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Review

Combinatorial biosynthesis of medicinal plant secondary metabolites

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Abstract

Combinatorial biosynthesis is a new tool in the generation of novel natural products and for the production of rare and expensive natural products. The basic concept is combining metabolic pathways in different organisms on a genetic level. As a consequence heterologous organisms provide precursors from their own primary and secondary metabolism that are metabolised to the desired secondary product due to the expression of foreign genes. In this review we discuss the possibilities and limitations of combining genes from different organisms and the expression of heterologous genes. Major focuses are fundamentals of the genetic work, used expression systems and latest progress in this field. Combinatorial biosynthesis is discussed for important classes of natural products, including alkaloids (vinblastine, vincristine), terpenoids (artemisinin, paclitaxel) and flavonoids. The role and importance of today's used host organisms is critically described, and the latest approaches discussed to give an outlook for future trends and possibilities.

Keywords: Combinatorial biosynthesis; Plant biotechnology; Bioconversion; Paclitaxel; Artemisinin; Carotenoids; Flavonoids; Herbal medicine; Herbals; Nutraceuticals; Biopharmaceuticals; Recombinant protein; Plant

Contents

1. Introduction

The approach to combine genes from different micro-organisms for the production of new and interesting metabolites has become known as combinatorial biosynthesis. Recent achievements with the polyketide biosynthesis from

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microorganisms, especially in *Streptomyces*, prove the potential of combinatorial biosynthesis (Hranueli et al., 2005; Moore et al., 2005; Weber et al., 2003; Pfeifer and Khosla, 2001). It also showed that this approach can be used to improve the biosynthesis capacity of known producing microorganisms like *Escherichia coli*, *Bacillus subtilis* or *Saccharomyces cerevisiae*. The heterologous expression of human genes in microorganisms is well known for more than 30 years now. Fundamental work on the expression of plant genes from biosynthetic pathways, performed since the 1980s, opens a way to similar research that may even be extended in the future by directed evolution. It is now possible to combine these genes and extend the realm of combinatorial biosynthesis far beyond the polyketide biosynthesis. The diversification of products will increase dramatically when genes of very different origins are used. However there is no need to concentrate on new compounds only; there are many interesting natural products, of which the application (e.g. as a drug or fine chemical) is hampered by its availability. This problem might be solved by using alternative production systems yet to be discovered, that are based on enzymes from other biosynthetic pathways.

Nature and its huge biodiversity harbours an endless source of compounds containing unique chemical structures. Even on a species level a given biosynthetic pathway adapts through the continuous selection pressure of its surrounding. Only those compounds that are highly favorable for the producing organism are accumulated, which is a delicate balance between energy cost and physiological/ecological benefit. There are many speculations about how evolution diverges biosynthetic pathways (Pichersky and Gang, 2000). Often the result is that specific compounds are produced by specific organisms. There are certainly products that will not be produced because they cost too much energy to synthesize, their activity is not beneficial enough or the organism lacks the enzyme machinery to perform a specific chemical reaction. In other words, the biodiversity is endless and there are still possibilities to enlarge the diversity from a chemical point of view, by combining genes and products from different sources that in nature would never meet. This strategy will deliver compounds that are not influenced by selection pressures, by a habitat, or the biochemical limitations of an organism (such as compartmentalization or storage). These compounds can be selected for a specific pharmaceutical mode of action or an activity can be adjusted to a more specific pharmaceutical demand.

There are several pharmaceuticals on the market that are highly expensive, due to the fact that these compounds are only found in rare plants and often in extreme low concentrations. Podophyllotoxin and paclitaxel (Fig. 1) are clear examples of pharmaceuticals that can only be produced through the isolation from plants. To achieve a sustainable source of such compounds scientists all over the world have been experimenting with biotechnological approaches aiming at the development of an alternative production system. With this aim in mind, combinatorial biosynthetic strategies are expected to yield interesting alternatives in the near future. With regard to the production of podophyllotoxin it has been shown that plant

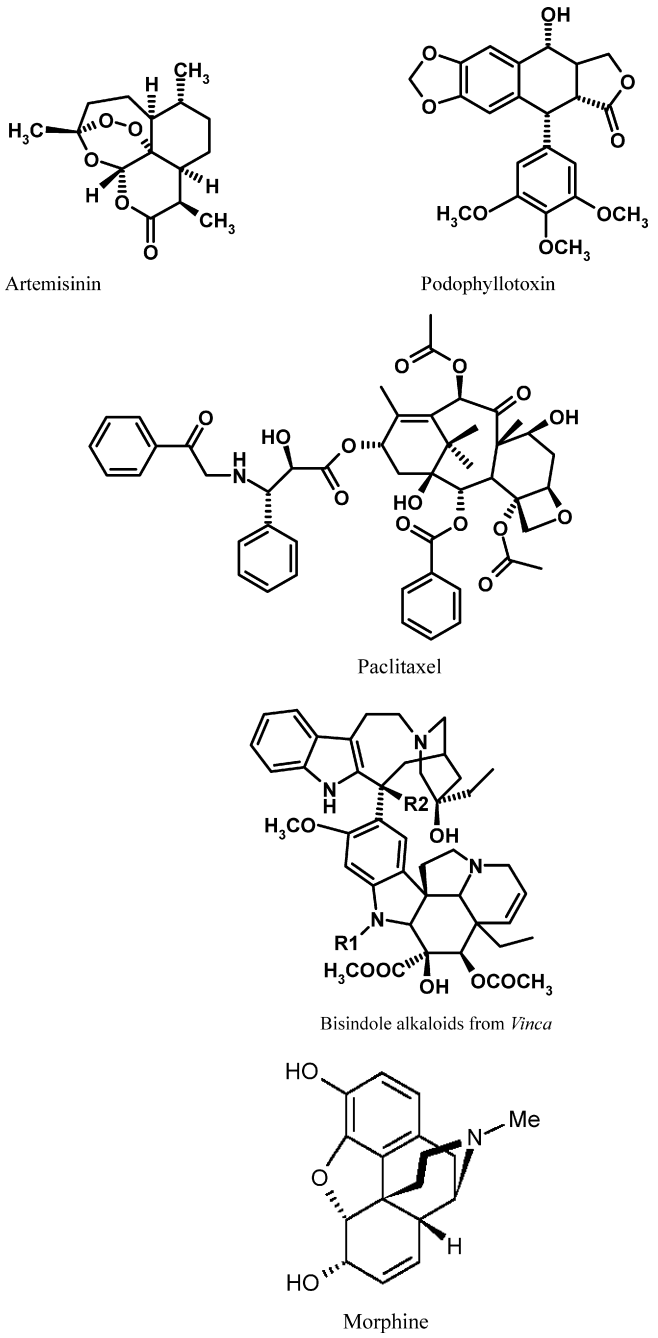

Fig. 1. Important natural plant products subject to combinatorial biosynthesis studies.

cell cultures of *Linum flavum* L. can be used to convert deoxypodophyllotoxin, a major lignan of *Anthriscus sylvestris* L. into 6-methoxypodophyllotoxin (Koulman et al., 2003; Van Uden et al., 1997). The combination of the product of one species and the enzymes of another species to yield a desired product is a good example of combinatorial biosynthesis. This topic will be extensively discussed in the following subchapters.

Not only can the expression of a single gene be of interest. The reconstruction of complete biosynthetic pathways by combining genes of the desired pathway in host organisms is the current aim of actual research projects. There are many