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Review Paper

A brief Review on Plant Type III Polyketide Synthases, an Important Group of Enzyme of Secondary Metabolism

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Abstract

From the ancient time, plants have been used for the treatment of several different types of human health disorders. The main bioactive compounds which exert pharmacological effect are derived from secondary plant metabolism. Several different groups of secondary metabolic compounds with different chemical structure and pharmacological properties are produced in plants. Among such different groups of secondary metabolic compounds, the compounds produced by simple modification of the basic chalcone or stilbene backbone have drawn interest of researcher working in this field. The enzyme(s) responsible for the formation of such chalcone or stilbene backbone structures are collectively known as type III polyketide synthase. These chalcone or stilbene backbone structures are further modified in enzymatic process within the plant cell and thus acts as the precursor substrate(s) in the biosynthesis of an array of compounds with significant pharmacological properties. The existence of enzyme(s) within this group producing functionally diversified products with immense pharmacological properties made them a good candidate for studying in molecular level. Research involving protein engineering approach to modify the enzyme architecture through modification in gene level for altering the substrate specificity, number of condensation reaction and cyclization of the intermediate product to alter the compound structure are getting priorities. A brief overview of this group of enzyme, their evolutionary significance, state of research in molecular level and recent interesting findings are presented here.

Keywords: Plant type III polyketide synthase, secondary metabolism, natural product, polyketide synthase, CHS/STS superfamily, chalcone synthase, stilbene synthase.

Introduction

Plants being the nature's most precious gift serve as the source of foods, raw materials and medicine. The cultivation of plants for medicinal purpose records long back. Plants or plant parts have been used to treat several human diseases from the early days of civilization. The use of traditional medicines using plants or plant parts has also been found in almost all human culture. The knowledge of traditional medicines was handed down orally or in the form of text book from generation to generation for millennia. Even in today's medicine practitioners in Homeopathy, Unani, Ayurvedic and also in Allopathic system, suggest many medicines that are prepared from plants or plant parts or from plant derived chemicals. Plant produces a plethora of compounds, some of which are important for its own metabolism and are likely to be present in almost all group of plant in the plant kingdom, the primary metabolites. While some other compounds that are also produced by plants, do not play any essential role for plant's survival and maintenance, but are helpful in protecting plants from pathogen infection, herbivore attack, UV irradiation and other biotic and physical stresses[1]. These groups of compounds are known as secondary metabolites. Plant produces a wide range of secondary metabolic compounds with different chemical structures and biochemical properties. They have been classified differently according to their structural and bio-chemical properties.

The widely accepted and well cited system is of J.B. Harbone's[2] classification system, according to which they fall in three distinct groups including phenolics, terpenoids, alkaloids and other nitrogen containing compounds. All these secondary metabolic compounds possess significant pharmacological properties and are responsible for the medicinal property of plants or any part of a plant. These are the key compounds that present collectively or in individual group or sometimes as a single compound in some specific parts of a medicinal plant or in whole plant rendering the significant pharmacological property. The biosynthetic pathways generating different types of phenolics have been well studied. All the phenolic metabolites are generated from the central phenylpropanoid pathway which starts with the deamination of phenylalanine or tyrosine which formed from in the shikimate pathway. This is the step which relates shikimate pathway to phenylpropanoid pathway allowing the flow of energy towards generating a large number of phenolic phytocompounds[3].

The two enzymes, phenylalanine ammonium-lyase and tyrosine ammonium-lyase are two most important enzymes catalyzing the deamination of phenylalanine and tyrosine, respectively. Enzymatic activities downstream the pathway, generates different CoA-esters which serve as the precursor molecule to generate the basic chalcone or stilbene backbone in an enzymatic reaction which is similar to fatty acid biosynthetic pathway. The enzymes generating chalcone or stilbene are known as chalcone synthase (CHS) or stilbene synthase (STS), respectively. These enzymes are very similar to each other and the enzymatic steps in generating basic chalcone and stilbene are also very similar. These enzymes are collectively known as type III polyketide synthases (PKS). This enzyme system has been well studied in molecular level and it was found that they have a lot of similarity in genetic level, resulting in the conclusion that they belong to the same super-family of enzyme known as chalcone or stilbene synthase superfamily. The basic chalcone or stilbene like backbone structures are further modified by several different groups of enzyme within the plant cell to generate a large number of phenolic phytoalexins, e.g. members of flavonoid subclasses, anthoayanins, xanthones, chalcones, stilbenes, stilbenoids etc. The enzymatic reaction that generates the basic chalcone or stilbene backbone is irreversible and thus considered to be the first committed step in this biosynthetic pathway. All of these phenolics phytoalexins have significant pharmacological potential.

Type III polyketide synthases in plants

All the different type III polyketide synthases which have been isolated and characterized from plants are homodimeric proteins. Each monomer of the dimeric protein contains its own active site and catalyze the sequencial condensation of starter CoA molecule and one acetyl unit from malonyl-CoA, independently. Each condensation step is associated with one decarboxylation step. The starter CoA molecules are generated as ester forms in the central phenylpropanoid pathway. The resulting polyketide backbone structure produced in the enzymatic reaction, serves as the precursor molecule of a large variety of phenolic phytoalexins. The downstream hydroxylation, glycosylation, acylation, O-methylation, prenylation, and conjugation reaction modify the basic molecule resulting in the generation of a wide variety of compounds in each step[4]. Among the starter substrates, *p*-coumaroyl CoA has the most significant role in generating the direct precursor of flavonoids, the basic chalcone backbone.

The first type III PKS enzymes discovered in plant is chalcone synthase which accepts 4-coumaroyl CoA as starter substrate to produce the basic chalcone backbone. Further studies revealed the occurrence of a natural enzyme which also accepts *p*-coumaroyl CoA and malonyl-CoA as starter substrates, but produce a structurally different end product. The tetraketide intermediate which is formed in both the enzymatic reactions cyclizes in a different way (figure-1) to form structurally different end product. The latter group of enzyme is known as stilbene synthase (STS) which forms anti-fungal phytoalexin resveratrol in an intramolecular aldol condensation reaction. Although there are several differences in reaction mechanism, but the enzyme reaction involving sequential addition of starter substrate fully resembles to that of fatty acid biosynthesis reaction of primary metabolism. All the polyketide synthases including those which have been reported from plants resemble to fatty acid synthase (FAS) and in all enzymatic reactions, the incorporation of one acetyl unit from malonyl-CoA in each step has been noted. Due to a high resembles to fatty acid synthases (FAS) which exist in nature from long ago, the evolutionary origin of plant type III PKSs from FAS has been hypothesized. In both CHS and STS catalyzed reaction, sequential addition of starter phenylpropanoid-CoA and malonyl-CoA results in tetraketide intermediate formation, but the final step of cyclization is different for these two enzymes. In CHS type reaction, cyclization occurs via intramolecular $C_6 \rightarrow C_1$ claisen condensation while in STS type reaction the cyclization occurs via intramolecular $C_2 \rightarrow C_7$ aldol condensation followed by a decarboxylative loss of one carbon molecule in the form of carbon-di-oxide[5].

The first report[6] on the isolation of gene encoding chalcone synthase came in the year 1983 and then some years later, in 1988 cDNA sequence of resveratrol synthase, a stilbene synthase was reported by Schröder *et al*.[7] After that, a huge number of genes similar in sequences to CHS and STS have been reported. This indicates the existence of a gene family in plants encoding CHS or STS like sequences resulting in the generation a large number of natural polyketide backbone. For this reason, all the type III PKSs isolated from different plant species are grouped under a superfamily. All the plant type III PKSs reported till date mainly differ in specifying starter substrates, determining the number of condensation reaction steps and the cyclization reaction pattern[8]. They accept a wide variety of starter substrates.

The variety ranges from small substrate like acetyl-CoA to somewhat bigger N-methylanthraniloyl-CoA as well as the NAC-esters of phenylpropanoid-CoA, aliphatic to aromatic CoAs and also polar substrates like malonyl CoA to nonpoplar substrates like hexanoyl CoA. The number of condensation step generally varies from one to three, but more than three condensation steps in polyketide biosynthesis are not uncommon. Sometimes, the number of condensation step used by some novel plant PKSs reaches up to eight. The cyclization reaction used varies in different enzymes. Some use $C_6 \rightarrow C_1$ claisen condensation as characterized by all CHS and CHS like PKSs, while some PKSs use $C_2 \rightarrow C_7$ aldol condensation, the characteristic nature of STS cyclization reaction. Sometimes,

C_5Oxygen→C_1 lactonization is noticed which results in the formation of derailment product in PKS reaction as well as in case of some novel PKSs[9]. Lactonization reaction has been noticed as by-default step when the enzyme can't proceed further in condensing acetyl units from malonyl-CoA that may be due to enzyme's three dimensational architecture.

Thus, the type III PKSs can generate large number of functionally divergent end products simply by altering substrate specificitiy, modifying condensation reaction steps and by adopting different cyclization pattern[10]. The example of pyrones, resorsinols, acridone, benzophenones, biphenyl, bibenzyle, phlorglucinol strongly supports this hypothesis. The PKSs which produce these end products that are structurally different from basic chalcone or stilbenes are considered to be unique in nature. It is a fact that these polyketide synthases have sequence similarity with basic chalcone synthase or stilbene synthase, but due to a few amino acid differences in sequence, these PKSs produce completely different type of products with specialized functions. These novel type III PKSs have become the central point of study. Studying the structure function relationship by site directed mutagenesis to generate novel type III PKS or polyketide bioengineering has become an interesting area of research aimed to generate new PKS enzyme that can produce pharmaceutically important natural product(s). Scientists are also interested to know the evolutionary significance of these novel type III PKSs as they share a good range of sequence similarity with chalcone synthase and stilbene synthases.

Novel type III PKS in plants

Although chalcone synthase is the most widely distributed type III PKS found in plants, the continued research in this area and homology based cloning as well as genome mining revealed several CHS like sequences which produce functionally divergent PKSs. Examples of such functionally diversified type III PKSs include acridone synthase (ACS)[11], 2-pyrone synthase (2-PS)[12,13], benzalacetone synthase (BAS)[14], phlorisovalerophenone synthase (VPS)[15], benzophenone synthase (BPS)[16], biphenyl synthase (BIS)[17], bibenzyl synthase (BBS)[18], olivetol synthase (OLS)[19], pentaketide chrome synthase (PCS)[20,21], alesone synthase (ALS)[22], octaketide synthase (OKS)[23], coumaroyl triacetic acid synthase (CTAS)[24], stilbenecarboxylate synthase (STCS)[13] and curcuminoid synthase(CURS)[25,26].

Continued discovery of increasing number of novel polyketide synthases inspires further extensive studies in determining the crystal structure and site specific mutagenesis study to understand the function of specific amino acid residue important in changing the function of the enzyme. The structure-functional relationship study by site directed mutagenesis has become more popular with the aim to identify specific amino acid residue (s) responsible for the generation of novel polyketide. The knowledge can be used in structure based engineering of novel polyketide synthases. Since after the first report of crystal structure of chalcone synthase from *Medicago sativa* by Ferrr *et al*.[5], the function of amino acids in determining substrate specificity, programming between the condensation reactions and choosing the cyclization pattern becomes more clear and in all most all studies, the new sequences have been compared with respect to *Medicago sativa* chalcone synthase (CHS2).

It is quite clear that in all type III PKSs reported in different plant species, three amino acids forming the catalytic triad inside the active site cavity is fully conserved. Among the three amino acids, cysteine 164 binds with the starter residues and intermediates with covalent bonding, while Histidine 303 and Asparagine 336 (Asn 336) stabilizes and or activates both starter as well as extender units[5]. The amino acid residues which are important and directly involved in the enzyme catalysis process in the initiation and elongation poket inside the active site cavity have been identified from the crystal structure of *M. sativa* chalcone synthase. The amino acid residues in initiation and elongation pocket of various different novel type III PKSs have been compared with the *Medicago sativa* chalcone synthase. This information helps to identify the specific amino acid residue responsible for the catalytic activity of the particular PKS. This is further confirmed by replacing the amino acid in the particular position by site directed mutagenesis and forms the basis of polyketide bioengineering.

Aspects of polyketide bioengineering

Changing in the catalytic property by changing a single amino acid residue is of great interest for the scientists and has significance in bioengineering of type III PKSs. Several attempts have been taken to change the function by changing a single amino acid residue. Several such examples are also available, among which the conversion of pentaketide chrome (PCS) to octaketide synthase (OKS) is well noticed. Both the enzymes exist in the same plant, *Aloe arborescens* and they also share a high sequence similarity, although their function is different[20].

The conserved amino acid residues in the active site of CHS (Thr197, Gly256 and Ser338) has been changed in natural gene sequence of PCS by Met, Leu and Val respectively, while in OKS by Gly, Leu and Thr respectively. A single point mutation (M207G) in the PCS sequence at the amino acid position M207 corresponding to the Thr197 of *M. sativa* chalcone synthase converts the enzyme into octaketide synthase, while a single point mutation in similar position in OKS, G207M, converts the enzyme into PCS[21]. Both the enzymes use malonyl-CoA as one of the substrates, but the number of condensation reactions they use differs, as in PCS five molecules of malonyl-CoA is used

while in OKS eight molecules of malonyl-CoA is used to generate the final end product. Thus, this example serves as good evidence that a single point mutation in the amino acid residue within the active site can change the enzyme's catalytic efficiency and increases or decreases the number of condensation steps in PKS catalysed reaction.

Similar replacement of amino acid residues in the active site of several type III polyketide synthases possessing novel properties, found in different plants, has also been noticed. The examples include 2-pyrone synthase (2-PS) found in *Gerbera hybrida*, aloesone synthase (ALS) found in *Rheum palmatum* and also in benzophenone synthase (BPS) found in *Hypericum androsaemum*. *G. hybrida* 2-PS contains there different amino acids (Leu202, Leu261 and Ile343) in the active site at similar position corresponding to conserved amino acid residues in *M. sativa* CHS2 active site.

It has been observed that replacing the amino acid residues in the active site of *M. sativa* chalcone synthase with the amino acids present similar in position to 2-PS, the CHS activity of the resulting triple mutant (T197L/G256L/S338I) changed to 2-pyrone synthase due to the reduction in the volume of active site cavity[27]. Although in *Rheum palmatum* aloesone synthase (ALS) in which Ala, Leu and they are present at the position similar to the conserved active site residues of *M. sativa* CHS, it has been observed that replacing the amino acids with that of *M. sativa* CHS conserved amino acid residues, changes the enzyme activity. Instead of producing heptaketide aloesone, the resulting triple mutant generated (A197T / L256G / T338S) in ALS, produce tetraketide coumaroyl tri-acetic acid lactone using *p*-coumaroyl-CoA as starter substrate[28]. Similar amino acid substitutions were also introduced at the corresponding position (T200L, A260L and G342I) of *Hypericum androsaemum* benzophenone synthase (BPS). The resulting triple mutant (T200L/A260L/G342I) was reported to be inactive with both benzoyl-CoA and acetyl-CoA[29].

The protein sequences of *H. androsaemum* CHS has nearly 60% amino acid similarity with the protein sequence of benzophenone synthase (BPS) found in the same plant. Site directed mutagenesis in *H. androsaemum* CHS to exchange the amino acid residues with the amino acid residues present in BPS at the position similar to the conserved active site residues of *M. sativa* CHS, resulting in the formation of a triple mutant (L263M/F265Y/S338G) (numbered corresponding to *M. sativa* CHS) with altered substrate specificity[16]. The triple mutant prefers benzoyl-CoA as substrate while coumaroyl-CoA is the best preferred substrate for *H. androsaemum* CHS.

The most significant result of site directed mutagenesis in *H. androsaemum* BPS is the single amino acid substitution T135L (numbered corresponding to *M. sativa* CHS) which transforms BPS into a functional phenylpyrone synthase, restricting the progress of the enzymatic reaction at the triketide level[29]. Chalcone synthase and stilbene synthase being the same type III polyketide synthase, use two different cyclization mechanism to form the final product using the same linear tetraketide intermediate. It was quite interesting to find out the scientific reason behind the phenomenon. After the publication of the crystal structure of *M. sativa* CHS2 and *Pinus sylvestris* stilbene synthase, several observation made by different group of scientists. Noel and Schröder proposed the formation of a hydrogen bond network involving Ser338-H_2O-Thr132-Glu192 (numbering in *M. sativa* CHS2) near the cysteine residue in the active site cavity that plays the key role in specifying the cyclization reaction.

Thus, Thr132 in this area is involved in determining and balancing the cyclization reaction in CHS and STS[9]. In case of *Ruta graveolens* acridone synthase (ACS), it has been observed that three amino acid residues serine, alanine and valine at position 132, 133 and 265 respectively (numbered corresponding to *M. sativa* CHS) play the vital role in specifying the starter substrate which is N-methylanthraniloyl-CoA, to produce acridone alkaloid. A triple mutation (S132T/A133S/V265F) in ACS, transformed it into functionally identical[30] CHS. Recently reported type III PKS cDNA encoding quinolone synthase from *Aegle marmelos* contains Ser and Ala at position 132 and 133 respectively, while Thr and Ser occupy the similar position, 132 and 133 respectively in *M. sativa* CHS. In site directed mutagenesis study, it has been observed that both the residues are important for correct functioning of the enzyme and replacement of Ser 132 and Ala 133 with Thr and Ser respectively results in the formation of chalcone utilizing coumaroyl- CoA as substrate due to the reduction of volume of active site cavity resulted from the constriction of the cavity[31]. A single point mutation in *R. graveolens* benzalacetone synthase (BAS), S33V, increases the enzyme activity as observed by the 2-fold increase in benzalacetone-forming activity[32,33].

Thus, the results of the mutagenesis study involving different plant polyketide synthases reveal much more information about the catalytic mechanism of the enzyme. A clearer conclusion can be drawn when the crystal structure of the enzymes will be available. Research in this area is being continued and new sequences of polyketide genes with novel functionality are being reported. In case of almost all genes, the correct amino acid residues responsible for the proper functioning of the enzyme are being determined through mutagenesis study.

All these information along with the detail knowledge of full crystal structure of novel type III PKSs are helpful in engineering novel enzymes and thus forms the basis of polyketide bioengineering.

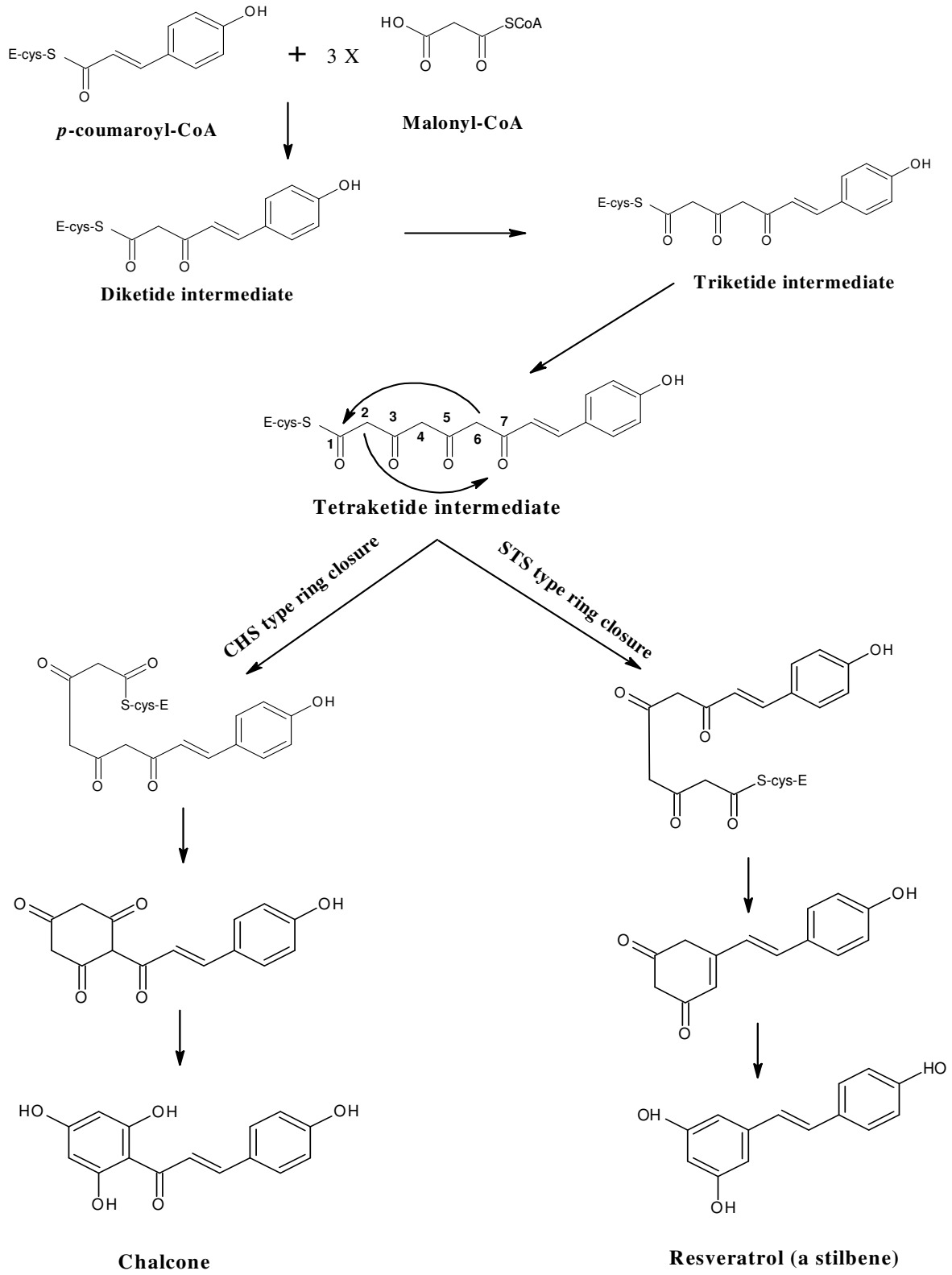

Figure-1
Enzymatic formation of chalcone and resveratrol in CHS and STS catalyzed reaction respectively using *p*-coumaroyl-CoA and malonyl-CoA as starter substrates. In both cases, the intermediate is formed, but cyclized pattern differs as evident from the figure, resulting in the formation of two different types of final products

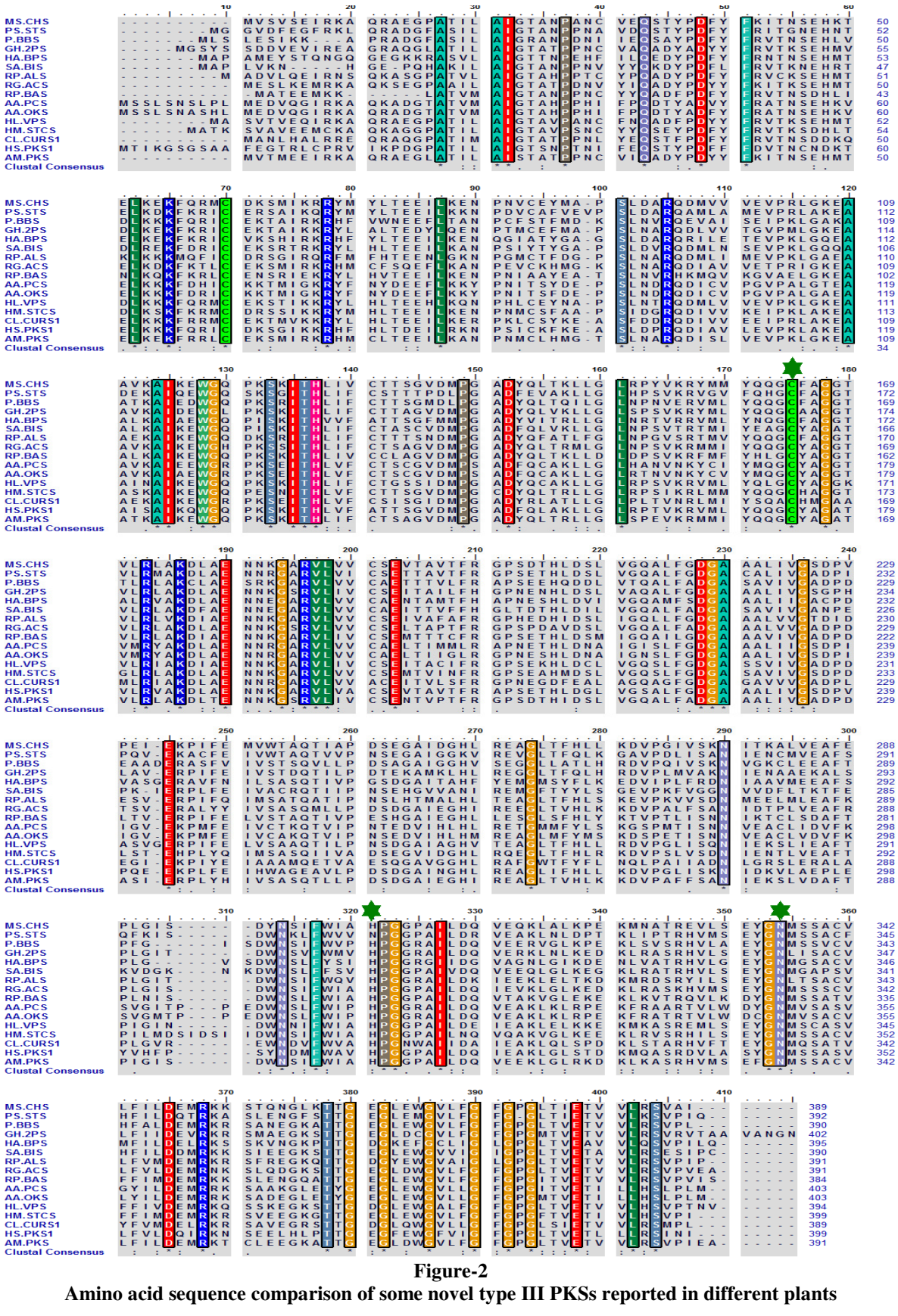

Figure-2
Amino acid sequence comparison of some novel type III PKSs reported in different plants

The sequences are aligned by Clustal W using Bio Edit (free version) software tool. The amino acid residues Cystein, Histidine and Asparagine that forms the catalytic triad are marked with a star symbol. The residues which are common and conserved are grouped. The clustal consensus has shown at the bottom. Abbreviations with Gene Bank accession numbers: MS.CHS, *Medicago sativa* chalcone synthase (P30074); PS. STS, *Pinus sylvestris* stilbene synthase (AAB24341); PS.BBS, *Phalaenopsis* sp. bibenzyl synthase (CAA56276); GH.2PS, *Gerbera hybrida* 2-pyrone synthase (P48391); HA.BPS, *Hypericum androsaemum* benzophenone synthase (AAL79808); SA.BIS, *Sorbus aucuparia* biphenyl synthase (ABB89212); RP.ALS, *Rheum palmatum* aloesone synthase (AAS87170); RG.ACS, *Ruta graveolens* acridone synthase (CAC14058); RP.BAS, *Rheum palmatum* benzalacetone synthase (AAK82824); AA.PCS, *Aloe arborescens* pentaketide chromone synthase (AAX35541); AA.OKS, *Aloe arborescens* octaketide synthase (AAT48709); HL.VPS, *Humulus lupulus* phlorisovalerophenone synthase (BAA29039); HM.STCS, *Hydrangea macrophylla* L. stilbenecarboxylate synthases (AAN76182); CL.CURS1, *Curcuma longa* curcuminoid synthase (AB495007); HS.PKS1, *Huperzia serrata* PKS1 (ABI94386); AM.PKS1, *Aegle marmelos* PKS1 (AGE44110).

Evolutionary significance of novel plant type III PKSs

Plant's secondary metabolic pathways, genes and the produced chemicals are always considered to be stress associated. Many of the metabolic pathways evolved to generate chemicals to form the cuticular layer; several different phenolic compounds giving protection against pathogen attack, desiccation and UV exist in nearly all the land plants. Gradually different pathways evolved to generate chemicals that constitute colour, flavor, scents and several other chemicals which plays important role in specific environmental conditions. Chalcone Synthase being the oldest enzyme reported, produce basic chalcone scaffold which serve as template for the production of several natural products which are used by the plant as antimicrobials to combat the microbial attack, UV screening agent to get rid of the deleterious effect of UV rays, floral pigment to attract insects facilitating pollination (e.g. anthocyanin pigment), inducer in root nodule formation and thus helpful in plant-microbe interaction. Genome wide extensive search has been carried in many plant species and it has been noticed that the chalcone synthase or CHS like sequences are present in duplicate or even triplicate form in plant's genome.

The theory of divergent evolution put forward after the close observation that the CHS gene present in more than one copy number in plant genome and has close resemblance with other novel type III polyketide synthases. Thus the formation of a large number of homologous enzymes having either the same function as CHS has been explained. The gradual divergence of the sequence results in the generation of new genes which does not exactly produce chalcone as end product, rather somewhat related and sometimes special end product with pharmaceutical importance[34, 35]. The existence of type III PKSs is not only limited to plant kingdom and bacteria, but their presence has also been reported in some filamentous fungi[36].

According to the bioinformatics analysis of protein sequences of type III PKSs done by Mallika[37] *et al*., type III PKSs protein of bacterial origin showed less than 50% sequence similarity, but in higher groups of plants they are more than 80% (sometimes >90%) similar to CHS and less than 70% to CHS like sequences. In a phylogenetic tree prepared by Mallika[37] *et al*., based on the sequence similarity of all the reported polyketide synthases from plants and some of fungal and bacterial PKS, it is evident that the bacterial, fungal and plant PKSs are present in separate lineage and the PKSs from lower plants (e.g. plants under the subdivision bryophyte and pteridophyta) are present just after the fungal cluster, suggesting the evolutionary origin of the CHS or CHS like enzymes from the early existed microbial community[37]. Bacterial CHS like enzymes do not have so much sequence divergence as compared to plants and it is an indication of their independent evolution from ancient type III PKSs or keto-acyltransferase III. It is believed that the ability of utilize the phenylpropanoid derived starter substrates or the ability of sequential condensation steps arose earlier in course of evolution and later additional CHS like activity evolved gradually. The presence of strylpyrone natural product in the primitive plant *Equisetum* (horsetail) supports the hypothesis[38]. Frequently found 6'-deoxychalcone and its glucoside which are often thought to be restricted to legumes are also found in *Selaginella doederleinii*.

More recent studies on *Psilotum nudum* type III PKSs reveal the presence of CHS, STS and phloro-isovalerophenone synthase (VPS) activity in this most primitive vascular plant[39]. All these reports indicate the early evolutionary origin of the novel polyketide synthases which are presently being reported in several different plants. Based on the report of a bioinformatic analysis which reveal that in grasses, duplication of CHS gene occurs in every 15-25 million years, some group of scientists believe that the evolution of present day novel type III polyketide synthases might have took place only in a few million years[10,37].

Duplication is not only the process to take part, scientists also consider the role of genetic drift which can introduce stop codons resulting in the disruption of open reading frame (ORF) which leads to the production of truncated proteins that are unable to fold in proper structure and lack catalytic property[10]. This theory can also be applied to explain the existence of inactive gene sequences within the genome as observed in case of *Psilotum nudum*[39]. Although there are many possible hypothesis explaining the origin of novel type III PKSs, but the formation of such gene from its early ancestor like fatty acid synthase is not yet fully understood. Chalcone synthase gene itself or CHS like genes may present in more copies within the genome due to the result of gene duplication. This may be

associated with further mutation in the open reading frame resulting in the generation of novel type PKSs and also inactive sequences which has generated in due course of evolution, keeping the original CHS activity undisturbed which allow the biosynthesis of flavonoids or downstream compounds to be continued required for the normal physiological functioning of the plant.

Conclusion

The varieties of bioactive compounds found in plant are plenty in number and thus the chemo diversity in nature is beyond our imagination. If we think about the biodiversity of plants on earth, then we can easily have an understanding of the situation. After the discovery of penicillin, there was a trend in mass screening of microorganisms for antibiotics. Gradually, natural products or natural product derived compounds become more popular in pharmacological market and effectively use in treating several human health disorders. Although only a minority of plant species have analyzed phytochemically, the varieties of the phytocompounds used in pharmacological market are huge in number. The compounds derived from the modification of basic polyketide backbone only contribute to a few among such bioactive compounds. Due to the broad spectrum functionality on human health, these compounds become pharmaceutically more important.

As a consequence, the study of the biosynthetic origin of the polyketide derived compounds, biochemically and molecular genetically becomes more popular and getting prioritized. Progress in the field of genomics, proteomics, transcriptomics, metabolomics and development of advanced bioinformatics tools become a boon for the human health care sector. With the help of these advanced techniques, there is an emerging trend in natural product research using system biology approach towards identifying the gene of interest, cloning in heterologous host and its further modification to produce new compounds with enhanced bioactivity. The crystal structure of several common and novel type III PKSs are now available. This knowledge will help to design and develop new enzymes with novel catalytic properties aiming to generate unnatural novel polyketide backbone. Recent success in generating unnatural novel polyketide-alkaloid scaffold using synthesized nitrogen containing substrate for an engineered enzyme[40] proves the possibility of generating novel pharmacological important unnatural natural product using the engineered polyketide enzyme system.

Further, using appropriate combination of modifying enzymes, new novel compounds with enhanced therapeutic potential can be produced using the polyketide backbone as base scaffold. The research in this field is not limited to this, further manipulation of plant's own metabolism by introducing genes encoding novel polyketide scaffold for designing pathogen resistant plants become more interesting and popular. So, it seems that there are enormous scopes of research in the area involving molecular biological and biochemical techniques to explore new and novel polyketides from different plants, isolating the genes, their further cloning and heterologous expression, identifying the particular amino acid residue responsible for the novel functionality and applying protein engineering approach to design new enzyme which can produce valuable pharmaceutical compounds of immense importance. Further investigating the effect of these compounds on plant pathogen, designer crops with pathogen resistance property may also be produced through genetic engineering. Such plant will have medicinal property due to the presence of the polyketide compound and also will be pathogen resistant. Thus the area of research becomes interdisciplinary involving the participation of plant biologists, plant molecular biologist, biochemists, protein engineers, genetic engineers, biotechnologists, pharmaceutical biologists and also from related background. The field of research is emerging due to the importance of the products in human health care sector and thus appears to be very promising in near future.

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