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Metabolic Pathways and Microbial Engineering for Vanillin Production

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Abstract. Vanillin, due to its unique aroma and widespread applications, has become an important molecule in food, medicine, and other fields. Currently, the main production methods include chemical synthesis, natural extraction, and biosynthesis. Although chemical synthesis is cost-effective, it is associated with environmental pollution and byproducts that affect purity. Natural extraction can maintain high purity and natural flavor, but due to low vanillin content, long cultivation cycles, and limited production sites, it is costly and yields low, making it difficult to meet market demand. In contrast, biosynthesis holds the greatest potential as it aligns with the principles of green chemistry and sustainable development. Common biosynthetic substrates include ferulic acid, glucose, and eugenol, and its metabolic pathways primarily involve the ferulic acid pathway, the tyrosine pathway, and the shikimate pathway. This article reviews the application of relevant microorganisms (such as Pseudomonas, recombinant Escherichia coli, and Bacillus subtilis) in vanillin synthesis and analyzes the advantages and limitations of biosynthesis. Results show that while biosynthesis offers significant advantages in terms of cost, safety, and environmental friendliness, it still faces challenges such as low conversion efficiency, byproduct formation, high substrate handling costs, and the toxicity of vanillin to the host microorganism. Current research is improving yields through metabolic engineering and tolerance modification, laying the foundation for future large-scale industrial production. In summary, biosynthesis of vanillin shows broad prospects for development in terms of economic and environmental benefits.

Keywords: Vanillin, Biosynthesis, Metabolic engineering.

1. Introduction

Vanillin is a widely used molecule. People love it deeply because of its unique fragrance. This is a natural substance. Vanillin has a wide range of applications in many fields in daily life, such as food and medicine. Now there are mainly three methods to produce vanillin. These are gained through extraction and biosynthesis. Now in the market, most of the vanillin is produced by chemical synthesis. This method not only causes pollution which can affect the environment, but also the by-products generated during the production process will reduce the purity of vanillin and this will causes the poor quality of vanillin. Although the vanillin that is synthesized by chemical process have a relatively low price, it has many significant drawbacks and is not in the line with the concepts

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of green chemistry and sustainable development. Another way to produce vanillin is natural extraction. This method is directly extracted from vanilla. Although this method can retain the natural fragrance and pure quality, due to the low content of vanillin in the plant itself, the ling cultivation cycle of plant, and the scarcity of suitable plating areas, this method leads to a high production costs and low yields. So this method can not satisfy the market request. By comparison, biosynthesis is a method with very broad prospects. This method conforms to the concepts of green production and sustainable development in modern society and meets the current market demand for high quality and green products. Several microorganisms are often used to biosynthesize vanillin: Pseudomonas sp., recombinant Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa [1].

This article will introduce various synthesis pathways, such as ferulic acid pathway, the tyrosine-utilizing production pathway, and the shikimic acid pathway for vanillin production. This article summaries the advantages of vanillin production by biosynthesis and analyzes the challenges faced in the biosynthesis process. Sorting out and summarizing these contents is not only of significant theoretical and practical significance for research on vanillin biosynthesis methods, also pointing out more exploration directions for related research.

2. Introduction to vanillin

2.1. Structural analysis of vanillin

Vanillin is an organic compound. Its molecular structure is C8H10O3. This substance appears as white to light yellow crystals or crystalline powder, with a fragrance similar to vanilla beans. It is slightly soluble in water and easily soluble in ethanol and ether, and has inhibitory effects on certain microorganisms.

Vanillin has a wide range of uses. In terms of food, vanillin can provide rich vanilla flavor to food, enhance the overall taste of food, and also mask some unpleasant taste. In the cosmetics industry., vanillin can provide fresh vanilla fragrance to the daily products of perfume, improve the use of products, and cover up some irritating smell of chemical pigments. In the field of medicine, vanillin can be used as an auxiliary ingredient to mask the bitter taste from the drug itself, making the patients to accept the medication. In addition, the experiment conducted by Ning et al. shows that In the rat liver fibrosis model induced by thioacetamide, it was found that vanillin can significantly enhance the regenerative ability of the liver. Further mechanistic studies suggest that the effect of vanillin may be related to the regulation of the cGAS/STING signaling pathway. Vanillin can improve liver function, IC, liver fibrosis indicators, and liver tissue damage in IC rats [2]. Studies have found that vanillin has some beneficial properties in neuroprotection and has been observed to have positive effects on some neuropathological and neurodegenerative diseases [3,4]. For example, vanillin can be used as a treatment for Alzheimer's disease because of its efficient scavenging activity against reactive oxygen species (ROS), which play a key role in the pathological progression of Alzheimer's disease. This means that vanillin has strong antioxidant activity and can play a positive role in Alzheimer's disease [5].

2.2. Source of vanillin

Currently, approximately 90% of vanillin on the market is produced through chemical synthesis methods [1]. Ferulic acid, coniferin, guaiacol, creosote, glucose, PHB, 3-methoxy-4-hydroxybenzyl alcohol, 3-bromo-4-hydroxybenzaldehyde, lignin, eugenol, and phenol are commonly used to synthesize vanillin. Eugenol and phenol are the most common substrates in commercial use. This

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method requires extensive multi-step chemical reactions such as oxidation, decarboxylation, and acidification. Approximately 85% of synthetic vanillin is synthesized from guaiacol compounds obtained from petroleum sources [1]. Compared with plant extracted vanillin, chemical synthesis has obvious disadvantages. For example, the aroma of chemically synthesized vanillin is relatively single and lacks the unique and rich layers of natural products. Some extra products during the chemical synthesis process can also lead to a deterioration of flavor, creating a gap between its flavor and flavor of naturally extracted vanillin. Furthermore, vanillin produced by this method must be labeled as artificial, which greatly reduces its appeal to consumers. There are three main methods for producing vanillin in the chemical industry [6].

Plant extraction can preserve the original flavor characteristics of vanillin through special processes and planting conditions, but natural extraction also has significant disadvantages. The vanillin content in vanilla raw materials is limited, and coupled with the scarcity of production areas, the growth process is time-consuming and requires a lot of manpower. Therefore, the vanillin obtained by natural extraction method is very rare and difficult to supply to the market constantly [7].

3. Production of vanillin

3.1. From ferulic acid

Ferulic acid is one of the main substrates for the biosynthesis of vanillin and is a sustainable raw material for vanillin production. Ferulic acid is widely distributed in nature, and most agricultural waste contains large amounts of ferulic acid. For example, corn cobs, a byproduct of corn production, have very low value, but alkaline hydrolysis can produce approximately 1,171 mg of ferulic acid per liter. Wheat bran, a byproduct of wheat production, contains large amounts of ferulic acid and can be extracted through various methods. Rice bran can also be used as a powerful raw material for the production of ferulic acid. Banana peels, pineapple peels, pomegranate peels, and orange peels are also sources of ferulic acid [8].Pseudomonas strains are one of the best transformants. In the experiment of 9, 9 developed an engineered yeast strain yVAN005, which can convert ferulic acid produced by lignin into vanillin. Through experiments at different temperatures, different brewer's yeast densities, different pH values and different glucose concentrations, and then analyzed, the results showed that the yVAN005 strain is suitable for the production of vanillin as a biocatalyst, but due to the inhibitory effect of vanillin on cells, the yield of vanillin from the strain is significantly limited [9]. In the experiment of Barghini P et al, they use engineered recombinant Escherichia coli cells as biocatalysts for vanillin production. The conclusion was drawn that E. coil JM109 cells expressing the gene from fluorescent pseudomonas BF13, which degrade ferlic acid. will not accumulating any adverse vanillin reduction and oxidation products., and also convert ferulic acid into vanillin effcienty [10]. The biosynthesis of vanillin via ferulic acid is prone to produce byproducts.

3.2. Vanillin production from glucose

Glucose can also be used as a substrate for the synthesis of vanillin. Glucose is the cheapest substrate for biosynthesis of vanillin and it has many advantages. It is non-toxic to microorganisms and therefore does not cause any adverse effects. In the experiment by Di Q et al. By constructing expression modules and analyzing gene expression, an engineered Saccharomyces cerevisiae strain was constructed. Using glucose as a substrate, the vanillin production was increased to 960

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micrograms per liter by feeding 40 grams per liter of glucose within 96 hours. This method successfully synthesized vanillin using synthetic biology [11].

3.3. Vanillin production via the eugenol/isoeugenol pathway

Eugenol/Isoeugenol can also serve as substrates for the biosynthesis of vanillin. In their study published on the Morahem Ashengroph et al. they isolated a strain that can grow on eugenol and found that it can convert eugenol into vanillin. Using resting cell reaction experiments, they examined the strain's main metabolites, including vanillin and vanillic acid. The strain, identified as Pseudomonas aeruginosa SPR1, produced 0.24 grams of vanillin per liter after 30 hours of bioconversion using eugenol as a substrate [12]. Eugenol/Isoeugenol use ferulic acid as an intermediate for biosynthesis, so they encounter the same by-product problem as ferulic acid.

4. Discussion

The use of biosynthetic vanillin has many advantages. First, it greatly reduces the cost of vanillin by using low-cost substrates. through biosynthesis, the dependence on petroleum for obtaining vanillin has been reduced, and this method avoids the use of toxic and harmful chemicals in the production process, reduces toxic waste emissions, and avoids harm to human health, in line with the principle of green environmental protection. Biosynthesis of vanillin has broad prospects. It has received attention from many fields. In this way, the entire strategy is in line with consumers' pursuit of natural, green, and healthy products, and the entire process has low product costs, high safety, and a wide range of product applications. At the same time, it has the advantages of environmental protection, economy, and sustainable development.

There are challenges. The cost of material pretreatment is relatively high. At present, chemical synthesis technology is basically mature and inexpensive. The biosynthesis methods has only been carried out in the laboratory, and the cost is relative high. This makes the biosynthesis relatively weak in the face mature chemical synthesis methods. But biosynthesis can be developed based in its high-quality products. Another challenge is vanillin can cause damage to cell membrane, inhibit cell growth and activity, affects protein synthesis related to amino acid metabolism. In addition, in the synthesis pathway of vanillin, the respiration and fermentation processes of cells are accompanied by the secretion of ethanol, which interferes with the intracellular redox state and thus affects the synthesis of vanillin. In the experiment of Meichen et al. when the concentration of vanillin was 1 g/L, there was little difference in the growth status of the strain compared to the control group. However, when the concentration of vanillin increased to 5 g/L and 10 g/L, the growth of the strain was significantly inhibited, with an inhibition rate of up to 96%, indicating that higher concentrations of vanillin had a strong growth inhibitory effect on the strain [13]. This is a very critical issue, and it is currently possible to cultivate tolerant strains through cultivation.

Vanillin can inhibit microbial growth and metabolism, affecting cell membrane integrity and inhibiting transmembrane transport. It also severely damages mitochondria and ribosomes, significantly impacting cellular translation. Current approaches to this problem involve genetically engineering vanillin-resistant strains, but this approach is extremely inefficient. Jia et al. proposed a strategy and conducted experiments using heavy particle technology (ALE) to cultivate tolerant strains. After testing, the newly cultivated strains showed significantly higher tolerance to vanillin across various metabolic processes, as well as transcription and translation. This experiment provides a new strategy for cultivating vanillin-resistant strains [14].

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5. Conclusion

This paper discusses some biosynthetic pathways that provide new production perspectives. By using renewable resources such as ferulic acid, glucose and eugenol as substrates, the biosynthesis of vanillin has lower costs, higher market acceptance and higher safety compared to other production methods. However, biosynthesis is still in the experimental stage and cannot be carried out in large-scale industrial production. Many shortcomings still need to be solved and optimized. For example, vanillin is toxic to microorganisms and will seriously affect their normal growth and metabolism at a certain concentration. In addition, the high cost of substrate treatment, low conversion efficiency and by-product problems also need further improvement. In response to these, current research has begun to use different technologies to improve the tolerance of microorganisms to vanillin and increase biosynthesis yield. This slightly improves the shortcomings of vanillin biosynthesis and lays the foundation for large-scale industrial production. In summary, the strategy of biosynthetic vanillin has significant economic and environmental advantages, conforms to the pursuit of green in the era, and has very broad development prospects.

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