An in-silico Approach for Enhancing the Lipid Productivity in Microalgae by Manipulating the Fatty Acid Biosynthesis

Bunushree Behera, S. Selvanayaki, R. Jayabalan and P. Balasubramanian

Abstract To fulfill the impetus of demands on alternative energy, microalgal biofuels have attracted significant attention due to the ease of cultivation, higher photosynthetic rate, as well as, the presence of significant quantity of lipids. However, from an energy perspective, the polyunsaturated fatty acids (PUFA) (substrate for transesterification to biodiesel) constitute only 10–20% of the total lipids. Approaches for increasing lipids include coercing the algal cells under nutrient depletion which also declines their growth rate. Improving the lipid accumulation without compromising growth requires strain modification via genomic or metabolic engineering which necessitates the core understanding of the critical regulators of *denovo* lipid biogenesis. Increase in activity of the enzyme acetyl-CoA carboxylase (ACCase) has been postulated to improve the lipid synthesis. Thus, the current study utilized the *Chlamydomonas reinhardtii* as the model organism for understanding the lipid metabolism. In-silico computational approach was used to design the 3D structure of ACCase, the key enzyme that catalyzes the rate-limiting step of lipid synthesis. The accuracy of the predicted structure was validated by the presence of 94% of amino acid residues in the favorable region of Ramachandran plot. The docking studies with four selected ligands (ACP, AMP, Biotin, and Glycine) showed biotin as the suitable ligand with a lowest binding affinity (−5.5 kcal/mol). The ligand–protein complex is expected to increase the enzyme activity driving lipid accumulation in vivo. Such in-silico studies are essential to design and decipher the role of different regulatory enzymes in improving the quantity and quality of microalgal biodiesel.

Keywords Microalgae · ACCase · Lipid production · Homology modeling
In-silico · Docking

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J. C. Bansal et al. (eds.), *Soft Computing for Problem Solving*,
Advances in Intelligent Systems and Computing 816,
https://doi.org/10.1007/978-981-13-1592-3_70

1 Introduction

Depleting fossil fuel reserves, increase in crude oil prices and growing environmental concern about greenhouse gas emissions have raised the stimulus for alternative fuels [1]. Biofuels derived from plant biomass popularly termed as energy crops are increasingly gaining attention. However, the food/feed versus fuel dilemma with the first-generation biofuels and the increased processing costs of second-generation biofuels delimit their commercialization on a large scale [2]. To sort out the problems mentioned above, microalgae have recently emerged as an attractive option for replacing the conventional fossil fuels due to the higher photosynthetic rate, faster growth rate, ease of cultivation of microalgae in wastewater or marine water and their ability to sequester as well as use carbon dioxide from the atmosphere as a nutrient [1, 3]. Biodiesel is one of the popular alternative fuels obtained from the polyunsaturated fatty acids (PUFAs) or triacylglycerols (TAGs) stored in microalgal biomass [3, 4].

In current decades, there has been an intense research in the arena of microalgal lipids. The lipids in microalgae are synthesized mainly via *denovo* fatty acid biosynthesis pathway. The primary step includes carboxylation of acetyl-CoA to malonyl-CoA and leads to the formation of palmitic and stearic acids, which undergo further saturation and elongation giving rise to oleic acid. All steps of fatty acid conversion and esterification occur in both plastids and the endoplasmic reticulum [4–6]. The lipids in algae exist in the form of lipid droplets and are used to provide structural membrane support, signaling, and as an energy source. Lipids utilized for a specialized function differ structurally from each other. From an energy perspective, most of the microalgal lipids are stored at the levels of 10–20% of total lipids in the form of TAGs which are formed by esterification of omega three fatty acids or PUFA [4]. The major drawback to produce PUFA rich lipid for use as biodiesel at field scale or industrial level is the low desirable lipid content in microalgae and the low biomass productivity in a photobioreactor that increases the harvesting cost considerably [7, 8].

Strategies for enhancing the lipid productivity include subjecting the microalgae to stress under environmental or operating conditions like nitrogen and phosphate limitations. Consequently, most of these approaches are time and energy consuming. Further, increase in the lipid productivity inhibits the growth and biomass productivity [8]. Strain selection is also an essential aspect of improving the lipid productivity. There is a need to understand and analyze the metabolism of lipid biosynthetic pathway to improvise the desirable lipid productivity in selected strains. Till recently, the biochemical understanding of the lipid metabolism in plants and unicellular algae is still lacking. Recently, Bellou et al. [5] have analyzed the biochemistry underlying the lipid metabolism in microalgae. Yu et al. [4] have discussed the modifications in the rate-limiting steps of TAGs formation to increase the quantum of lipids. A comprehensive review of the various databases and bioinformatics tools along with the omics approaches to improve the algal lipid metabolism has been discussed by different researchers [6, 8–10]. The molecular dynamic simulations have been used

by Kumar et al. [11] to predict the effect of nitrogen and phosphorous on the activity of acetyl-CoA carboxylase (ACCase) activity in cyanobacteria. Studies have also postulated that an increase in the cytosolic concentration of ACCase enhances the oil content [12, 13]. Nevertheless, there still lies a knowledge gap between the available omics data and the metabolic pathways underlying the lipid biosynthesis.

The current study utilizes the in-silico computational modeling approach to design a 3D model of ACCase enzyme/protein which is the key enzyme of the lipid biogenesis pathway. The ligands responsible for enhancing lipid production are then identified through docking with the protein/enzyme. Computational biology approaches like homology modeling, annotating the function of modeled protein, and docking were used to study the ligand–protein binding effects. The best and stable protein–ligand docked molecule could be taken into system biology studies for network analysis and some high-throughput experiments to realise the microalgal biofuel in the market.

2 Computational Methodology

2.1 Selection of Enzyme from the Lipid Biosynthesis Pathway

The model organism selected for study is *Chlamydomonas reinhardtii* as it is one of the algae whose genome has been completely sequenced and is the most common algae for studying the metabolic pathways [14]. The lipid synthesis pathway in microalgae (*C. reinhardtii*) consists of two essential enzymes, i.e., type II fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACCase), occurring in chloroplasts [4, 6]. The enzyme ACCase determines the rate-limiting pathway for fatty acid biosynthesis, converting acetyl-CoA to malonyl-CoA which then elongate in endoplasmic reticulum giving rise to PUFAs or TAGs [6]. Since ACCase is the enzyme that determines the fatty acid pools in microalgae, it was selected for homology modeling to predict the 3D structure that is unavailable in the literature.

2.2 Design of the Three-Dimensional Structure of ACCase Protein

The target protein sequence of enzyme ACCase from *E. coli* in FASTA format was retrieved from NCBI database (http://www.ncbi.nlm.nih.gov/). The template structure of the retrieved sequence was identified using BLASTP (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins). The sequence of the protein and the template structure was fed into MODELER software (http://www.salilab.org/modeller/) using python scripts for obtaining the 3D structure of the protein. Discrete Optimized Protein Structure (DOPE) score and GA341 values were considered for finding the

best structure from the various modeled structures. Further structural analysis of the protein chain, folds, family, and domain was done using PHYRE software (http://www.sbg.bio.ic.ac.uk/phyre2/). Sequence-level annotations (position-specific annotations) for finding the binding sites in the protein were carried out using amiGO gene ontology tool (http://www.amigo.geneontology.org/) using the BLAST link. The UniProtKB ID was used to search the unique gene product. Structural validation of the model in PDB format was done using the RAMPAGE tool (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) to obtain the phi–psi torsion angles which were then analyzed using the Ramachandran plot.

2.3 Docking of Ligands to the Protein and Analysis of Ligand–Protein Interaction

Four different ligands like ACP (phosphomethyl phosphonic acid adenylate ester), AMP (adenosine monophosphate), biotin and Gly (glycine) as mentioned in the literature were selected for docking with the protein modeled using homology modeling. The structure of the ligands was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Docking was done using the AutoDock Vina (http://vina.scripps.edu/) after finding the docking site of protein using metaPocket (http://metapocket.eml.org/) tool. The interaction was viewed using PYMOL software (https://pymol.org/). The ligand–protein stability was studied in terms of the binding affinity of the docked structures.

3 Results and Discussion

3.1 Identification of Target Amino Acid Sequence and Template Structure

The target sequence of the ACCase enzyme (acetyl-CoA carboxylase, EC 6.4.1.2, Alpha subunit) (https://www.brenda-enzymes.org/) obtained from *Escherichia coli* strain. K-12 substrain MG1655 is given in FASTA format as in Table 1. Submitting the target sequence to BLASTP (protein–protein BLAST) resulted in a series of template structures as shown in Fig. 1. Color indications obtained were as follows: <40% black, 40–60% blue, 60–80% green, 80–200% pink, and ≥200% red. The identity should be more than 30%, and the *e*-value must be minimum for the structure to be chosen as the target structure [15]. The list of different PDB structures with their description, total scores, *e*-value, and similarity index retrieved from BLASTP is illustrated in Fig. 2. Four PDB structures were taken as a template which are having scores greater than 30%. The structures selected for modeling were 2F9Y, 2F9I, 27AS, and 2BZR.

Table 1 Sequence of amino acid for the enzyme ACCase in FASTA format

Source	FASTA sequence:
Escherichia coli str. K-12 substr. MG1655	gil16128178|ref|NP_414727.1| acetyl-CoA carboxylase, carboxytransferase, alpha subunit [Escherichia coli str. K-12 substr. MG1655]
Accession no. NP_414727 **Sequence length** 319 aa	MSLNFLDFEQPIAELEAKIDSLTAVSRQD EKLDINIDEEVHRLREKSVELTRKIFADL GAWQIAQLARHPQRPYTLDYVRLAFD EFDELAGDRAYADDKAIVGGIARLDG RPVMIIGHQKGRETKEKIRRNFGMPAPE GYRKALRLMQMAERFKMPIITFIDTPG AYPGVGAEERGQSEAIARNLREMSRL GVPVVCTVIGEGGSGGALAIGVGDKV NMLQYSTYSVISPEGCASILWKSADKAPL AAEAMGIIAPRLKELKLIDSIIPEPLGGA HRNPEAMAASLKAQLLADLADLDVLSTED LKNRRYQRLMSYGYA

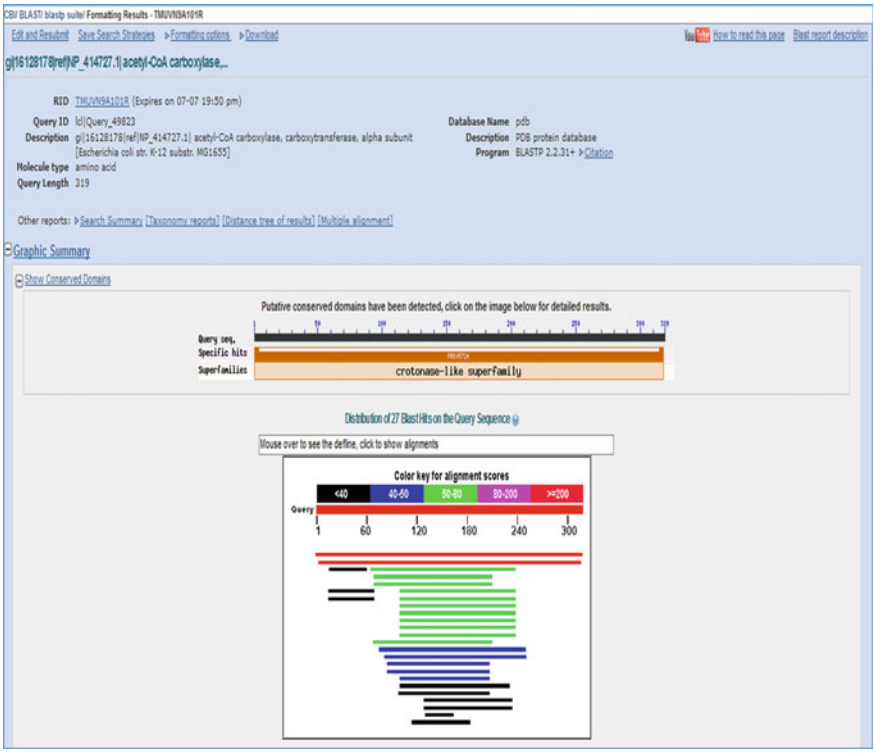
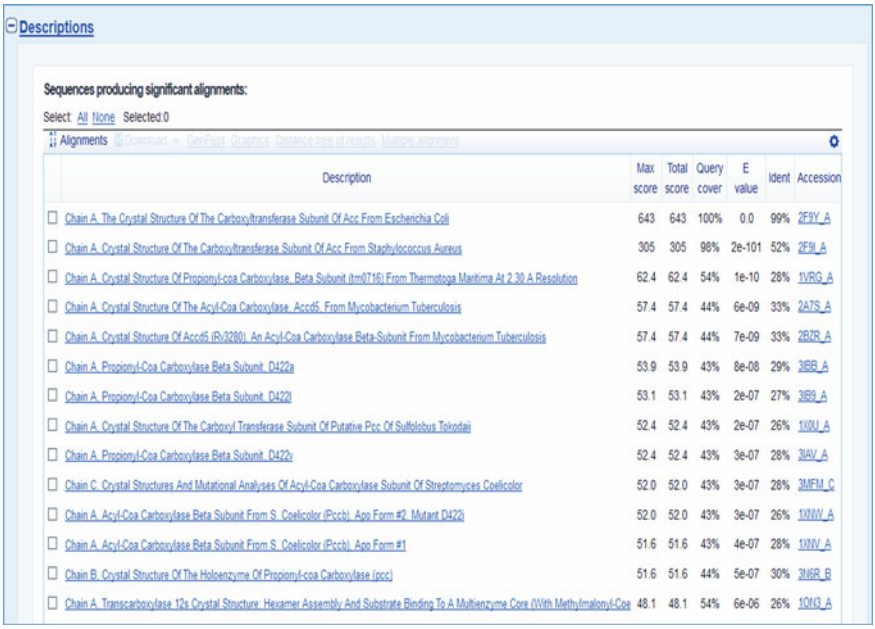


Fig. 1 Template structures with color codes obtained by BLASTP



Descriptions

Sequences producing significant alignments:

Select: All None Selected 0

Alignments

Description	Max score	Total score	Query cover	E value	Ident	Accession
Chain A, The Crystal Structure Of The Carboxyltransferase Subunit Of Acc From Escherichia Coli	643	643	100%	0.0	99%	2F9Y_A
Chain A, Crystal Structure Of The Carboxyltransferase Subunit Of Acc From Staphylococcus Aureus	305	305	98%	2e-101	52%	2F9I_A
Chain A, Crystal Structure Of Propionyl-coa Carboxylase, Beta Subunit (tm0716) From Thermotoga Maritima At 2.30 A Resolution	62.4	62.4	54%	1e-10	28%	1VRG_A
Chain A, Crystal Structure Of The Acyl-Coa Carboxylase, Accd5, From Mycobacterium Tuberculosis	57.4	57.4	44%	6e-09	33%	2A7S_A
Chain A, Crystal Structure Of Accd5 (Rv3280), An Acyl-Coa Carboxylase Beta-Subunit From Mycobacterium Tuberculosis	57.4	57.4	44%	7e-09	33%	2BZR_A
Chain A, Propionyl-Coa Carboxylase Beta Subunit, D422a	53.9	53.9	43%	8e-08	29%	3IBB_A
Chain A, Propionyl-Coa Carboxylase Beta Subunit, D422I	53.1	53.1	43%	2e-07	27%	3IB9_A
Chain A, Crystal Structure Of The Carboxyl Transferase Subunit Of Putative Pcc Of Sulfolobus Tokodaii	52.4	52.4	43%	2e-07	26%	1X9U_A
Chain A, Propionyl-Coa Carboxylase Beta Subunit, D422v	52.4	52.4	43%	3e-07	28%	3IAV_A
Chain C, Crystal Structures And Mutational Analyses Of Acyl-Coa Carboxylase Subunit Of Streptomyces Coelicolor	52.0	52.0	43%	3e-07	28%	3MFM_C
Chain A, Acyl-Coa Carboxylase Beta Subunit From S. Coelicolor (Pccb), Apo Form #2, Mutant D422i	52.0	52.0	43%	3e-07	26%	1XNW_A
Chain A, Acyl-Coa Carboxylase Beta Subunit From S. Coelicolor (Pccb), Apo Form #1	51.6	51.6	43%	4e-07	28%	1XNV_A
Chain B, Crystal Structure Of The Holoenzyme Of Propionyl-coa Carboxylase (pcc)	51.6	51.6	44%	5e-07	30%	3N6R_B
Chain A, Transcarboxylase 12s Crystal Structure: Hexamer Assembly And Substrate Binding To A Multienzyme Core (With Methylmalonyl-Coe	48.1	48.1	54%	6e-06	26%	1ON3_A

Fig. 2 Template sequences obtained via alignment using BLASTP

3.2 Predicting the Three-Dimensional Structure of Protein

The template and the target protein sequence of amino acids were fed into the MODELER software using python scripts. The templates were analyzed for resolution values (Fig. 3). The template structure 2F9I showed 52% similarity with the target protein structure and with a lower resolution value of 2.0, while compared to others was selected for further analysis [15]. The target sequence and the chosen template were aligned in MODELER along with the DOPE score, and the GA341 was used to screen the predicted model. The five predicted model structures as given by MODELER software have been illustrated in Fig. 4. The structure with the lowest DOPE score was selected, and the 3D structure of the protein was then analyzed by PHYRE software [16, 17]. The analysis of the folds, families of the selected structure was done by PHYRE software (Fig. 5). The fold of the protein is identified by PHYRE using Structural Classification of Protein (SCOP) 3D-BLAST server [18]. The structure with the identity of 99% was selected with fold corresponding to c14:clpP/crotonase core with four turns of (beta beta-alpha in superhelix) as illustrated in Fig. 6. The 3D structure of the protein was viewed in RASMOL (Fig. 7). The sequence-level annotation of the predicted 3D model structure with 319 amino acids was done using amiGO gene ontology tool (as shown in Fig. 8) which was further used in docking studies [19].

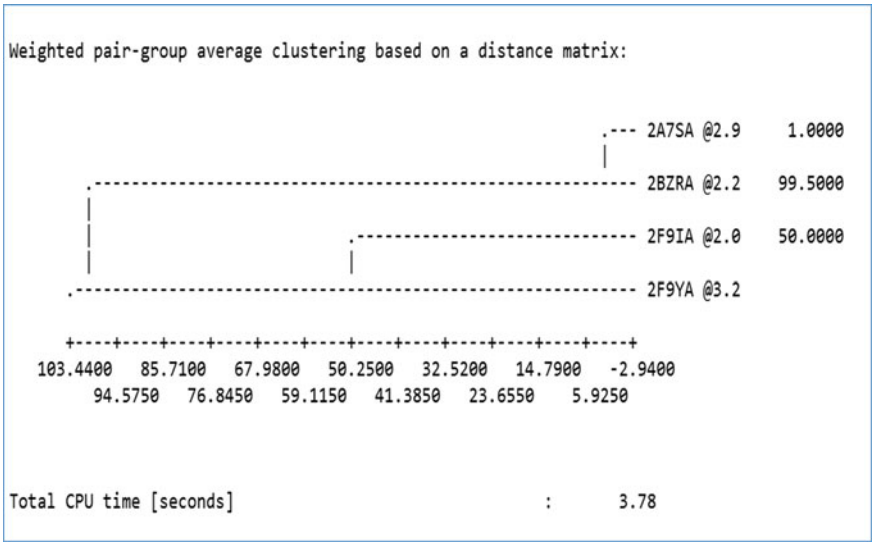


Fig. 3 Screening of templates using MODELER software and python script

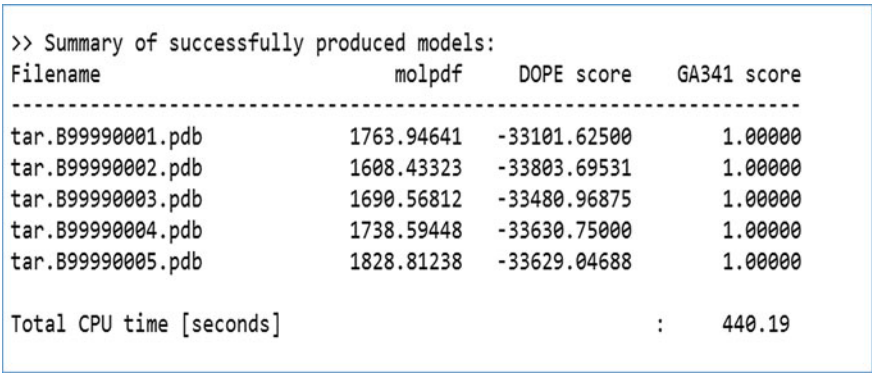


Fig. 4 Results of DOPE score for selecting the desired templates

3.3 Validation of Protein Structure Using Ramachandran Plot

The 3D predicted protein structure from MODELER was validated using RAMPAGE. The stereochemical properties of protein and the accuracy of the predicted model were analyzed via Ramachandran plot using RAMPAGE tool (Fig. 9). The analysis of the Ramachandran plot (Fig. 10) showed that 94% of the residues of

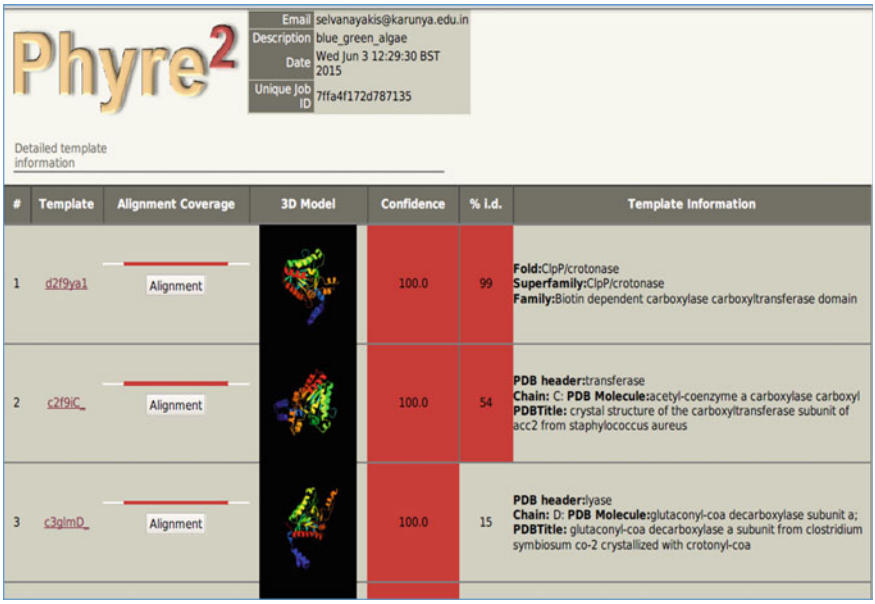


Fig. 5 Structural analysis of the 3D structure of protein in PHYRE

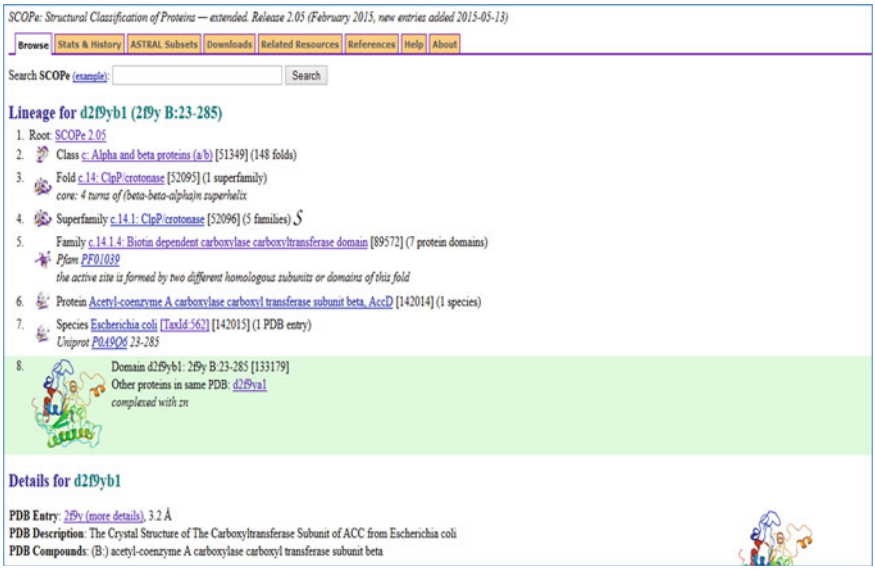


Fig. 6 Structural analysis of the folds and families of 3D protein structure in PHYRE using SCOP

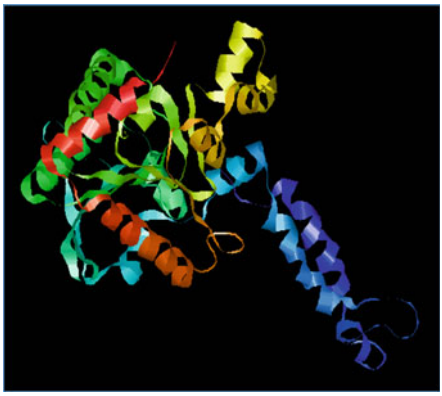


Fig. 7 3D structure of the protein as viewed in RASMOL software

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>UNIPROTKB|P0ABD5 [details] [associations]
            symbol:accA species:83333 "Escherichia coli K-12"
            [GO:0006633 "fatty acid biosynthetic process" evidence=IEA;IGI;IMP]
            [GO:0042759 "long-chain fatty acid biosynthetic process"
            evidence=NAS] [GO:0005737 "cytoplasm" evidence=IC] [GO:2001295
            "malonyl-CoA biosynthetic process" evidence=IEA] [GO:0005524 "ATP
            binding" evidence=IEA] [GO:0000166 "nucleotide binding"
            evidence=IEA] [GO:0009329 "acetate CoA-transferase complex"
            evidence=IDA] [GO:0016874 "ligase activity" evidence=IEA]
            [GO:0005515 "protein binding" evidence=IPI] [GO:0003989 "acetyl-CoA
            carboxylase activity" evidence=IEA] UniPathway:UPA00655
            HAMAP:MF_00823 InterPro:IPR001095 InterPro:IPR011763 Pfam:PF03255
            PRINTS:PR01069 PROSITE:PS50989 GO:GO:0005524 EMBL:U00096
            EMBL:AP009048 GenomeReviews:AP009048_GR GenomeReviews:U00096_GR
            GO:GO:0003989 GO:GO:2001295 GO:GO:0042759 eggNOG:COG0825
            HOGENOM:HOG000273832 KO:K01962 PANTHER:PTHR22855:SF3
            TIGRFAMs:TIGR00513 OMA:QLTKDIY ProtClustDB:PRK05724 EMBL:M96394
            EMBL:D49445 EMBL:U70214 EMBL:D87518 EMBL:M19334 PIR:A43452
            RefSeq:NP_414727.1 RefSeq:YP_488487.1 PDB:2F9Y PDBsum:2F9Y
            ProteinModelPortal:P0ABD5 SMR:P0ABD5 DIP:DIP-35897N IntAct:P0ABD5
            MINT:MINT-1228651 PaxDb:P0ABD5 PRIDE:P0ABD5
            EnsemblBacteria:EBESCT00000001533 EnsemblBacteria:EBESCT00000014303
            GeneID:12930759 GeneID:944895 KEGG:ecj:Y75_p0181 KEGG:eco:b0185
            PATRIC:32115481 EchoBASE:EB1600 EcoGene:EG11647
            BioCyc:EcoCyc:CARBOXYL-TRANSFERASE-ALPHA-MONOMER
            BioCyc:ECOL316407:JW0180-MONOMER
            BioCyc:MetaCyc:CARBOXYL-TRANSFERASE-ALPHA-MONOMER SABIO-RK:P0ABD5
            EvolutionaryTrace:P0ABD5 Genevestigator:P0ABD5 GO:GO:0009329
            Uniprot:P0ABD5
          Length = 319
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Fig. 8 Results of sequence annotation of the 3D structure of protein using amiGO

the predicted 3D structure from MODELER is in favored regions. It signifies the accuracy of the predicted model and stereospecific stability of the 3D protein [20].

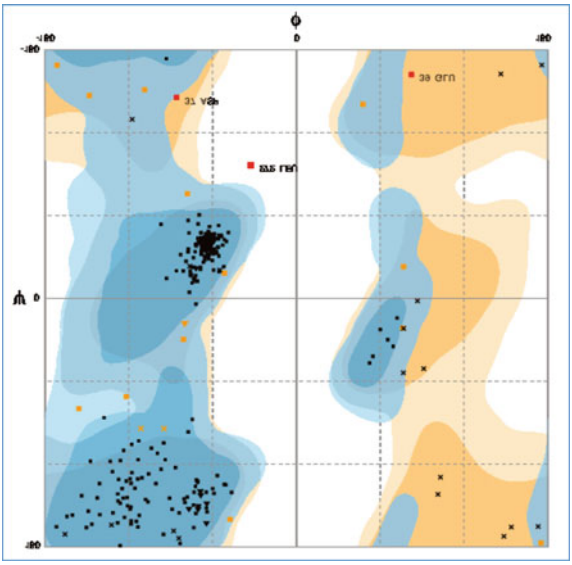

Fig. 9 Analysis of protein residues in the favored regions of Ramachandran plot

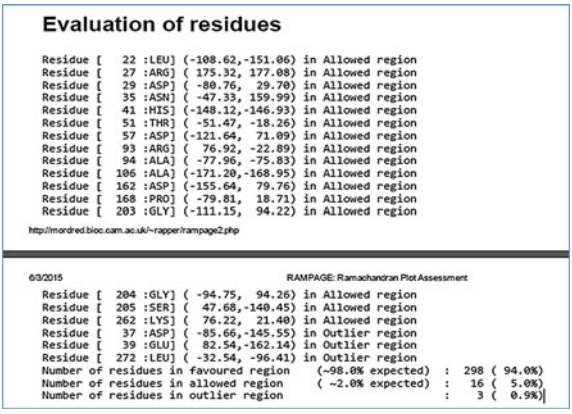

Fig. 10 Evaluation of the model accuracy based on the number of favored regions

3.4 Analysis of Stability of Ligand-Protein Interaction via Docking

The selected ligands were successfully docked into 3D protein structure. Docking of the ligands with protein resulted in significant ligand–protein interaction. The recognition surface for ligand–protein interaction was studied using the PYMOL software (Fig. 11). The stability of these interactions depends on the interaction energy which is usually denoted in terms of the binding affinity (Table 2). Since the interaction of biotin had the lowest binding affinity, binding of biotin is expected to

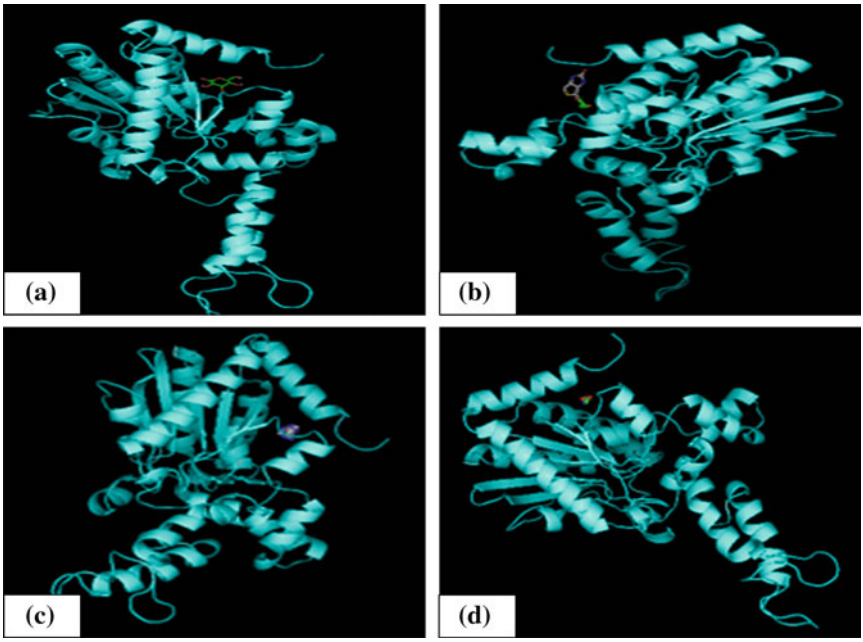

Fig. 11 Structure of the protein docked with **a** ACP; **b** BIO; **c** AMP; **d** GLY

Table 2 Binding affinities of various ligands used in docking studies

Ligand		Binding affinity (kcal mol^{-1})
Phosphomethyl phosphonic acid adenylate ester	ACP	−5.2
Biotin	BIO	−5.5
Adenosine monophosphate	AMP	−4.8
Glycine	GLY	−2.6

remodel the configuration of substrate–enzyme complex resulted in increasing the activity of ACCase. Further increase in ACCase activity is expected to increase the accumulation of PUFA/TAGs in microalgae. Interaction of biotin with ACCase could transfer the carboxyl group to acetyl-CoA to form malonyl-CoA, which further got esterified and elongated to form PUFAs and then stored as TAGs [21]. Blatti et al. [22] had also highlighted the significance of protein–protein interaction in increasing the lipid biogenesis in algae. These principles provide a fundamental understanding of the role of ACCase in algal fatty acid biosynthesis, paving the way for future metabolic engineering. Nevertheless, further system biology-based network analysis along with the experimental studies is required to validate the results mentioned above.

4 Conclusion

Though microalgae are considered to be the promising feedstocks for alternative fuels, relatively, the lower lipid accumulation necessitates the strain modification via genomics and metabolic engineering. It is essential to understand the primary biochemical pathways of their lipid accumulation in vivo in microalgae. Improvising the activity of the critical enzyme ACCase via ligand–protein interaction could enhance the accumulation of lipids. Homology modeling was used to design the 3D structure of enzyme ACCase successfully. The Ramachandran plot analysis showed that the accuracy of the predictive structure as 94% of the amino acid residues was in the favored regions. The docking studies confirmed the stability of interaction of the selected ligands (ACP, AMP, biotin, and glycine). The interaction of biotin with the enzyme having the least binding affinity (−5.5 kcal/mol) showed a stable enzyme–substrate complex formation, which could favorably reconfigure the structure, and thus enhances the enzyme activity for lipid accumulation via *denovo* lipid biosynthesis pathway. However, the experimental analysis is essential to validate the predicted results further. Such in-silico studies are essential for increasing the fundamental understanding of in vivo lipid regulation and biosynthesis before proceeding for metabolic or genetic engineering experiments at laboratory scale.

Acknowledgements The authors are thankful to the DBT sponsored Bioinformatics Infrastructure Facility (BIF) at Department of Biotechnology and Medical Engineering of NIT Rourkela for their support during the research work. The authors are grateful to Ministry of Human Resources and Development of Government of India (MHRD, GoI) for sponsoring the first author's Ph.D. program.

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