S, Sarkar A, Mitra S, Maiti TK. Genet Eng. Biotechnol J. 2017; 15(2):527-37.
[17] Mukherjee K,RungtaD.International Journal of Computational Bioinformatics and In Silico. Modeling.2014; 3(3): 398-406
[18] Green ML, Karp PD. Nucleic Acids Res.2006; 34: 3687–3697
[19] KhatriP , SirotaM, ButteAJ. Plos One. 2012; https://doi.org/10.1371/journal.pcbi.1002375
[20] BieblH, MenzelK, ZengAP, DeckwerWD.Appl Microbiol Biotechnol. 1999; 52: 289-297