A chemical library by engineered polyketide synthase

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The type III PKSs have been recognized as a member of the plant chalcone synthase superfamily involved in flavonoid and stilbene biosynthesis, catalyzing decarboxylative condensation of three acetate units from malonyl-CoA to a p-coumaroyl-CoA starter molecule and then Claisen or aldol cyclization to form the chalcone orstilbene skeletone, respectively. Following CHS and STS, the type III PKS for aromatic heptaketide aloesone was identified from rhubarb. Then, the pentaketide synthase for chromone (PCS) was obtained from aloe. Replacement of a single aminoacid residue, Met 207 with less bulky Gly formed a mutant to produce aromatic octaketide, SEK4 and SEK4B, products of the minimal *act* Type I PKS. An octaketide synthase(OKS) bearing Glycine at 207 was also found from aloe, the mutation of the Gly207 to more bulky aminoacid resulted in loss of the OKS activity and concomitant formation of shorter chain length polyketides including a hexaketide. A triple PCS mutant F80A/Y82A/M207G, was constructed and shown to produce unnatural novel naphthopyrone by condensation of nine acetate units. Homology modeling predicted that the active-site cavity volume of the triple mutant is 4 times larger than that of the wild type PCS.

Lifestyle-related illness could be aborted or relieved by oral intake of nutraceuticals and pharmaceuticals, which are mainly derived from plants. To enrich plants with those substances, we have applied a promoter sequence of gene for actin 2 (*AtACT2*) of *Arabidopsis* to accumulate polyphenols by enhanced expression of gene for *Arabidopsis* chalcone synthase (*AtCHS*) in *Arabidopsis*, where transcript levels of *AtCHS* have increased approx. 20 times higher in rosette leaves. This strategy has also been applied to expression of genes for benzalacetone synthase (*RpBAS*) from *Rheum palmatum* and for pentaketide chromone synthase (*IpPCS*), a type-III polyketide synthase from *Ipomoea purpurea*. We are indebted for constructs for transformation to Yasuo Niwa, Aftab Ahmad and Izumi Kaji, and for analysis of polyphenols to Nana Funato, Tsutomu Nakayama, Hiroyuki Sakakibara, Toshimasa Toyo'oka.