

Alkylresorcinol biosynthesis in plants

New insights from an ancient enzyme family?

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Alkylresorcinols are members of an extensive family of bioactive compounds referred to as phenolic lipids, which occur primarily in plants, fungi and bacteria. In plants, alkylresorcinols and their derivatives are thought to serve important roles as phytoanticipins and allelochemicals, although direct evidence for this is still somewhat lacking. Specialized type III polyketide synthases (referred to as 'alkylresorcinol synthases'), which catalyze the formation of 5-alkylresorcinols using fatty acyl-CoA starter units and malonyl-CoA extender units, have been characterized from several microbial species, however until very recently little has been known concerning their plant counterparts. Through the use of sorghum and rice EST and genomic data sets, significant inroads have now been made in this regard. Here we provide additional information concerning our recent report on the identification and characterization of alkylresorcinol synthases from *Sorghum bicolor* and *Oryza sativa*, as well as a brief consideration of the emergence of this intriguing sub-family of enzymes.

Introduction

Alkylresorcinols are members of a family of compounds referred to as phenolic lipids, which have been identified in numerous plant, fungal and bacterial taxa, but relatively few animal species. Among the major classes of phenolic lipids, which

include alkylphenols, alkylresorcinols, anacardic acids and alkylcatechols, the alkylresorcinols are by far the most prevalent in nature.¹ In higher plants, alkylresorcinols typically occur as mixtures of homologues possessing side chains of 13 to 27 carbons with varying degrees of saturation. Fungi and bacteria similarly accumulate mixtures of alkylresorcinols with varying chain lengths, however microbial homologues all possess saturated side chains.² The potent antimicrobial activity demonstrated for many naturally occurring alkylresorcinols and alkylresorcinol derivatives in in vitro assays has led to the widely held view that these compounds primarily serve a defensive role in plants.^{1,3}

The highly localized pattern of deposition within regions surrounding plant structures coupled with their demonstrated antimicrobial activity, could indicate a role for alkylresorcinols in the formation of defensive chemical barriers. For example, significant accumulation of alkylresorcinols occurs within grains of cereals such as wheat, rye, triticale and barley, existing within a thin cuticle layer external to the seed coat.^{4,5} Alkylresorcinols have also been found to be concentrated within the cuticles of rye leaves, with similar homolog compositions occurring on the adaxial and abaxial leaf surfaces.⁶ Root systems of *Oryza* spp. exude an alkylresorcinol mixture,⁷ and likewise, *Sorghum* spp. exudates contain the alkylresorcinol derivative sorgoleone, which is an allelochemical also possessing antifungal activity.^{8,9} The amphiphilic (surfactant-like)

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characteristics of alkylresorcinols and derivatives such as sorgoleone² could promote the formation of thin exudate layers completely covering root systems, thus providing a continuous defensive boundary. This would be analogous to the situation proposed to occur with leaf trichome exudates of *Lycopersicon* spp., where the formation of continuous antifungal chemical barriers may be facilitated by the presence of amphiphilic acylglucose exudate constituents.¹⁰

An Emerging Picture of Alkylresorcinol Biosynthesis in Poaceae

Alkylresorcinols, as well as all other classes of phenolic lipids, are thought to be derived predominantly from type III polyketide synthase (PKS)-associated pathways.¹¹ In plants, type III PKS enzymes are involved in the biosynthesis of a vast array of natural products, including flavonoids derived from the important branch metabolite 2',4',4',6'-tetrahydroxychalcone, the product of the enzyme chalcone synthase. Type III PKSs which produce 5-*n*-alkylresorcinols from fatty acyl-CoA starter units, termed alkylresorcinol synthases (ARSs), have been described from several microorganisms, including *Azotobacter vinelandii*, *Streptomyces griseus* and *Neurospora crassa*,¹²⁻¹⁴ however information concerning the genes and enzymes responsible for alkylresorcinol production in higher plants has been limited. In our recent study, we have described the sequences and activities of 5 enzymes from Poaceae (2 from *Sorghum bicolor* and 3 from *Oryza sativa*) which possess 5-*n*-alkylresorcinol synthase activity and accept a range of medium- to long-chain fatty acyl-CoA starter units.¹⁵

While inroads have now been made concerning the genetics of alkylresorcinol production in sorghum and rice, this story is far from complete. For example, BLAST analyses of the rice *ssp. japonica* genome indicate that additional ARS-encoding genes likely exist. As shown in **Figure 1A**, a cluster of potential ARS genes occurs within an approximately 30 kb region on chromosome 5, which includes LOC_Os05g12180, a locus previously shown to encode an ARS

enzyme by recombinant enzyme studies.¹⁵ This region also includes LOC_Os05g12190, LOC_Os05g12210 and LOC_Os05g12240, which are predicted to encode type III PKS-like polypeptides sharing approximately 92, 94 and 96% amino acid identity, respectively, with Os05g12180 (**Fig. 1A**). Like Os05g12180, these predicted proteins share the unusual Thr132 → Tyr, Met137 → Ala and Gly256 → Met substitutions (based on *M. Sativa* CHS2 numbering; **Fig. 1B**) conserved in the characterized rice and sorghum ARS sequences, which likely play a role in their preference for medium- to long-chain acyl-CoA substrates.¹⁵ Several additional promising ARS-like sequences exist within the *ssp. japonica*, cv. Nipponbare genome (not shown), thus the genetic complexity of alkylresorcinol biosynthesis is likely much greater in rice than in sorghum. A major question requiring attention for rice is determining the tissue- and developmental stage-specific expression patterns for these demonstrated and putative ARS sequences. Surprisingly, among the approximately 1.2 million *O. sativa* ESTs deposited in dbEST (<http://www.ncbi.nlm.nih.gov/dbEST>), only 2 entries (accession numbers EE590603.1 and D42548.1) match with known or putative ARSs and both ESTs correspond to LOC_Os05g12210 (**Fig. 1A**). Some preliminary information can however be gleaned from public microarray data repositories such as the Rice Array Database (RAD; <http://www.ricearray.org>). For example, one rice GeneChip study performed by Jain and co-workers (RAD experiment ID number GSE6893)¹⁶ using targets prepared from developing panicles, seeds, leaves, roots and shoot apices of *ssp. indica*, cv. IR64, indicates that the expression of the ARSs encoded by LOC_Os10g08620 and LOC_Os05g12180 are enhanced in seeds at 11–20 DAP and 21–29 DAP, respectively, whereas the expression of LOC_Os10g07040 appears to be less seed developmental stage-specific (**Fig. 1C**). Given that alkylresorcinol accumulation in Poaceae appears to be highly localized (see above), a more detailed analysis of gene expression would likely provide valuable clues concerning the physiological roles played by each of the various rice ARS enzymes.

Ancient Evolutionary Origin of Plant ARSs?

The diversity of natural products produced by various type III PKSs is due in part to differences in the type of intramolecular cyclization reactions performed by specific enzymes. The details of these reactions have attracted much interest in recent years, as PKSs involved in the biosynthesis of aromatic ring-containing intermediates mainly utilize either an aldol condensation-based mechanism (a.k.a., stilbene synthase or “STS-type”) or a Claisen condensation-based mechanism (a.k.a., chalcone synthase or “CHS-type”) for ring folding.^{11,17} ARSs use a STS-type cyclization mechanism, and may also generate pyrone by-products from specific acyl-CoA starters via intramolecular C5 oxygen → C1 lactonization (**Fig. 2**).^{12-15,18} This distinction among ring folding mechanisms provides a very useful context for considering the evolutionary history of ARSs, as well as type III PKSs in general.

Our phylogenetic analyses performed on type III PKS sequences from higher as well as primitive plant species¹⁵ revealed two points of particular interest. The first being that SbSTS1, the only known stilbene synthase (STS) from Poaceae, clustered together with Poaceae CHS-type enzymes which utilize a different ring folding mechanism (Claisen condensation) than SbSTS1 (aldol condensation). This suggests that SbSTS1 diverged fairly recently from CHS-type enzymes, as was previously proposed¹⁹ for plant STS-type enzymes from peanut (*Arachis hypogaea*), Scots pine (*Pinus sylvestris*) and eastern white pine (*Pinus strobus*). An additional insight gained from these analyses is that the ARSs identified in sorghum and rice fall within a cluster very distant from the cluster containing the Poaceae CHS-type enzymes, suggesting an ancient divergence of these PKS subtypes.

The plant-specific CHS and STS enzymes were the first type III PKSs identified, however as discovered later, the protein family is actually much older, as type III PKSs have now been identified in numerous bacteria and fungi. Among the functionally identified microbial enzymes, there is only one utilizing a Claisen condensation,²⁰ but several utilizing aldol

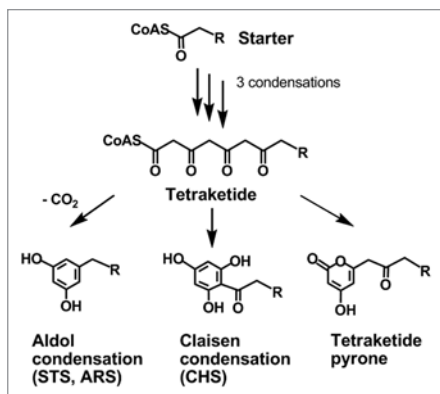


Figure 2. Reactions catalyzed by stilbene synthase, alkylresorcinol synthase and chalcone synthase-type III PKS enzymes. All of these PKSs utilize a starter unit and perform three condensation reactions with malonyl-CoA as the extender unit, yielding a tetraketide intermediate. Different ring-folding mechanisms are then employed, as discussed in the text. Alkylresorcinol synthases can also generate tetraketide pyrone side-products via C5 oxygen \rightarrow C1 intramolecular lactonization. STS, stilbene synthase; ARS, alkylresorcinol synthase; CHS, chalcone synthase.

The limited number of plant ARS examples which currently exist renders inferences into the origin/ancestry of this PKS subfamily somewhat circumspect, however a report concerning a type III PKS from the moss *Physcomitrella patens* (PpCHS11) that prefers long-chain acyl-CoA substrates seems to provide the first substantial hints.²² In the phylogenetic analyses performed by Jiang et al.²² it was observed that PpCHS11 consistently clustered with a *Synechococcus* (cyanobacterial) type III PKS also exhibiting ARS and alkylpyrone synthase activities. Could this suggest that pyrone formation and ARS activities represent the original functions of type III PKSs? In such a scenario, the now ubiquitous plant CHS-type (Claisen condensation-utilizing) enzymes could have been re-invented, perhaps concomitantly with the evolution of phenylpropanoid metabolism. The recently identified sorghum and rice ARS sequences will undoubtedly play a role in answering this question, as well as advance the analysis of alkylresorcinol biosynthetic pathways in other plant species.

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