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Biotechnological production of aromatic compounds of the extended shikimate pathway from renewable biomass

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ABSTRACT

Aromatic chemicals that contain an unsaturated ring with alternating double and single bonds find numerous applications in a wide range of industries, e.g. paper and dye manufacture, as fuel additives, electrical insulation, resins, pharmaceuticals, agrochemicals, in food, feed and cosmetics. Their chemical production is based on petroleum (BTX; benzene, toluene, and xylene), but they can also be obtained from plants by extraction. Due to petroleum depletion, health compliance, or environmental issues such as global warming, the biotechnological production of aromatics from renewable biomass came more and more into focus. Lignin, a complex polymeric aromatic molecule itself, is a natural source of aromatic compounds. Many microorganisms are able to catabolize a plethora of aromatic compounds and interception of these pathways may lead to the biotechnological production of value-added aromatic compounds which will be discussed for *Corynebacterium glutamicum*. Biosynthesis of aromatic amino acids not only gives rise to L-tryptophan, L-tyrosine and L-phenylalanine, but also to aromatic intermediates such as dehydroshikimate or chorismate from which value-added aromatic compounds can be derived. In this review, we will summarize recent strategies for the biotechnological production of aromatic and related compounds from renewable biomass by *Escherichia coli*, *Pseudomonas putida*, *C. glutamicum* and *Saccharomyces cerevisiae*. In particular, we will focus on metabolic engineering of the extended shikimate pathway.

1. Introduction

Aromatic chemicals are among the most important petroleum-based feedstocks produced from BTX (benzene, toluene, and xylene) in the chemical industry (Krömer et al., 2013). In addition, aromatic compounds obtained from plants constitute a major group of natural products with various biological functions (Ananga et al., 2013). These compounds have numerous applications in the food, feed, cosmetics, pharmaceutical, and chemical industries. The biotechnological production of aromatics from renewable biomass has received great attention due to various issues, including petroleum depletion, environmental and health compliance, global warming, and limited availability and yield from plant (Gallage and Moller, 2015; Okai et al., 2016; Polen et al., 2005; Thompson et al., 2016).

Aromatic compounds are generally derived from the shikimate pathway known only in bacteria, fungi, and plants (Rodriguez et al., 2014). This pathway starts from phosphoenolpyruvate and erythrose 4-phosphate which are converted to chorismate via shikimate. The shikimate pathway is regarded as a key metabolic pathway with many branches, providing precursors for a variety of valuable aromatic products. All genes, corresponding enzymes, and metabolites of the shikimate pathway as well as their regulation have been analyzed and characterized in *E. coli* and many other bacteria (Ikeda, 2006). Of these intermediates, chorismate is used as a common precursor for the biosynthesis of aromatic amino acids (AAA; tryptophan, phenylalanine, and tyrosine), folate, menaquinone, ubiquinone, and siderophores. Aromatic amino acids are mainly used as food and feed additives, pharmaceutical intermediates, and sweetener precursors. To date, the AAA have been commercially manufactured by bacterial fermentation with recombinant *Escherichia coli* and *Corynebacterium glutamicum* (Ikeda, 2006). Shikimate, another intermediate, is a main starting

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building block for the synthesis of oseltamivir which is an antiviral medication used to treat influenza infection. Shikimate-producing *E. coli* and *C. glutamicum* were constructed by metabolic engineering (Kogure et al., 2016; Martinez et al., 2015).

A wealth of aromatic compounds useful for potential building blocks in the chemical polymer industry or with other functional applications has been derived from their precursors in the shikimate pathway or from related compounds (Gosset, 2009; Rodriguez et al., 2014). Recently, novel biosynthetic routes have been generated by integrating metabolic engineering strategies for strain development with extensive omics-related information of organisms and synthetic biology, resulting in de novo biosynthesis of compounds from an extended shikimate pathway (Liu et al., 2014; Rodriguez et al., 2014). Several dehydroshikimate-derived compounds such as protocatechuate (PCA), catechol, *cis,cis*-muconic acid (ccMA), and vanillin as well as chorismate-derived compounds including 4-hydroxybenzoate (4HBA), 4-aminobenzoate, PCA, ccMA, salicylic acid, phenol, gastrodin, and coenzyme Q_{10} have been produced in recombinant microbial systems (Cluis et al., 2011; Curran et al., 2013; Gallage and Moller, 2015; Li et al., 2005; Liu et al., 2014; Niu et al., 2002) (Fig. 1). In particular, ccMA would provide a high interest because of its potential use as a platform chemical that serves as the precursor of adipic acid and terephthalic acid, which are used as bulk feedstocks for making polyamide (nylon-6,6), lubricants, polyurethane, and polyethylene terephthalate (PET) (Polen et al., 2013; van Duuren et al., 2011). Vanillin is an important flavoring and aromatic component and currently manufactured by chemical synthesis and extraction from the plant *Vanilla planifolia*. The biotechnological production of vanillin by fermentation through the extended shikimate pathway provides an alternative access to this important flavor in food industry (Gallage and Moller, 2015). Furthermore, recent progress based on systems metabolic engineering demonstrated that the extended shikimate pathway is an attractive platform to produce diverse aromatics and related compounds with great commercial interest.

In this paper, we review the recent strategies and achievements employed for the biotechnological production of aromatics and related compounds from renewable biomass. Efforts to alleviate the toxicity of aromatic compounds to cells are discussed and an overview of aromatic catabolism in *C. glutamicum* and the potential to make use of catabolic reactions for production of aromatics is presented.

2. A treasure trove of microbial hosts for production of aromatic compounds

Metabolic engineering for the production of aromatic compounds concentrated on few microorganisms, mainly on *E. coli, S. cerevisiae, P. putida,* and *C. glutamicum* (Becker and Wittmann, 2015). The flexible feedstock concept for access to a wide range of renewable carbon sources as substrates has been realized for these microorganisms (Wendisch et al., 2016a). These microorganisms are able to degrade aromatic compounds to various extent and, moreover, they differ with respect to tolerance to aromatic compounds, to pH and temperature.

E. coli is a Gram-negative bacterium and considered the best studied microorganism. Since it is a model organism of molecular biology, the genetic toolbox is the best developed for any individual organism, which drove and is still driving its use as potent microbial cell factory for industrial application (Becker and Wittmann, 2015). The fact that very early on biotechnological processes using *E. coli* have been commercialized, facilitated subsequent applications due to regulatory considerations. Moreover, *E. coli* grows on a variety of monomeric carbon sources under aerobic as well as under anaerobic conditions. The product portfolio of *E. coli* ranges from organic acids (Wendisch et al., 2006) and biofuels (Liao et al., 2016) to building blocks for subsequent chemical conversion (Nielsen et al., 2013), polymers (Wubbeler and Steinbuchel, 2014) and active pharmaceutical ingredients (APIs) (Huang et al., 2001; Paddon and Keasling, 2014).

S. cerevisiae or Baker's yeast has the longest tradition in biotechnology dating back thousands of years with respect to baking and brewing and is the best studied eukaryotic microorganism (Becker and Wittmann, 2015). Baker's yeast can grow aerobically and anaerobically on a number carbon substrates. In particular, *S. cerevisiae* can grow at acidic pH. *S. cerevisiae* has a long-standing history of safe production in the food industry at large scale. Due to these characteristics and exceptionally well developed genetics, *S. cerevisiae* has been engineered for the production of organic acids, biofuels, materials, and pharmaceutically relevant products (Leavell et al., 2016) (Krivoruchko and Nielsen, 2015).

P. putida shows very good tolerance towards organic solvents (de Bont, 1998) and bears potential in bioremediation of aromatic compounds. Strain KT2440 possesses GRAS status and has been intensively studied with regard to possible applications in industrial biotechnology since this Gram-negative soil bacterium copes well with xenobiotics and other often toxic chemicals (Poblete-Castro et al., 2012), e.g. with arsenic and other metalloids and heavy metals (Chen et al., 2013; Molina-Henares et al., 2010; Paez-Espino et al., 2015). It has been used for the production of chemicals that often are toxic to other hosts, e.g. cinnamate (Nijkamp et al., 2005), *p*-coumarate (Nijkamp et al., 2007), *p*-hydroxybenzoate (Verhoef et al., 2007) or phenol (Wierckx et al., 2005).

C. glutamicum is a Gram-positive bacterium used as a GRAS organism in the food and feed industries with a history of six decades of safe production of amino acids. The biotechnological production of L-glutamate and L-lysine amounts to about five million tons per year (Wendisch et al., 2016b). *C. glutamicum* has been engineered for the production of many chemicals (Becker and Wittmann, 2015), e.g. amino acid precursors and amino acid derived products (Wendisch, 2014), such as diamines (Kind and Wittmann, 2011; Schneider and Wendisch, 2011), organic acids (Litsanov et al., 2012; Okino et al., 2005; Zahoor et al., 2014), terpenoids (Heider et al., 2014), polyphenols (Kallscheuer et al., 2016b), keto acids (Buchholz et al., 2013; Buckle-Vallant et al., 2014; Krause et al., 2010), isobutanol (Blombach et al., 2011; Smith et al., 2010), propanediol, *n*-propanol (Siebert and Wendisch, 2015), γ-amino butyrate (Jorge et al., 2016a,b; Takahashi et al., 2012), δ-amino valerate, glutarate (Rohles et al., 2016), pipecolic acid (Perez-Garcia et al., 2016) and polyhydroxyalkanoates (Matsumoto et al., 2011).

3. Production of *cis,cis*-muconic acid via dehydroshikimate or chorismate

Adipic acid and terephthalic acid are platform chemicals for the production of nylon-6,6 fiber and PET, respectively, with annual global production volumes of 2.8 and 71 million tons, respectively (Sengupta et al., 2015). Currently, their industrial production relies exclusively on chemical synthesis using petroleum-based starting feedstocks such as benzene and *p*-xylene. Since these petrochemical manufacturing processes have encountered many challenges due to increasing environmental concern and limited fossil fuels, the bio-based production of these chemicals from renewable biomass would provide a feasible alternative. ccMA is a naturally occurring unsaturated dicarboxylic acid with extensive industrial applications, e.g. as precursor of adipic acid, terephthalic acid, and caprolactam as well as in pharmaceuticals, functional resins, and agrochemicals (Sengupta et al., 2015). In fact, adipic