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Research review paper

Recent advances in biotechnological production of 2-phenylethanol

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ABSTRACT

2-Phenylethanol (2-PE) is an important aromatic alcohol with a rose-like fragrance. It has been widely applied in the cosmetic, perfume, and food industries and is mainly produced by chemical synthesis. An alternative method for the production of natural flavors and fragrances is the microbial transformation process, which is attracting increasing attention because it is an environmentally friendly process and the products are considered "natural". The production of 2-PE from L-phenylalanine by biotransformation is possible through the Ehrlich pathway and considerable progress has been made in the development of this process. The present report reviews recent advances in biotechnological production of 2-PE, with emphasis on the strategies used to increase production and the applications of in situ product removal techniques. Future research should focus on product scale-up and product recovery processes for the industrialization of microbial processes.

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1. Introduction

2-Phenylethanol (2-PE) is an aromatic alcohol with a rose-like fragrance that is considered an important component of several products. It is widely applied in the cosmetics, perfumery, and food industries. The main use of 2-PE in the world market is as a flavor ingredient to modify certain flavor compositions (Hua et al., 2010; Huang et al., 2000). 2-PE is also used as a substrate for the synthesis of other flavors or pharmaceutical compounds such as phenylethyl acetate ester, which is also a valuable chemical (Etschmann et al., 2002).

The world's annual production of 2-PE was estimated to be approximately 10,000 tons in 2010, most of which was obtained by chemical synthesis from benzene or styrene with a price of about US$ 5/kg (personal communication, Apple Flavor & Fragrance Group Co., Ltd, China). Chemical synthesis processes are often environmentally unfriendly (high temperature, high pressure, and strong acid or alkali) and are associated with the production of unwanted byproducts, thus reducing efficiency and increasing downstream costs. Moreover, according to US and European legislations, the use of chemically

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synthesized flavor compounds is restricted in food, beverages, and cosmetics (Xu et al., 2007a). Natural 2-PE can be extracted from the essential oils of certain flowers (e.g., rose). However, the concentration of 2-PE in flowers is very low, and the extraction process is therefore complicated and costly (about US$ 1000/kg, personal communication, Apple Flavor & Fragrance Group Co., Ltd, China). The harvest of flowers is also influenced by weather conditions. Therefore, natural 2-PE from botanical sources cannot meet the large market demands and is significantly more expensive than its chemically produced counterpart (Mei et al., 2009).

In recent years, the US Food and Drug Administration and European legislations determined that products obtained by biotechnological methods can be considered natural if the substrate used for the production process is of natural origin (Hua et al., 2007a, 2007b). Whole-cell microbial transformation has several advantages such as mild reaction conditions, high substrate selectivity, and few by-products, and is considered to be a promising strategy for the production of high-valued compounds (Xu et al., 2007a, 2007b). Many studies have focused on the biotechnological production of natural flavors, such as benzaldehyde, vanillin, γ-decalactone, acetoin, tetramethylpyrazine, and 2-PE (Etschmann et al., 2002; Xu et al., 2007a, 2007b; Hua et al., 2007a, 2007b, 2010; Krings and Berger, 1998; Longo and Sanromán, 2006; Xiao et al., 2006, 2007, 2009, 2010; Zhang et al., 2006). Among these natural fragrances, 2-PE has received increasing attention for its value as a flavor compound, and considerable progress in the production of 2-PE has been made. In this review, we summarize recent advances in the biotechnological production of 2-PE with emphasis on strategies for higher production and applications of in situ product removal (ISPR) techniques.

2. Ehrlich pathway: the route for 2-PE production

Although 2-PE can be de novo synthesized in microbes, the final concentrations obtained are usually very low, which are not economically viable or applicable to a bioprocess (Etschmann et al., 2002). However, 2-PE is an intermediate in the microbial transformation of L-phenylalanine (L-Phe), which is an essential amino acid in humans. This can be applied for the large-scale production of 2-PE by enzymatic transformation or microbial fermentation with a low production cost, in a process that can be considered a natural process. As shown in Fig. 1, L-Phe is transaminated to phenylpyruvate by a transaminase, decarboxylated to phenylacetaldehyde by phenylpyruvate decarboxylase (PDC), and then reduced to 2-PE by a dehydrogenase (Etschmann et al., 2002; Wittmann et al., 2002). The 2-PE produced can be further transformed to phenylaldehyde and then phenylacetate (2-PEAc). This pathway was found by Ehrlich (1907) and therefore named the Ehrlich pathway. When L-Phe is the sole nitrogen source in the medium, large amounts of 2-PE accumulate. Several of the biotechnological processes for the production of 2-PE were thus based on this pathway. For the detailed information, please see the review article by Etschmann et al. (2002).

The discovery of the Ehrlich pathway to explains the transformation of L-Phe to 2-PE stimulated recent studies that focused on identifying the enzymes and genes involved in this process. In *Saccharomyces cerevisiae*, the activity of PDC was present in cultures grown with L-Phe as the sole nitrogen source but was absent from ammonia-grown cultures. Five candidate genes with sequence similarity to genes encoding thiamine diphosphate-dependent decarboxylases were identified (Vuralhan et al., 2003). A novel 2-PE dehydrogenase was purified from *Brevibacterium* sp. KU1309 and shown to grow on 2-PE as the sole carbon source. This enzyme has broad substrate specificity and catalyzes the reversible oxidation of various primary alcohols to aldehydes, due to which it is classified into a group of NAD^+-dependent primary alcohol dehydrogenases (Hirano et al., 2005). In a subsequent study, the same group purified and characterized phenylacetaldehyde dehydrogenase (PADH), which catalyzes the oxidation of aryl (benzaldehyde, phenylacetaldehyde, 3-phenylpropionaldehyde) and aliphatic (hexanal, octanal, decanal) aldehydes to their corresponding carboxylic acids, using NAD^+ as the electron acceptor (Hirano et al., 2007).

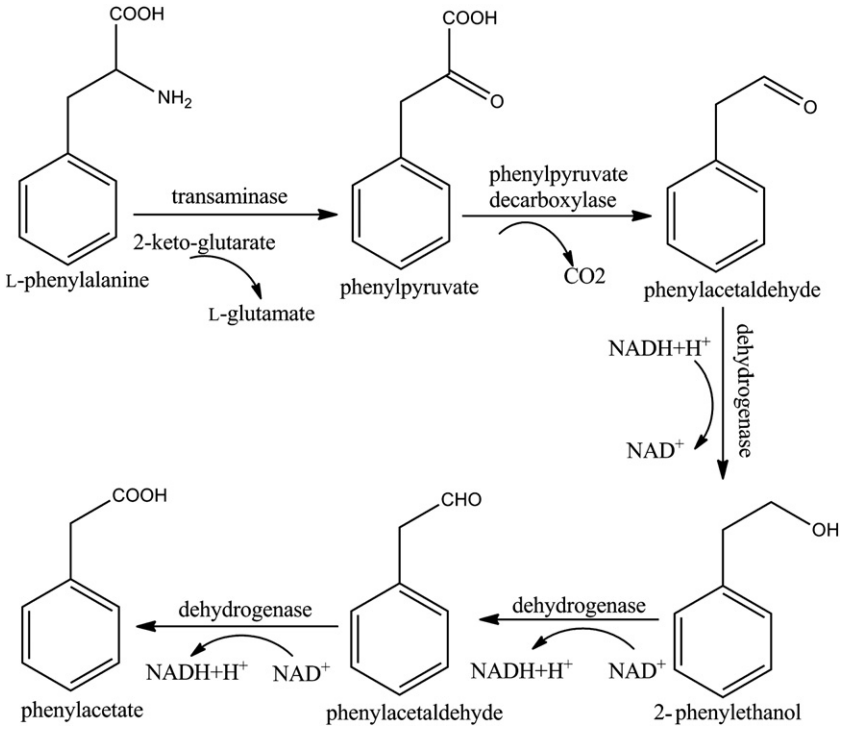

Fig. 1. Ehrlich pathway for 2-PE production from L-Phe. L-Phe is transaminated to phenylpyruvate by a transaminase, decarboxylated to phenylacetaldehyde by phenylpyruvate decarboxylase, and then reduced to 2-PE by a dehydrogenase (Etschmann et al., 2002). 2-PE also can be transformed to phenylaldehyde and phenylacetate in a reaction catalyzed by a dehydrogenase.