

## **Heterologous Polyketide Production in *Saccharomyces cerevisiae*.**

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Most organisms produce secondary metabolites of some sort, i.e. substances that are not part of the organism's vital metabolism but are secondary in the organism's ontogeny and employed as colour, smell or self defence for example. Among secondary metabolites are chemical groups as varied as alkaloids, terpenes and, the group discussed here: polyketides. These natural products are of great significance for the food-, chemical- and pharmaceutical industries.

Polyketides are a very diverse family of secondary metabolites. They are produced by large, multifunctional enzymes termed polyketide synthases (PKS) in a decarboxylative condensing process similar to fatty acid synthesis. The difference is that polyketide synthases implement a varying state of ketoreduction and use the oxide groups for various changes on the carbon chain, ring formation for example. An additional level of complexity comes from the use of different acyl CoA ester starter units. Polyketides are found almost everywhere and among organisms that produce polyketides are bacteria, fungi and plants as well as many species of invertebrates.

The primary hindrance to the utilization of polyketides is that many organisms that produce exciting substances are impossible to grow or harvest so a more efficient method would be to clone PKS genes and move their production into a more convenient organism such as *Saccharomyces cerevisiae*. Efforts to this end have been successful in some cases but more often it has proved harder than expected to get yeast to express the foreign gene. Possible reasons include raw material deficiency, codon bias or different mRNA signals for example. It is also important to ensure that a functional tailoring enzyme is present, in most cases 4' phosphopantetheine transferase (PPT) that activates the PKS by transferring a phosphopantethein group onto a serine residue in the PKS's acyl carrier protein (ACP), the part of the enzyme that holds the carbon chain as it is being extended.

To study heterologous production of polyketides in *Saccharomyces cerevisiae*, we have made use of two variants of a simple PKS, 6-methylsalicylic acid synthase. Both were cloned from the fungus *Penicillium patulum* (*P. griseofulvum*). One of these (PP-6MSAS) has been shown to produce 6-methylsalicylic acid in *S. cerevisiae* but attempts to express the other (PG-6MSAS) have thus far been unsuccessful. 6-methylsalicylic acid is a simple polyketide and therefore a promising candidate for research of this sort. To produce it a sufficient supply of acetyl CoA and malonyl CoA is needed as well as a functional PPTase. To this end we made use of two PPTases, one from *Bacillus subtilis* and the other from *Aspergillus nidulans*, both of which have been used successfully for 6-methylsalicylic acid production in *S. cerevisiae*. Our aim is to use this material to find answers to the question why some PKS's are harder to express in *S. cerevisiae* than others and to seek solutions.