Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(2): 785-790

Received: 03-01-2018
Accepted: 04-02-2018

Meenu Goyal
Department of Biotechnology, Central University of Haryana, Mahendergarh, Haryana, India

Sugandh Chauhan
Department of Biotechnology, Central University of Haryana, Mahendergarh, Haryana, India

Ankush
Department of Biotechnology, Central University of Haryana, Mahendergarh, Haryana, India

Preeti Goyal
Faculty of Life Sciences, PDM University, Bahadurgarh, Haryana, India

Jyoti Prabha Bishnoi
Amity institute of Biotechnology, Amity University Rajasthan, Jaipur, Rajasthan, India

Correspondence
Jyoti Prabha Bishnoi
Amity institute of Biotechnology, Amity University Rajasthan, Jaipur, Rajasthan, India

Structural modeling of shikimate pathway enzymes for herbicide and drug development: A review

Meenu Goyal, Sugandh Chauhan, Ankush, Preeti Goyal and Jyoti Prabha Bishnoi

Abstract
The shikimate pathway is the biosynthetic route for aromatic amino acids in microbes and plants but not in animals. Due to the absence of this pathway in animals, it is the main target for action of herbicides and antimicrobial agents. All the enzymes of this pathway have been targeted for herbicide and drug development. The EPSP (5-enolpyruvylshikimate-3-phosphate) synthase is one of the important enzymes of the shikimate pathway which is also involved in the biosynthesis of various secondary metabolites essential for survival. DAHP (3-Deoxy-D-arabinoheptulosonate 7-phosphate synthase) is the first enzyme of this pathway, which is involved in the condensation of PEP (Phosphoenolpyruvate) and E4P (D- erythrose 4-phosphate) to produce DAHP. Chorismate synthase and shikimate kinase are other enzyme targets of the pathway. To develop new herbicides and drugs targeting this pathway, three dimentional (3D) structure of the target enzymes must be known. But still a large number of protein structures are not available due to difficulties in wet lab determination of protein structures. This review highlights the importance of computational techniques for structural modeling of enzymes of shikimate pathway and subsequent applications for developing new herbicides and drugs.

Keywords: shikimate, herbicide, modeling

Introduction
Due to exponential increase in the rate of protein sequencing, more than one million protein sequences are available in the sequence databases (SwissProt, Protein Information Resource). The primary database for protein structure information is the Protein Data Bank (PDB) [1]. The Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) provides an information for structures of biological macromolecules. Various tools and resources would required for understanding the relationship between function of biological macromolecules, sequence and structure [2]. The prediction of the 3D structure of a protein sequence is a difficult task. Wet lab techniques for determination of protein structure by X-ray crystallography or Nuclear Magnetic Resonance (NMR) is expensive and complex. Therefore, protein threading, homology modeling and ab initio were developed under computational methods and the most simple and reliable method is homology modeling. The goal of protein modeling is to predict the structure of a protein from its amino acid with an accuracy that is comparable to the best results achieved experimentally [3]. Homology modeling refers to construct a protein 3D structure using an already existing experimentally determined structure that closely relating at the sequence level [4]. 3D structure of a given protein is predicted via homology modeling based on its alignment to one or more proteins of known structure (templates).The prediction process includes identification of template, target-template alignment, model building, model refinement and model validation. There are several computer programs and Web servers that may be used for modeling [5]. The sequence with similarity greater than 30% can act as template [6]. Threading method compare a target sequence against a library of structural templates, producing a list of scores. According to the rank, the best fold is assumed to be one adopted by the sequence. *Ab initio* prediction method assumes that the native structure of a protein is at the Gibbs free energy minimum [7].

Shikimate Pathway
The shikimate pathway is an important and common pathway for the biosynthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan and also serve as precursors

for a wide range of secondary products (auxin, folic acid, phytoalexins, flavonoids, alkaloids etc.) that are essential for plant survival [8] as well as for human nutrition and health [9]. This pathway is found in algae, higher plants, bacteria and fungi, as well as in apicomplexan parasites [10] but absent from mammals thus the pathway is an attractive target for the development of herbicides and antimicrobial agents against number of diseases [11]. Figure 1 outlines the seven steps of the shikimate pathway.

The shikimate pathway initiates from phosphoenolpyruvate (PEP) and erythrose 4-phosphate (E-4P) and comprises seven reactions catalyzed by seven enzymes to produce chorismate [9]. The seven enzymes of the shikimate pathway were originally discovered through studies on bacteria, mainly *Escherichia coli* and *Salmonella typhimurium*. In the first step, there is condensation of the glycolytic intermediate phosphoenolpyruvate and the pentose phosphate pathway intermediate erythrose- 4-phosphate to form a seven-carbon six-membered heterocyclic compound, 3-deoxy-D-arabino-heptulosonate7-phosphate (DAHP). The reaction is catalyzed by DAHP synthase [12]. Further steps involve six other enzymes which result in the formation of an aromatic compound, chorismate that is the final product of the shikimate pathway. Chorismate is the precursor for several anabolic metabolites leading to primary and secondary compounds [11]. In addition to performing essential roles in plant growth and development, the shikimate pathway has important therapeutic and biotechnological applications. Many tyrosine-derived alkaloids such as morphine have medical applications and a variety of phenylalanine derived phenylpropanoids have been shown to have wide ranging health benefits [13].

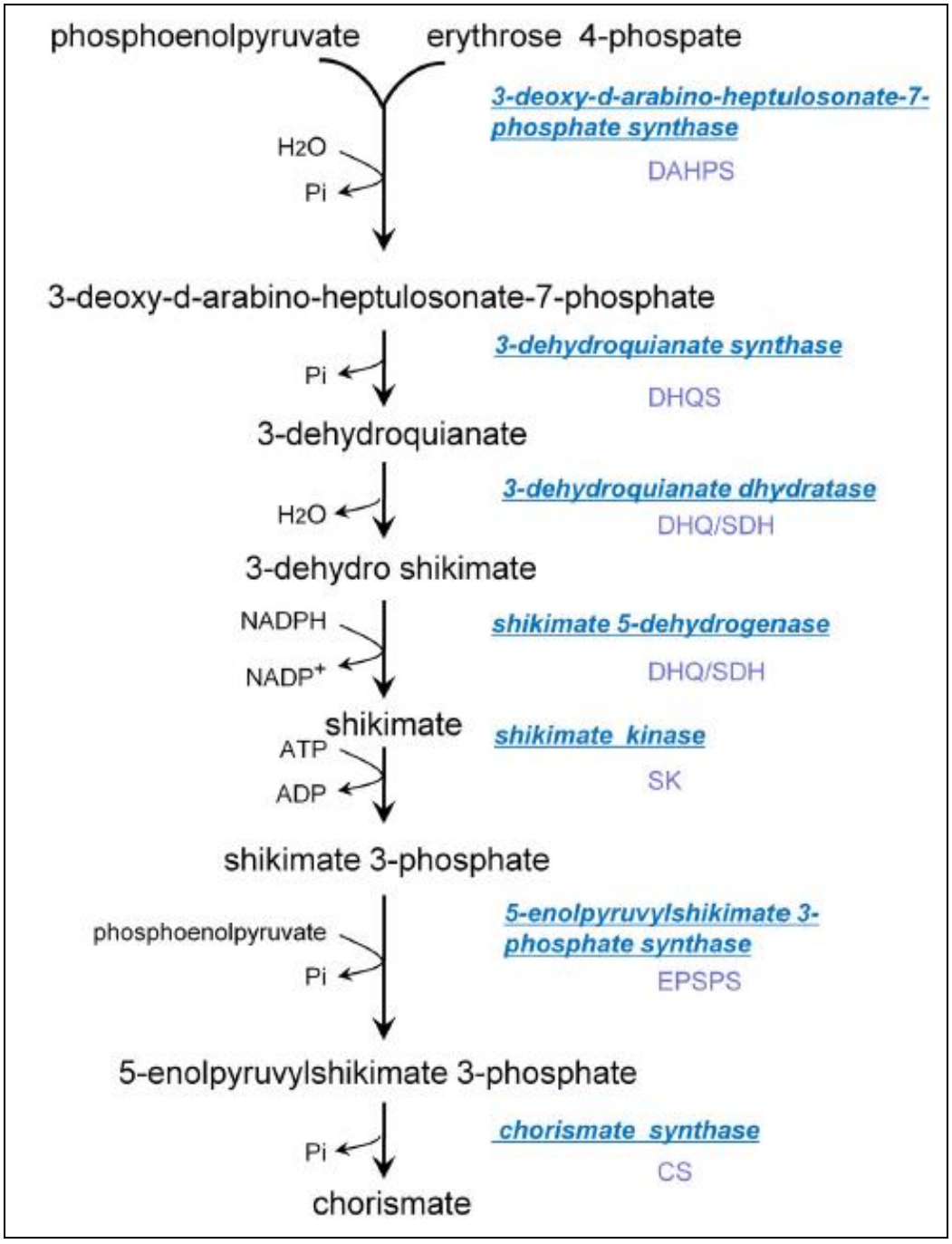

Fig 1: The shikimate pathway

A. Homology modeling of shikimate pathway enzymes in plants

Homology modeling of EPSP Synthase

The enzyme EPSP synthase is the sixth enzyme of the shikimate pathway. EPSP synthase (also referred to as 3-phosphoshikimate1-carboxyvinyltranferase) catalyzes the penultimate step of the shikimate pathway by transferring the enolpyruvyl moiety of PEP to the 5-hydroxyl position of shikimate 3-phosphate forming EPSP and inorganic phosphate. The reaction is chemically unusual because it proceeds via C-O bond cleavage of PEP rather than via P-O bond cleavage [14]. EPSP synthase is a monomeric enzyme with a molecular mass of 46-48 Kda. The enzyme has been purified from prokaryotes and eukaryotes and the *E. coli* EPSP synthase has been crystallized [15]. The enzyme is encoded by a single gene called *aro*A, in the majority of the organisms in which its activity has been characterized [16].

EPSP synthase is the primary target of broad spectrum herbicide glyphosate [17]. Glyphosate (N-phosphonomethyl glycine, 'Roundup') is a relatively non selective postemergence herbicide, which has proven as potent and specific inhibitor of EPSP synthase [18]. Gyphosate cause the inhibition of EPSP synthase by a slowly reversible reaction and shows competitive behavior with PEP while uncompetitive behavior with shikimate-3-phosphate [14]. Glyphosate has been shown to inhibit the growth of several apicomplexan parasites, including those that cause malaria and toxoplasmosis [19]. EPSP synthases are mainly categorized into two classes i.e.type I and type II. Type I EPSP synthases are glyphosate sensitive and mainly present in all types of plants and bacteria While type II EPSP synthase are usually present in naturally occurring specific forms of microbes with glyphosate tolerance ability [20]. Naturally occurring glyphosate tolerant microbes were identified including *Agrobacterium* sp. Strain CP4, *Achromobacter*sp. Strain LBAA and *Pseudomonas* sp. Strain PG2982 [21]. The presence of Ala100 is the main cause of tolerance towards glyphosate in some species, including *Agrobacteriumtumefaciens*strain CP4 [16].

The two basic strategies that have been successful in introducing glyphosate resistance into crop species--Expression of an insensitive form of enzyme and Detoxification of the glyphosate molecule. The strategy used is the former, employing a microbial (CP4) or glyphosate is not able to inhibit a mutated form of EPSP [22]. Therefore, mutated form of EPSPS have been identified which does not allow the binding of glyphosate. Mutagenesis of EPSPS was done to obtain glyphosate-tolerant EPSPS in various species like proline-106 to serine in *E. indica* [23], proline-106 to leucine in *N. tabacum* [24], glycine-100 to alanine in *Agrobacterium* sp. strain CP4 [21] (Funke *et al*., 2006), proline 101 to serine in *Salmonella typhimurium* [20]. 2D and 3D structure of EPSP synthase from different plants i.e. *Nicotiana tabacum, Vitis vinifera, Amaranthus palmeri, Gossypium hirsutum, Brassica napus, Eleusine indica, Convolvulus arvensis* and *Capsicum annu was revealed through* In silico approach via homology modeling [10, 25]. Yaqoob *et al* [8] revealed the homology model of *Oryza sativa* EPSPS (OsEPSPS) protein using the structure of *E. coli* EPSPS as template. The EPSPS protein of rice is composed of α-helices (42.52%), extended strands (17.86%) and beta turn (10.10%). Glyphosate resistant corn was developed via expression of a glyphosate insensitive EPSP synthase enzyme (CP4-EPSP) [26]. Roundup Ready soybean was produced by introduction of the glyphosate tolerant CP4 EPSPS coding sequence derived from the common soil bacterium *Agrobacterium* sp. strain CP4 into the soybean genome using particle-acceleration transformation [16].

Homology modeling of Dahp Synthase

3-Deoxy-D-arabino-heptulosonate7-phosphate (DAHP) synthase catalyzes the first step of the shikimate pathway, an aldol condensation of PEP and E4P to produce DAHP [27]. DAHP synthase is ubiquitous in archaebacterial, fungi and plants and in plant is known to undergo metabolic regulation. DAHP synthases are classified into two types on the basis of primary structure homology and molecular mass - Class I and Class II. Class I is found in prokaryotic and archae organisms (*S. cerevisiae, N. crassa)*. Class II DAHP synthases are found in plants and some microbes (e.g., *Mycobacterium tuberculosis*). The sequences of both the classes of DAHP synthases are complementary to each other. The 3D structure of DAHP synthase from *Brachypodium distachyon* has been studied by using the structure of DAHPS from Mycobacterium tuberculosis as template [11].

Homology modeling of Chorismate Synthase

The 3D structure of the yeast chorismate synthase was resolved by X-ray crystallography using selenomethionine-labeled crystals at 2.2-Å resolution. The structure was consist of βαβα fold with alternate arrangement of two α-helical and two β-sheet layers [28].

Homology modeling of Shikimate Kinase

The crystal structure of *Mycobacterium tuberculosis* (MtSK) and *Aquifexaeolicus* (AaSK) was used as template for the construction of 3D structure of shikimte kinase from *Cassia obtusifolia.* The resulted 3D structure of CoSK was furnished with α-β-α fold with five parallel β-sheets flanked by 12 α-helices and endowed with the ability to phosphorylate shikimate [29].

B. Homology Modeling Of Shikimate Pathway Enzymes In Microbes

Homology modeling of Epsp Synthase

The first crystal structure of EPSPS was determined for the *E. coli* enzyme in its ligand free state by a research group of Monsanto in 1991 [30]. The 3-D structure of *E. coli* EPSP synthases enzyme consists of six aligned parallel alpha-helices in each of two similar EPSPS I domains. In *E. coli,* the EPSPS protein is composed of 38.88% α-helices, 20.61% extended strands and 11.48% beta turn. The crystal structure was further reported for EPSPS from *Streptococus pneumoniae* and Agrobacterium sp. Strain CP_4 [21, 30]. The overall topology of both (*S. pneumoniae* and *E. coli*) the EPSP synthase is similar but according to amino acid sequence alignment both the enzymes are 25% Xerox of each other [30, 14]. Microbial EPSP synthase variants derived from *Agrobacterium tumefaciens* sp. CP4 are not inhibited by glyphosate and provide the basis for tolerance to this herbicide in the most widely planted transgenic crops. The three-dimensional structure of CP4 EPSP synthase revealed that the enyzme exists in an open, unliganded state [20]. The double mutation Thr97Ala and Pro101Ser in class I EPSP synthase from *E. coli* produces a catalytically efficient, glyphosate-resistant enzyme and also provides the mode of action for the first commercial varieties of glyphosate tolerant maize [31].

The first glyphosate-insensitive enzyme reported was a Gly96Ala mutant of EPSP synthase from *Klebsiella*

pneumonia [32]. In mycobacteria, the shikimate pathway leads to the biosynthesis of aromatic amino acids, naphthoquinones, menaquinones and myobactin. Homologues to enzymes in the shikimate pathway have been identified in the genome sequence of *Mycobacterium tuberculosis* H37RV strain [33].

Pereira *et al* [34] describes the two molecular models of *M. tuberculosis* EPSP synthase, one without any ligand and another in complex with 3-phosphoshikimate (S3P) and glyphosate. The homology modeling was performed by using the structure of *E. coli* as template. MtEPSP synthase is an α/β protein consisting of a mixed β sheet surrounded by α helices. Bhattacharya [19], describes the molecular model of *Bordetella pertussis* EPSP synthase (BpEPSPS) in complex with 3- phosphoshikimate (S3P) and glyphosate. The homology modeling was performed using the crystallographic structure of EPSPS from *Escherichia coli*, solved to resolution 1.5 Å, as template.

Sutton *et al* [35] describe the crystal structure of 5-enolpyruvylshikimate3-phosphate (EPSP) synthase from the ESKAPE pathogen *Acinetobacter baumannii*.

Homology modeling of Dahp Synthase

DAHP synthase is main site for negative-feedback regulation of the pathway with metal based activity. The isoforms of DAHPS are specifically inhibited by one of the three aromatic amino acids in *Escherichia coli*.The first crystal structure of DAHPS was determined for *E. coli* enzyme in complexed with PEP and Pb^{2+} [36].

The three-dimensional structure of *Pseudomonas fragi*-DAHPS was predicted by homology modeling based on the crystal structure of Mt-DAHPS. Pf-DAHPS model consisted of a (β/α) (8) TIM barrel structure. Sequence alignment, phylogenetic analysis and 3D structure model classifies Pf-DAHPS as a type II DAHPS [37].

Homology modeling of chorismate synthase

On the basis of crystal structure of chorismate synthase from *Helicobacterpylori*, Marla *et al* [38] studied the comparative modeling of *Prevotell arumnicola* chorismate synthase. The predicted homology of CS from *Prevotellarumnicola* possess a tetramer consist of three layered "β-α-β sandwich fold" with highly conserved regions forming the active site with a unique FMN binding pocket. The homology of CS for *B. Melitensis* was generated using *H. pylori* CS a template. The resulted model is a tetramer with β-α-β sandwich fold in each monomer [39]. A number of anti-parasitic agents can be designed for the treatment of malaria after studying the shikimate pathway in *Plasmodium falciparum.* The crystal structure of *Helicobacter pylori* chorismate synthase (HpCS) was used to predict the structure of *Plasmodium falciparum* chorismate synthase (PfCS) along with cofactor FMN via modeling. The model was improved through structure analysis and molecular dynamics. Dimeric form of PfCS has been generated along with the proposed mechanism of FMN binding involving movement of loop near active site. The identification of active site pocket has been done and the docking of substrate 5-enolpyruvylshikimate 3-phosphate (EPSP) along with screened potent inhibitors has been studied [37].

The 3D structure of *Mycobacterium tuberculosis* chorismate synthase (MtBCS) and the geometric docking of the coenzyme FMN and the substrate EPSP were studied using the crystal structure of CS from *Streptococcus pneumoniae* as template [40]. The homology model of the *Shigella flexneri* chorismate synthase has been proposed using homology modeling method with the Flavin mononucleotide (FMN) bounding based on the known CS crystal structure [24] (Zhou *et al*., 2006).

Homology modeling of Shikimate Kinase

Cheng *et al* [41] determined the basic structure of shikimate kinase from *Helicobacter pylori.* The study of crystal structure shows a three-layer alpha/beta fold furnished with a central sheet parallel β-strands flanked by seven α-helices. 3D model of Shikimate Kinase (SK) (6 helices and 5 sheets) of *Shigella flexneri* was introduced by Arora *et al* [42] using shikimate kinase of E.coli (PDBID: 1KAG A) as template. Arora *et al* [43] also described the 3D structure of SK from 3 different strains of *Yersinia pestis* (the causative organism of Plague). The molecular model of *M. tuberculosis* shikimate kinase (MtSK) and shikimate complex was studied using docking simulations [44]. The homology modeling was performed using *Erwinia chrysanthemi* shikimate kinase as a template at a resolution better than 2.6 Å.

Structural analysis of *Bacillus anthracis* Shikimate kinase (BaSK) was done via molecular modeling approach and molecular dynamics simulations which describes main residues involved in ATP and SKM-binding. The way of interaction between ligand and protein helps in finding new inhibitors for SK and understanding the catalytic activity and rational drug design for BaSK [45].

References

1. Zhang Y. Protein structure prediction: when is it useful?. Current opinion in structural biology. 2009; 19(2):145-55.
2. Breda A, Valadares NF, de Souza ON, Garratt RC. Protein structure, modelling and applications. In Bioinformatics in Tropical Disease Research: A Practical and Case-Study Approach, Bethesda, MD, USA: Nat. Center Biotechnol. Inf. 2008. Available: http://www.ncbi.nlm.nih.gov/books/NBK6824/.
3. Dhurigai N, Daniel RR, Auxilia LR. Structure determination of leghemoglobin using homology modeling. Int. J. Curr. Microbiol. App. Sci. 2014; 3(10):177-87.
4. Sohpal VK, Dey A, Singh A. Z-score for homology modeling and structure prediction of globular protein of homosapiens & plant. International Journal of Advance Research in Science and Engineering. 2013; 2(6):56-63.
5. Mishra S. Fundamentals of Homology Modeling Steps and Comparison among Important Bioinformatics Tools: An Overview Akansha Saxena Halberg Hospital and Research Center, Civil Lines, Moradabad 244 001, UP, India Rajender Singh Sangwan Central Institute of Medicinal and Aromatic Plants, Near Kukrail Picnic Spot, PO CIMAP, Lucknow 226 015, UP, India. Science International. 2013; 1(7).
6. Brindha S, Sailo S, Chhakchhuak L, Kalita P, Gurusubramanian G, Kumar JN. Protein 3D structure determination using homology modeling and structure analysis. InColloquium www. science. 2011; 11(3):125-133.
7. Bernasconi A, Segre, AM. Ab initio methods for protein structure prediction: A new technique based on Ramachandran plots. ERCIM News. 2000; 43:13-14.
8. Yaqoob U, Kaul T, Pandey S, Nawchoo IA. In-silico characterization, structural modelling, docking studies and phylogenetic analysis of 5-enolpyruvylshikimate-3-phosphate synthase gene of *Oryza sativa* L. Med Aromat

Plants (Los Angel). 2016; 5(274):2167-0412.
9. Tzin V, Galili G. New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants. Molecular Plant. 2010; 3(6):956-72.
10. Thakur A, Gogate O, Chaudhari N, Jagtap DN. *In silico* analysis, homology modeling and evolutionary analysis of EPSP synthase enzyme from different plants. International Journal of Scientific and Research Publications. 2014; 4(2):1-8.
11. Dev A, Bodra N, Kumar P, Pratap S, Kumar P. Homology modeling and functional characterization of three-dimensional structure of DAHP synthase from *Brachypodium distachyon*. Journal of Proteins & Proteomics. 2013; 4(1).
12. Herrmann KM. The shikimate pathway: early steps in the biosynthesis of aromatic compounds. The Plant Cell. 1995; 7(7):907.
13. Poulin J. The role of 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase 1 in *Arabidopsis thaliana* metabolism (Doctoral dissertation). 2011.
14. Schönbrunn E, Eschenburg S, Shuttle worth WA, Schloss JV, Amrhein N, Evans JN, Kabsch W. Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail. Proceedings of the National Academy of Sciences. 2001; 98(4):1376-80.
15. Herrmann KM, Weaver LM. The shikimate pathway. Annual review of plant biology. 1999; 50(1):473-503.
16. Reis LF, Van Sluys MA, Garratt RC, Pereira HM, Teixeira MM. GMOs: Building the future on the basis of past experience. Anais da Academia Brasileira de Ciências. 2006; 78(4):667-86.
17. Funke T, Healy-Fried ML, Han H, Alberg DG, Bartlett PA, Schönbrunn E. Differential inhibition of class I and class II 5-enolpyruvylshikimate-3-phosphate synthases by tetrahedral reaction intermediate analogues. Biochemistry. 2007; 46(46):13344-51.
18. Lin M, Tang K, Wang Y, Wang J, Zhu Y, inventors; Sichuan Heben Biotic Engineering Co Ltd, assignee. EPSP synthase highly tolerant to glyphosate. United States patent US 7, 238,508. 2007.
19. Bhattacharya S. Combatting the whooping cough bacteria: *In-silico* approaches. Nature & Environment. 2015; 20(2):64-72.
20. Garg B, Vaid N, Tuteja N. In-silico analysis and expression profiling implicate diverse role of EPSPS family genes in regulating developmental and metabolic processes. BMC research notes. 2014; 7(1):58.
21. Funke T, Han H, Healy-Fried ML, Fischer M, Schönbrunn E. Molecular basis for the herbicide resistance of Roundup Ready crops. Proceedings of the National Academy of Sciences. 2006; 103(35):13010-5.
22. Pollegioni L, Schonbrunn E, Siehl D. Molecular basis of glyphosate resistance–different approaches through protein engineering. The FEBS journal. 2011; 278(16):2753-66.
23. Baerson SR, Rodriguez DJ, Tran M, Feng Y, Biest NA, Dill GM. Glyphosate-resistant goosegrass. Identification of a mutation in the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Plant physiology. 2002; 129(3):1265-75.
24. Zhou M, Xu H, Wei X, Ye Z, Wei L, Gong W, Wang Y, Zhu Z. Identification of a glyphosate-resistant mutant of rice 5-enolpyruvylshikimate 3-phosphate synthase using a directed evolution strategy. Plant physiology. 2006; 140(1):184-95.
25. Goyal M, Chauhan S, Kumar P. In silico analysis, structural modeling and phylogenetic analysis of EPSP synthase of *Phaseolus vulgaris*. Agricultural Science Digest. 2017; 37(3):185-90.
26. Feng PC, Qi Y, Chiu T, Stoecker MA, Schuster CL, Johnson SC, Fonseca AE, Huang J. Improving hybrid seed production in corn with glyphosate-mediated male sterility. Pest management science. 2014; 70(2):212-8.
27. Maeda H, Dudareva N. The shikimate pathway and aromatic amino acid biosynthesis in plants. Annual review of plant biology. 2012; 63:73-105.
28. Zhou CZ, de La Sierra-Gallay IL, Quevillon-Cheruel S, Collinet B, Minard P, Blondeau K, Henckes G, Aufrère R, Leulliot N, Graille M, Sorel I. Crystal structure of the yeast Phox homology (PX) domain protein Grd19p complexed to phosphatidylinositol-3-phosphate. Journal of Biological Chemistry. 2003; 278(50):50371-6.
29. Liu Z, Zhu Q, Li Y, Yu J, Wang W, Tan R, Zhou J, Liao H. Isolation and *in silico* characterization of a shikimate kinase from *Cassia obtusifolia*. Acta physiologiae plantarum. 2015; 37(4):85.
30. Stallings WC, Abdel-Meguid SS, Lim LW, Shieh HS, Dayringer HE, Leimgruber NK, Stegeman RA, Anderson KS, Sikorski JA, Padgette SR, Kishore GM. Structure and topological symmetry of the glyphosate target 5-enolpyruvylshikimate-3-phosphate synthase: a distinctive protein fold. Proceedings of the National Academy of Sciences. 1991; 88(11):5046-50.
31. Funke T, Yang Y, Han H, Healy-Fried M, Olesen S, Becker A, Schönbrunn E. Structural basis of glyphosate resistance resulting from the double mutation Thr97→ Ile and Pro101→ Ser in 5-enolpyruvylshikimate-3-phosphate synthase from *Escherichia coli*. Journal of Biological Chemistry. 2009; 284(15):9854-60.
32. Eschenburg S, Healy ML, Priestman MA, Lushington GH, Schönbrunn E. How the mutation glycine96 to alanine confers glyphosate insensitivity to 5-enolpyruvyl shikimate-3-phosphate synthase from *Escherichia coli*. Planta. 2002; 216(1):129-35.
33. Cole S, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry Iii CE, Tekaia F. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature. 1998; 393(6685):537.
34. Pereira JH, Canduri F, de Oliveira JS, da Silveira NJ, Basso LA, Palma MS, de Azevedo Jr WF, Santos DS. Structural bioinformatics study of EPSP synthase from *Mycobacterium tuberculosis*. Biochemical and biophysical research communications. 2003; 312(3):608-14.
35. Sutton KA, Breen J, Russo TA, Schultz LW, Umland TC. Crystal structure of 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase from the ESKAPE pathogen *Acinetobacter baumannii*. Acta Crystallographica Section F: Structural Biology Communications. 2016; 72(3):179-87.
36. Shumilin IA, Kretsinger RH, Bauerle RH. Crystal structure of phenylalanine-regulated 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase from *Escherichia coli*. Structure. 1999; 7(7):865-75.
37. Tapas S, Patel GK, Dhindwal S, Tomar S. *In silico* sequence analysis and molecular modeling of the three-dimensional structure of DAHP synthase from *Pseudomonas fragi*. Journal of molecular modeling.

2011; 17(4):621-31.
38. Marla S, Yalamanchili HK, Gelli P, Singh HK, Praveen G, Ghatta G, Srikanth S, Goutham K. Comparative structure analysis of chorismate synthase. OJB. 2006; 7:35-45.
39. Maskar AU, Meshram RJ. Homology modeling of chorismate synthase from *Brucella melitensis*: A novel target molecule. Research & Reviews: Journal of Microbiology and Biotechnology. 2013; 2(3):7-18.
40. Fernandes CL, Breda A, Santos DS, Basso LA, de Souza ON. A structural model for chorismate synthase from *Mycobacterium tuberculosis* in complex with coenzyme and substrate. Computers in biology and medicine. 2007; 37(2):149-58.
41. Cheng WC, Chang YN, Wang WC. Structural basis for shikimate-binding specificity of *Helicobacter pylori* shikimate kinase. Journal of bacteriology. 2005; 187(23):8156-63.
42. Arora N, Banerjee AK, Murty US. In silico characterization of Shikimate Kinase of *Shigella flexneri*: A potential drug target. Interdisciplinary Sciences: Computational Life Sciences. 2010; 2(3):280-90.
43. Arora N, Narasu ML, Banerjee AK. Shikimate Kinase of Yersinia pestis: A sequence, structural and functional analysis. Int J Biomed Data Min. 2016; 5(119):2.
44. de Azevedo WF, Canduri F, de Oliveira JS, Basso LA, Palma MS, Pereira JH, Santos DS. Molecular model of shikimate kinase from *Mycobacterium tuberculosis*. Biochemical and Biophysical Research Communications. 2002; 295(1):142-8.
45. Pauli I, Caceres RA, de Azevedo Jr WF. Molecular modeling and dynamics studies of Shikimate Kinase from *Bacillus anthracis*. Bioorganic & medicinal chemistry. 2008; 16(17):8098-108.