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Review Ariticle

A Summary of Producing Acetoin by Biological Method

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Abstract: Acetoin (3-hydroxy-2-butanone) is an important frankincense flavor and 4-carbon platform compound, which is widely used in food, daily chemical, chemical and pharmaceutical industries. With the continuous improvement of people's living standards, it has proposed more food safety High requirements. At present, the US JM Company, German BASF Company and Japan's Xinda Company mainly produce 3-hydroxybutanone in the world. At present, its production method is mainly based on chemical synthesis. The process has serious pollution, complex process, unstable product quality, and limited raw material sources. Biological method has abundant raw material sources and can be regenerated, environmentally friendly process, mild conditions, and products. High quality and other advantages have attracted attention. At present, there is no report on the scale production of 3-hydroxybutanone by biological method at home and abroad. Coming from the pressures of population, resources, environment, etc., the traditional chemical industry using fossil resources as raw materials is bound to be gradually replaced by new and environmentally friendly biochemical industries using renewable resources as raw materials. Carrying out environmentally friendly, abundant sources of raw materials, mild conditions, products can be regarded as pure natural microbial fermentation technology to produce 3-hydroxybutanone technology has broad prospects for promotion and application. This article summarizes the research status of the production of 3-hydroxybutanone by biological methods, including 3-hydroxybutanone producing strains, 3-hydroxybutanone synthesis pathway, 3-hydroxybutanone decomposition pathway, metabolic mechanism and related enzymes, metabolic regulation Mechanism and efficient accumulation strategy of 3-hydroxybutanone.

Keywords: Producing Acetoin, Biological Method, Strains, Metabolic Mechanism, Accumulation Strategy

1. Introduction

Acetoin is widely distributed throughout corn, grape, cocoa, apple, banana, cheese and meat such food, which is a kind of widely used flavouring with pleasant cream flavor. It is mainly used in the production of essence such as cream, dairy, yogurt and strawberry. Acetoin is related to the flavor of beer and glucose, and plays a key role in the flavor of producing cheese during the fermentation of butter and cream [1, 9-12]. China's standard GB2760-86 stipulates that it is a flavouring which is

allowed to be used [2]. The US FEMA safety number is 2008, and the recommended dosages are shown in Table 1 [3].

The demand for acetoin for milk flavouring continues to increase with the growth of people's living standards and the sales of dairy products. In addition, in 2004, the US Department of Energy listed acetoin as one of the 30 platform compounds that were developed and utilized preferentially [4, 13-19], which is also sufficient to illustrate the potential use and importance of acetoin. The production and application research of acetoin, especially the research on the producing acetoin by biological method which is environmentally friendly and the product can be regarded as pure natural has attracted people's attention [5, 20-24].

Table 1. *Recommended dosage of acetoin.*

Food category	Recommended dosage (mg/kg)
Soft drink	7.4
Cold drink	3.3
Candy	1.8
Baked food	32
Pudding	21
Couda cheese	7.0
Margarine	50
Butter	8.0

The chemical synthesis of acetoin was reported as early as 1906 by the partial reduction of diacetyl by zinc and acid [6, 10-16]. At present, the producing acetoin by chemical synthesis at home and abroad is mainly based on three processes of partial hydrogenation reduction of butanedione, selective oxidation of 2,3-butanediol and chlorination of butanone. Processes are generally shortcomings such as low product recovery and low yield, serious environmental pollution, and the quality and safety of products are questioned [7, 8, 11-13]. Another serious problem is that the raw materials used in the chemical synthesis process, such as diacetyl or 2,3-butanediol, are mainly derived from non-renewable fossil resources, and these raw materials are also important chemical products, petroleum resources. The growing shortage and rising oil prices, limited sources of raw materials, and higher product costs have become the main bottlenecks limiting the large-scale development of acetoin by chemical synthesis. Since acetoin contains asymmetric carbon atoms and has two chiral isomers (left-handed R, right-handed S), it is not currently possible to synthesize products with high optical purity and certain optical rotation, using conventional physics. Methods (eg fractionation, fractional crystallization, etc.), it is very difficult to carry out chiral resolution of a racemic product of equal enantiomers or directly synthesize a product with a specific optical rotation, which must be carried out by means of microbial methods. The special method such as induced crystallization can achieve the purpose of chiral separation, which also limits the development and application of acetoin by chemical synthesis [9-11, 17-21, 25-29].

With the development of biotechnology, biotechnology plays an increasingly important role in the synthesis of flavor substances. Chemical or petrochemical synthetic flavor substances are gradually replaced by enzymatic synthesis or fermentation technology production. It is well known that biological processes are environmentally friendly. In addition, food regulations in many countries have recognized that natural wind agents can be obtained by biological method. The research and development of bio-productive technologies with commercial value of natural compounds has attracted people's attention [8, 12-19]. In recent years, there have been many reports on the technical research on the production of acetoin by biological methods. Although most of the research is still in the laboratory research stage or small batch production stage, there are still many technical or economic problems, and industrial large-scale applications are still not feasible, but the advantages of bio-process environmental friendliness, product safety and renewable raw materials will become the leading direction of producing acetoin in the future [13-18, 30-33].

2. Producing Acetoin by Enzymatic Conversion

With the development of enzyme engineering technology, there have been many successful examples of enzyme conversion technology replacing traditional chemical processes. The key to this process is to obtain a large number of specific enzymes [19-22, 34-38]. In 1992, Hummel et al. [9] in the United States applied butanone reductase (diacetyl reductase) in *Lactobacillus* or *Yeast* cells as a biocatalyst to catalyze the reduction of butanone to acetoin. The method firstly isolates and purifies the diacetyl ketone reductase from the cells by culturing *Lactobacillus* or *Yeast*, and catalyzes the reduction of the butyl diketone reductase and the coenzyme NAPH at pH 5.0 and 70°C. The advantage of this method is that the yield is up to 100%, and no other by-products are produced. Due to the specificity of the biological enzyme, a product with a certain optical rotation can be obtained. In 1996, Zhang et al. [6] reported that it can be used to prepare acetoin with 2,3-butanediol using Sorbitan, or to ferment sugarcane juice by fungus such as *Aspergillus and Penicillium*, but there are still many problems with the scale application of these processes. In 2003, DeFaveri et al. [10] used a membrane reactor adsorbed with alcohol dehydrogenase to synthesize acetoin from 2,3-butanediol catalyzed by *Acetobacter hansenii*. The effects of dissolved oxygen level and P/O ratio on the catalytic reaction of the enzyme were investigated. Under the optimum reaction conditions, the maximum molar conversion rate of 2,3-butanediol to acetoin is 71.6%, and the highest is acetoin. The mass concentration reached 8.93 g/L, and a mathematical model of the material and energy balance of the transformation process was established.

Although the yield of acetoin in the enzymatic conversion process is higher, the by-products are less, and the product has optical rotation, the enzymatic conversion to produce acetoin is mainly based on diacetyl or butanediol. Its source is limited and the price is higher, which is similar to the chemical synthesis method. In addition, the production cost and performance of the enzyme are the key to the process

technology. It is difficult to obtain a large number of specific enzymes, therefore, the preparation of raw materials and enzymes restricts the development and application of acetoin in the enzyme conversion process.

3. Producing Acetoin by Biological Method

The biological method for producing acetoin has the advantages of simple process, mild conditions and renewable raw materials, and is the most promising method. Acetoin is an intermediate in the metabolism of many microbial sugars, which is the basis for the production of acetoin by fermentation. The development of modern biotechnology, metabolic control fermentation technology, metabolic engineering technology and advanced fermentation parameter control technology, separation technology and other supporting technologies have laid a theoretical foundation and technical support for the establishment of acetoin production technology by fermentation. Since the discovery of some microbial metabolism of sugars to secrete acetoin in the early 20th century, studies on the biosynthesis and metabolic regulation of acetoin have been the focus of attention. There are many reports on the microbial metabolism of sugars to secrete acetoin, but most of them are related to microbial metabolic mechanisms and gene regulation [10-13, 21-23, 39-42]. A few studies involving acetoin fermentation are mainly as diacetyl and By-products of 2,3-butanediol fermentation have been mentioned, and there have been few reports on the production of acetoin by microbial fermentation of saccharide raw materials, and most of them are still in the preliminary research stage such as strain selection [42-46].

3.1. Acetoin-producing Microbial Strains

Acetoin is a metabolite of important physiological functions secreted by many microorganisms. The Voges Proskauer test to diagnose whether a strain secretes acetoin as a marker for microbial classificationin. The secretion of acetoin has important physiological significance for the microorganism itself [11, 25-27]. Acetoin is an intermediate of most microbial sugar metabolism. There are many microorganisms that can convert sugar to produce acetoin in nature. For fungi and bacteria, more bacteria are found in the research [21-26, 47-49]. The bacterial strains reported mainly include *Enterobacter, Bacillus, Klebisella, Serratia, and Lactococcus*. The acetoin producing strains reported in the literature are shown in Table 2. In 1948, Olson et al. [28] reported that the fermentation of 100 mM glucose with *Aerobacter aerogenes* produced up to 80 mM 2,3-butanedione and 1.8 mM acetoin. In 1971, Braneni et al. [29] reported that in the study of the effects of stirring, citric acid and pyruvate on the growth of *Lactobacillus* and the accumulation of 2,3-butanedione, it was found that this strain accumulates 2,3-butanedione while also accumulating acetoin and 2,3-butanediol. In 1987, Jeffrey et al. [30] reported that the conversion of glucose can produce up to 10.8 mM acetoin by using of Streptococcus mutans wild-type strain and lactate dehydrogenase-deficient mutant under aerobic conditions. In 1991, Zeng et al. [17] reported that the 2,3-butanedione and acetoin with a final mass concentration is 110 g/L by *A. aerogenes*. The yield of the 2,3-butanedione and acetoin of *Enterobacter aerogenes* engineering bacteria which efficiently constructs hemoglobin (VHb) gene (vgb) was increased by 83% which was constructed by Hikmet et al. [31]. In 1996, Hespell [32] reported that when investigating the substrate of *Bacillus polymyxa*, some strains were found to produce 2,3-butanedione and acetoin with xylose, etc., with the substrate of 5% glucose and 5% arabinose, the yield of acetoin reaches 11.3 g/L at a temperature of 30°C, pH 7.0, and 250 r/min, and the yield of 2,3-butanediol is 2.3 g/L. There are many reports on the simultaneous production of 2,3-butanediol by strains to produce acetoin, but most strains have low concentrations of acetoin. It is impossible to produce acetoin as a production strain for industrial fermentation. Zhao et al. [25] and Liu et al. [26] obtained a high-yield acetoin-producing *Bacillus subtilis* SFA-H31 (CGMCC1869) with two Chinese patents and one US patent (US9315874B2), which yield of acetoin reached 55.67 g/L, and this strain does not produce by-products such as diacetyl and 2,3-butanediol. Hui Xu and Jianjun Liu constructed a high-yield acetoin-producing *Bacillus subtilis* HB-32. The yield of acetoin in the 50 L fermenter reached 63.55 g/L [1-4]. This strain is currently reported as a relatively high-yield acetoin-producing strain, and is the most likely to be applied to the industrialization of acetoin in fermentation technology, especially the strain does not produce or accumulate a small amount of by-products such as butanedione and 2,3-butanediol, and is essential for its application to acetoin fermentation production.

Table 2. *Recommended dosage of acetoin.*

Strains	Carbon source	Yield (g/L)	References
Bacillus subtilis HB-32	Glucose	63.55	[1]
Bacillus subtilis SFs-4	Glucose	55.7	[3]
Klebsiella pneumoniae NRRL B-199	Glucose	17~19	[12]
Enterobacter cloacae ATCC 27613	Glucose	<14	[13]
Bacillus subtilis AJ 1992	Sucrose	<20	[14]
Serratia marcescens IAM1022	Molasses	6.62	[15]
Lactococcuslactis subsp. lactis 3022	Glucose	9.28	[16]
Enterobacter aerogenes DSM 30053	Glucose	10~12	[17]
Bacillus subtilis CICC10025	Glucose	37.19	[20]
Klebsiella pneumoniae CICC 10011	Molasses	13.1	[21]
Klebsiella oxytoca DSM 3539	Glucose	4.13	[22]
Bacillus pumilus DSM 16187	Molasses	63.0	[24]
Bacillus subtilis CGMCC 1869	Glucose	55.5	[25, 26]
Hanseniaspora guilliermondii CCT3800	Glucose	0.365	[27]

Compared with chemical synthesis and enzymatic conversion, the production of acetoin by microbial fermentation has the advantages of simple process, mild conditions, environmental friendliness, abundant raw material source and renewable, and the product can be regarded as pure natural and high security, which is the most economically

viable production method for acetoin. With the deepening of research on the production of acetoin by fermentation, advanced biotechnology and traditional breeding techniques are combined to breed and construct high-yield, high-performance acetoin-producing strains, while focusing on the supporting research on the upstream and downstream technologies for the production of acetoin by fermentation, it is the key to realizing the producing acetoin by bio-fermentation as soon as possible [36-39, 50-55].

3.2. Biosynthesis Pathway of Acetoin

Since the discovery of some microbes having the ability to secrete acetoin, the research on the synthesis and decomposition pathways of acetoin in the organism has attracted wide interest [12-14, 56-59]. The formation mechanism of acetoin was studied as early as the 1940s, and the ways to synthesize acetoin in bacteria, yeast, plants, and animal livers have been found in the subsequent studies. In bacteria, acetoin is synthesized only from pyruvate, in yeast and animal tissues, it is synthesized by pyruvic acid and acetaldehyde, but in plants and certain animal tissues, it is only synthesized by acetaldehyde [30-33, 60-63]. There are large differences in the routes of synthesis of acetoin between different microorganisms and in plants or animal organs. Mizuno et al. [82] onfirmed the transformation process of bacterial strain pyruvate to acetoin by isotope tracer method, it is shown in Figure 1. Juni [33] have reported the mechanism of acetoin by bacteria taking aerogens as the research object, and it was confirmed that the bacterial extract capable of forming acetoin from pyruvic acid can convert α-acetolactate to form acetoin, in contrast, bacterial extracts that are unable to form acetoin are unable to convert alpha-acetolactate to form acetoin. Two kinds of enzyme components are separated from the cell extract of *Aerogenophilus*, one component acts on pyruvate to form α-acetolactate, and the other component catalyzes decarboxylation of α-acetolactate but has no effect on pyruvic acid. It was later confirmed that the former is α-acetolactate synthase, and the latter is α-acetolactate decarboxylase. In 1954, Harold et al. [34] used bacterial cell extracts to study the relationship between the pyruvate oxidation system and the formation of acetoin, the formation of acetoin is not related to aerobic oxidation of pyruvic acid (into the tricarboxylic acid cycle) or anaerobic metabolism (produce lactic acid or acetaldehyde), instead, there is another way to form acetoin. Isabel et al. [51] studied the formation and properties of α-acetolactate synthase in *Bacillus subtilis*. Its pH value is between 6.0 and 8.0, which is regulated by the feedback inhibition of proline and the activation of pyruvate. The metabolic regulation mechanism of branched-chain amino acids is not identical in different strains. In *Serratia marcescens*, the metabolic regulation mechanism of branched-chain amino acids and the synthesis of acetoin are shown in Figure 2 [25-29, 64-69].

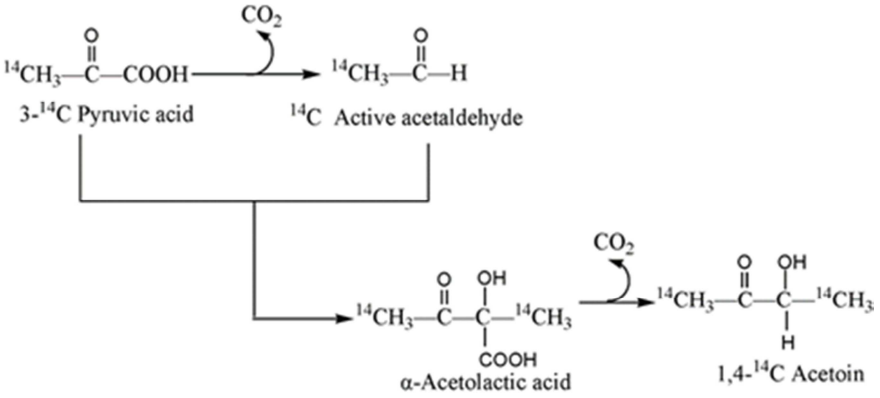

Figure 1. *Conversion of β-14C pyruvate to acetoin.*

Although there are differences in the structure and properties of some enzymes related to the metabolism of acetoin in different microorganisms, different scholars have different opinions, but so far, there are a large number of studies in biochemical and molecular organisms on the synthesis of acetoin pathway by bacteria. it have been have confirmed that the metabolic pathway of acetoin mainly involves the glycolysis (EMP) pathway of glucose metabolism (glucose to pyruvate) and the conversion of pyruvate to acetoin, as shown in Figure 3 [31,36-38, 70-74]. There are two main ways to synthesize acetoin in microorganisms: Two molecules of pyruvic acid synthesize a molecule of α-acetolactate under the action of α-acetolactate synthetase, alpha-acetolactate is naturally oxidatively decarboxylated under acidic conditions to form 2,3-butanedione, 2,3-butanedione is reduced to produce acetoin by the action of butanedione reductase or 2,3-butanediol dehydrogenase, Another way, Two molecules of pyruvic acid synthesize a molecule of α-acetolactate under the action of α-acetolactate synthase, the α-acetolactate is converted to acetoin by the action of α-acetolactate decarboxylase. The reaction process of conversion from glucose to acetoin is shown in Figure 4 [16-19, 41, 63, 75-82]. The theoretical conversion of glucose to acetoin is: (88÷180) ×100%=48.89%.

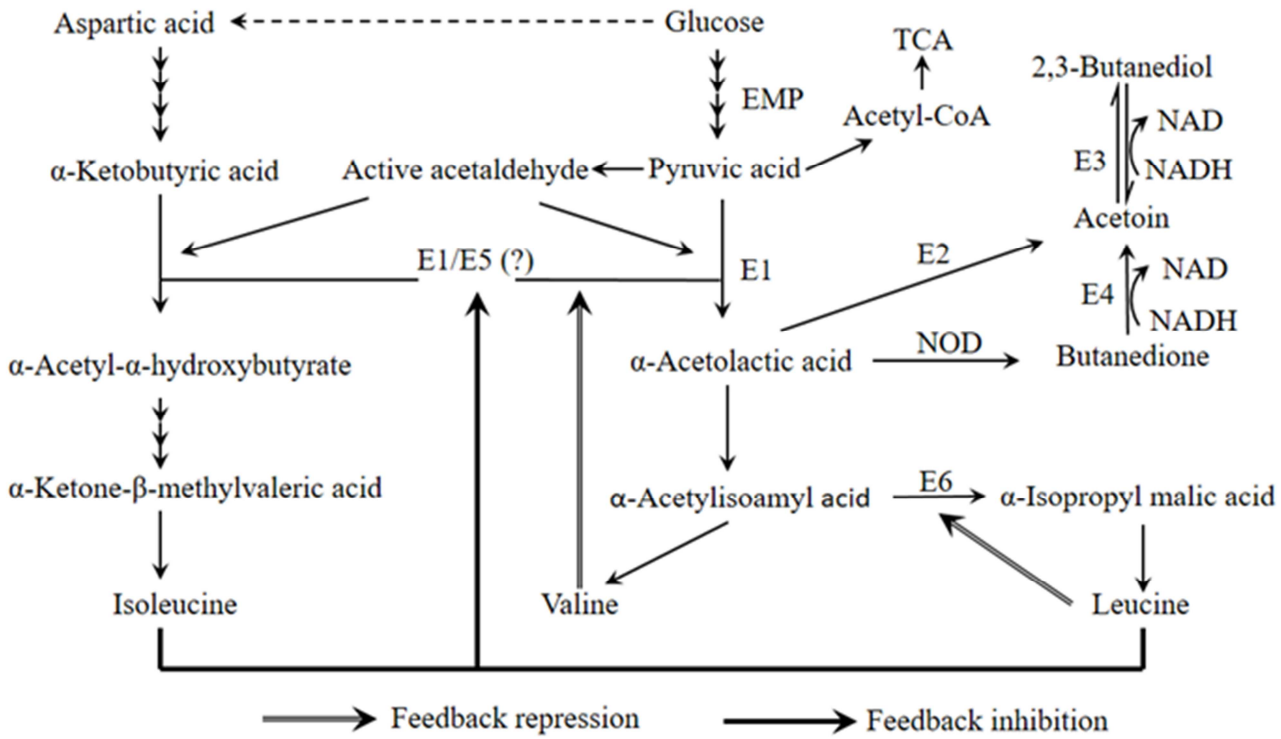

Figure 2. *Metabolism of Branched-chain amino acid and Accumulation of acetoin.*

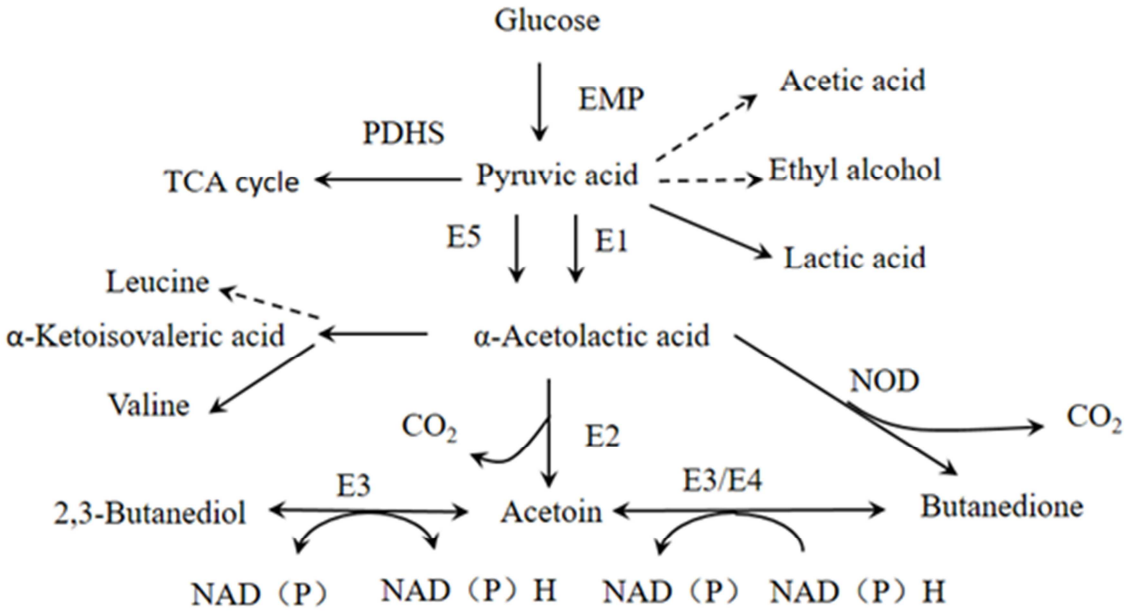

Figure 3. *Synthesis route of acetoin. Note: EMP-glycolysis pathway; TCA-tricarboxylic acid cycle; PDHS-pyruvate dehydrogenase system; E1-α-acetolactate synthase (catabolic type); E2-α-acetolactate decarboxylase; E3-2,3-butanediol dehydrogenase; E4-butanedione reductase; E5-α-acetolactate synthase (anabolic); NAD (P)-oxidized coenzyme I (II); NAD (P) H-reduced coenzyme I (II); NOD-non-enzymatic oxidative decarboxylation.*

With the development of biology and related technologies, there are many stuies on the isolation and purification of related enzymes related to acetoin metabolism, as well as the study of the catalytic behavior of enzymes *in vitro*, it further reveals the anabolic pathway of acetoin *in vivo*, which lays a foundation for gradually revealing the metabolic regulation mechanism of acetoin at the genetic level in the past decade. At the same time, it has laid a theoretical foundation for the construction of high-yield acetoin-producing strains by modern biotechnology such as genetic engineering and metabolic engineering [40, 42-44, 85].

$$CHO(CHOH)_4CH_2OH + O_2 \longrightarrow 2CH_3COCOOH + 2H_2O$$

$$2CH_3COCOOH \longrightarrow CH_3COH(COCH_3)COOH + CO_2$$

$$CH_3COH(COCH_3)COOH \longrightarrow CH_3CHOHCOCH_3 + CO_2$$

$$CHO(CHOH)_4CH_2OH + O_2 \longrightarrow CH_3CHOHCOCH_3 + 2H_2O + 2CO_2$$

Figure 4. *Conversion from glucose to acetoin.*

3.3. Decomposition Pathway of Acetoin

Under normal circumstances, acetoin cannot be accumulated in microorganisms. Microorganisms accumulate acetoin as a storage energy source in order to withstand adverse environments, after the easy-to-assisize carbon source such as glucose is exhausted, it continues to consume acetoin to maintain its own life activities, which fully indicates the existence of acetoin catabolic system in microorganisms. Although the catabolism of acetoin has been studied since the 1920s, it has been more than 80 years old [11, 33, 57, 67, 86-88], but compared with the biosynthesis of acetoin, the research report about the catabolism of acetoin is still relatively small. There are still different opinions on the catabolism of acetoin in living organisms. Early studies suggested that the degradation of acetoin was achieved by a circular pathway (2,3-butanediol ring). As early as the 1950s, Juni et al. [35-37, 89-95] studied the pathways of bacterial metabolism of 2.3-butanediol, acetoin and diacetyl. It is proposed that a compound capable of growing with 2,3-butanediol or acetoin as the sole carbon source has a circular pathway to convert these compounds to produce acetic acid, and clarified the composition of the circular pathway and the transformation process between related substances, in addition to 2.3-butanediol, acetoin and diacetyl, the cyclic pathway is mainly involved in two new intermediates, 3-acetyl-3-hydroxybutanone and 3-acetyl-2,3-butanediol, in the ring pathway, diacetyl is converted to 3-acetyl-3-hydroxybutanone and acetic acid by a ketamine condensation catalyzed ketol condensation reaction, and 3-acetyl-3-hydroxybutanone is reduced to 3-acetyl Base-2,3-butanediol, which in turn is hydrolyzed to 2,3-butanediol and acetic acid, the effect of one cycle of the circular pathway is that one molecule of acetoin forms two molecules of acetic acid, which is a compound that can be rapidly oxidized by microorganisms, thereby reaching the microorganisms using 2.3-butanediol, acetoin as growth and the purpose of the energy substrate. Many scholars have studied this approach [38-39, 69, 96-98] and have proposed different views afterwards. The composition of the 2,3-butanediol cycle is shown in Figure 5 [35, 33]. López et al. [39, 40] confirmed that although *Bacillus subtilis* is capable of degrading all intermediates in the 2,3-butanediol ring, for the catabolism of acetoin, a complete ring is not required, blocking the mutant strain in the two-step reaction in the loop can metabolize acetoin as well as the standard strain. Later, López et al. [41] studied the degradation of acetoin in *Bacillus subtilis* by direct oxidative cleavage. The direct oxidative decomposition products of acetoin detectable in *Bacillus subtilis* cell extracts are acetic acid and acetaldehyde. No diacetyl was detected in the reaction system. Direct oxidative cleavage of acetoin explains that the catalysis of acetoin does not necessarily require the oxidation of acetoin to diacetyl, which requires thiamine pyrophosphate as a cofactor. It was also confirmed that the mutant strain lacking the acetoin cleavage ability could not utilize the acetoin accumulated after the glucose sugar was depleted, which further confirmed the true importance of the acetoin cleavage reaction to the acetoin catabolism. The 3-acetyl-3-hydroxybutanone synthase which catalyzes the formation of 3-acetyl-3-hydroxybutanone from diacetyl may be identical to the enzyme which catalyzes the cleavage of acetoin. Their conclusions indicate that B. subtilis directly cleaves acetoin into two molecules of acetaldehyde, one of which is an activated form which is subsequently further oxidized to acetic acid. This conclusion is clearly inconsistent with the degradation of acetoin proposed by Juni et al. [35-37] through the 2,3-butanediol ring pathway. The rapid development of molecular biology provides a guarantee for the gradual revealing of the acetoin catabolism mechanism at the molecular level. In the past 20 years, there have been many reports on the gene regulation mechanism of acetoin catabolism, such as Grundy et al. [42] proposed the catabolic products of *Bacillus subtilis* acetic acid and acetoin utilization genes from CcpA (spherical Regulatory protein) regulation. Huang et al. [43] proposed that the decomposition of acetoin is related to the deletion of the strain *aco*A gene (alpha-subunit encoding acetoin dehydrogenase dependent on thiamine pyrophosphate). The view that the degradation of acetoin is carried out through the 2.3-butanediol ring is considered to be a false assumption. A growing number of studies have shown that acetoin is more commonly catalyzed by the direct oxidative cleavage pathway of acetoin dehydrogenase (AoDH ES) in bacteria [44–47, 66-69, 24]. In summary, the degradation pathway of acetoin *in vivo* is shown in Figure 6.

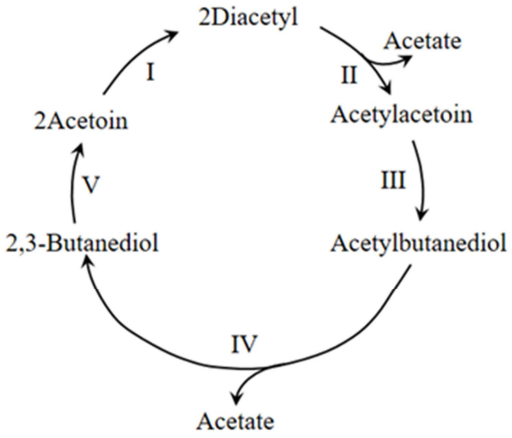

Figure 5. *Constitute of 2,3 - butanediol cycle.*

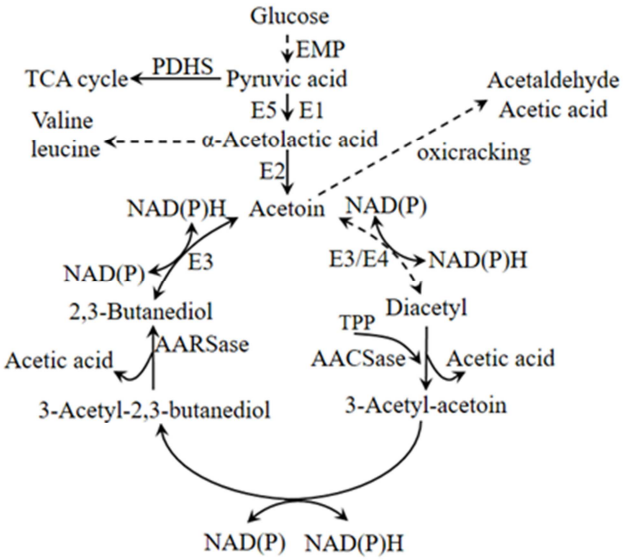

Figure 6. *Catabolic pathway of acetoin. Note: AACSase-acetylacetoin synthase; AACRase-acetylacetoin reductase; TPP-pyrophosphate pyrophosphate; other abbreviations annotated with Figure 1, E3/E4 catalyzed diacetyl and 3-hydroxy Whether the reaction between butanone (dotted line connection) is reversible remains to be confirmed.*

3.4. Acetoin Metabolism-related Enzymes

According to Figure 1 and Figure 3, the enzymes involved in the metabolism of acetoin mainly involve α-acetolactate synthase (E1) which catalyzes pyruvate to α-acetolactate, and catalyzes the production of acetoin by α-acetolactate. Ketone alpha-acetolactate decarboxylase (E2), 2,3-butanediol dehydrogenase (E3) catalyzing the conversion of 2,3-butanediol and acetoin, and catalyzing diacetyl to acetoin Ketone 2,3-butanediol dehydrogenase (E3) and butanedione reductase (E4). With the development of biochemical separation and purification technology, there are many studies on enzymes related to acetoin metabolism [43-45]. The enzymes related to acetoin metabolism in many microorganisms have been isolated and purified and their properties studied [33, 48-51, 57, 67, 85-88], the results show that there are differences in the structure and properties of enzymes catalyzing the same reaction in different microorganisms, and there are still some problems to be further confirmed.

The earliest study by Juni et al. [37] found that 2,3-butanediol dehydrogenase extracted from bacteria cultured in 2,3-butanediol and mineral media lost substrate specificity and it can catalyze several reactions in the process of 2,3-butanediol dissimilation. 2,3-butanediol dehydrogenases of several microorganisms cultured from glucose have different substrate specificities and different substrate affinities, when *Aerogenes* are grown in glucose and acetoin as a carbon source. In the base, different 2,3-butanediol dehydrogenases are formed. Klaus et al. [52] purified the enzyme (E4) which catalyzes the reduction of diacetyl in *Aerobacteria* and the enzyme (E3) which reacts reversibly with acetoin and 2,3-butanediol to homogeneity. The ratio E3/E4 is kept constant, and it is inferred that both reactions are catalyzed by the same enzyme and it is recommended to name the enzyme diacetyl (acetoin) reductase. Øyvind et al. [53] studied the diversity of diacetyl (acetoin) reductase in aerobacteria, and confirmed that the pure enzyme of diacetyl (acetoin) reductase of aerogenic bacteria consists of at least 12 different protein components. All of these components are enzymatically active. Liv et al. [54] studied the kinetics of the amphetamine-dihydroxy (acetoin) reductase catalyzing the reduction of diacetyl to acetoin. It was confirmed that the reaction of diacetyl (acetoin) reductase to catalyze the reaction of acetoin to 2,3-butanediol and diacetyl to acetoin is a reversible reaction. The enzyme is a tetramer composed of four subunits of the same size. The reaction of 2,3-butanediol to acetoin is inhibited by acetate and found at pH 5.8, the K_m value of 2,3-butanediol in acetic acid buffered liquid was increased by 10 times compared with other buffer systems. Forlani et al. [55] examined the mechanism of acetoin production in suspension culture of carrot, tobacco, corn and rice plant cells. The extract can catalyze the synthesis of acetoin from pyruvate and/or acetaldehyde. Three acetoin forming enzymes were isolated and purified by adsorption chromatography and gel filtration chromatography, and the diversity of acetoin forming enzymes in plant cells was also studied. Halpern [56] confirmed that there are two different enzymes that catalyze the synthesis of a-acetolactate in *Aerobacteria*, one related to the synthesis of proline and the other related to the formation of acetoin. In 1976, Zahler et al. [57] confirmed that there are also two enzymes that can catalyze the synthesis of α-acetolactate in *Bacillus subtilis*, one is related to the synthesis of branched chain amino acids, catalyzing one molecule of active acetaldehyde and one molecule of acetone acid condensation to form α-acetolactate (precursor of branched chain amino acids), another α-acetolactate synthase catalyzes the condensation of two molecules of pyruvate to form α-acetolactate, which is related to the production of acetoin in the stationary phase of *Bacillus subtilis*. It has been confirmed that the synthesis of acetoin and the synthesis of branched-chain amino acids share a precursor α-acetolactate, and the presence of two enzymes catalyzing the formation of α-acetolactate has also been confirmed, which is related to the synthesis of branched-chain amino acids is called anabolic α-acetolactate forming enzyme or α-acetohydroxy acid synthase (AHAS, E5), which is related to the formation of acetoin is called catabolic alpha-acetolactate forming enzyme or alpha-acetolactate synthase (ALS) [11]. Thomas et al. [58] studied the mechanism of synthesizing acetoin from carboxylase. High purity wheat embryo a-carboxylase catalyzes the synthesis of acetoin from pyruvate and acetaldehyde and only acetaldehyde. The synthesized products consisted of 72% (+) dextrorotatory and 28% (-) left-handed optical isomers, respectively. Holtzclaw et al. [59] purified *Bacillus subtilis* degrading acetolactate synthase and studied the properties of the enzyme. In-depth study of acetoin synthesis and decomposition enzymes and their regulatory mechanisms provides a theoretical basis for the targeted transformation of acetoin production strains to achieve efficient product accumulation. The question about acetoin

metabolism is still inconclusive, such as whether 2,3-butanediol dehydrogenase or diacetyl reductase-catalyzed reaction from diacetyl to acetoin is reversible is still to be confirmed by further research.

3.5. Acetoin Metabolism Regulation and Accumulation Mechanism

Acetoin is the product of the metabolism of many prokaryotic and eukaryotic microorganisms. The microbial metabolism of carbohydrates to secrete acetoin has important physiological significance for the microorganism itself. The more accepted meaning is to resist environmental acidification, participate in the regulation of NAD/NADH ratios, and act as a storage carbon source. Recognizing that bacteria can use acetoin as a carbon source and energy source for growth for more than 80 years [41, 60, 61, 38, 82-83]. The acetoin combination metabolic pathway involves many enzymes such as α-acetolactate synthase and α-acetolactate decarboxylase. Many people's attention has always focused on their research. At present, great progress has been made on the molecular mechanism of the regulation of the metabolism of bacteria acetoin. In-depth understanding of the catabolism of acetoin stems from the study of the diversity of the use of bacteria acetoin, for example, *Pelobacter carbinolicus* [62,63], *Clostridium magnum* [49], *Klebsiella pneumoniae* [46], *Bacillus subtilis* [64], *Alcaligenes eutrophus* [44] and *Pseudomonas putida* [45], the catabolism of acetoin was found to be catalyzed by the acetoin dehydrogenase enzyme system (AoDH ES) in these bacteria, including acetoin dehydrogenase (AoDH E1), dihydrolipoamide acetyltransferase (AoDH E2), and dihydrolipoamide dehydrogenase (AoDH E3), which are dependent on thiamine pyrophosphate, and The structural gene *aco*A of AoDH E (alpha-subunit encoding AoDH E1), *aco*B (β-subunit encoding AoDH E1), and *aco*C (encoded AoDH E2) are linearly arranged on the chromosome. In P. carbinolicus, C. magnum and K. pneumoniae, the *aco*L gene (encoded AoDH E3) is also part of the *aco* gene cluster. The relevant genes in the AoDH ES system reported in the current study are shown in Table 3. Renna et al. [65] cloned and sequenced the acetolactate synthase gene (alsS), acetolactate decarboxylase gene (alsD) and regulatory gene alsR of *Bacillus subtilis*, and found that alsS and alsD belong to the same operon. The transcription of this operon is regulated by the alsR gene product, and alsS cannot be expressed if alsR is disrupted. Acetoin is stored and secreted as an additional carbon source in the logarithmic growth phase of the strain, and is utilized in a still unclear pathway during the stable growth phase, acting during sporulation [43, 66]. Grundy et al. [42, 67] proposed that *Bacillus subtilis* was regulated by CcpA protein using acetic acid and acetoin genes, and the acu gene cluster downstream of ccpA gene was isolated and identified, and it was confirmed that if the acuA gene was knocked out, the strain Growth with acetoin or 2,3-butanediol as carbon sources and formation of spores were affected. Huang et al. [43] reported the biochemical and molecular characteristics of the catabolism of acetoin of *Bacillus subtilis*. Different views were put forward, it is believed that the enzyme that catabolizes acetoin by *Bacillus subtilis* is encoded by the acu gene cluster, which is completely different from that found in all other bacterial strains that can grow using acetoin as a carbon source, a multi-component acetoin dehydrogenase enzyme system (AoDH ES) encoded by the *aco* gene cluster. Clostridium magnum contains AoDH ES *aco*A and *aco*B gene fragments as probes, and hybridized with *Bacillus subtilis* genome to isolate homologous genes such as *aco*A, *aco*B, *aco*C, *aco*L and *aco*R. It was also found that if the acoA gene (alpha-subunit encoding AoDH E1) was knocked out, the cells could not be grown using acetoin in the medium or acetoin as the sole carbon source. It was shown that the use of acetoin by *Bacillus subtilis* was also controlled by the *aco* gene cluster. According to the molecular regulation mechanism of acetoin metabolism, the alsR gene mutation can block the transcription of the alsSD operon, and the ALSS gene deletion mutation does not accumulate acetoin [65]. *Aco*A gene deletion mutant cells cannot grow using acetoin as the sole carbon source [43]. Ali et al. [47] also confirmed that the cumulative acetoin will not continue to be consumed when the strain *aco*A gene is deleted. A growing number of studies have shown that 3-hydroxybutanone is primarily degraded by the direct oxidative cleavage pathway of the acetoin dehydrogenase system (AoDH ES) in bacteria [38, 83, 86, 95]. In summary, the relevant pathways for bacterial metabolism of acetoin are shown in Figure 7 [92-98]. The results of this series of theoretical studies laid the foundation for the construction of high-yield acetoin strains by genetic engineering.

Table 3. *Related genes of AoDH ES system.*

Genes	Strains	Main function	References
acoA	*Pelobacter carbinolicus*	Structural gene, encoding AoDH, ES, E1α-	[63, 77]
acoB	*Clostridium magnum*	Structural gene, encoding AoDH, ES, E1β-	[49,85]
acoC	*Klebsiella pneumoniae*	Structural gene, encoding AoDH, ES, E2	[46, 76]
acoL	*Bacillus subtilis*	Structural gene, encoding AoDH, ES, E3	[38]
acoR	*Alcaligenes eutrophus Pseudomonas putida*	The regulatory gene of *aco* operon, encoding regulatory protein	[44]
acoD	*Bacillus subtilis*	Encoding acetaldehyde dehydrogenase II, relative to AC catabolism	[43]
acoE	*Clostridium magnum*	Encoding acetyl CoA synthase, relative to AC metabolism	[83]
acoK	*Alcaligenes eutrophus*	Relative to AC expression induced by *aco* operon	[75]
acoS	*Alcaligenes eutrophus*	Encoding Zinc sulfate synthase, locating in the downstream of operon *aco*C, sharing the same operon with *aco*L	[74, 74]
acoX	*Klebsiella pneumoniae*	Encoding unknown protein, relative to AC catabolism	[73, 74]

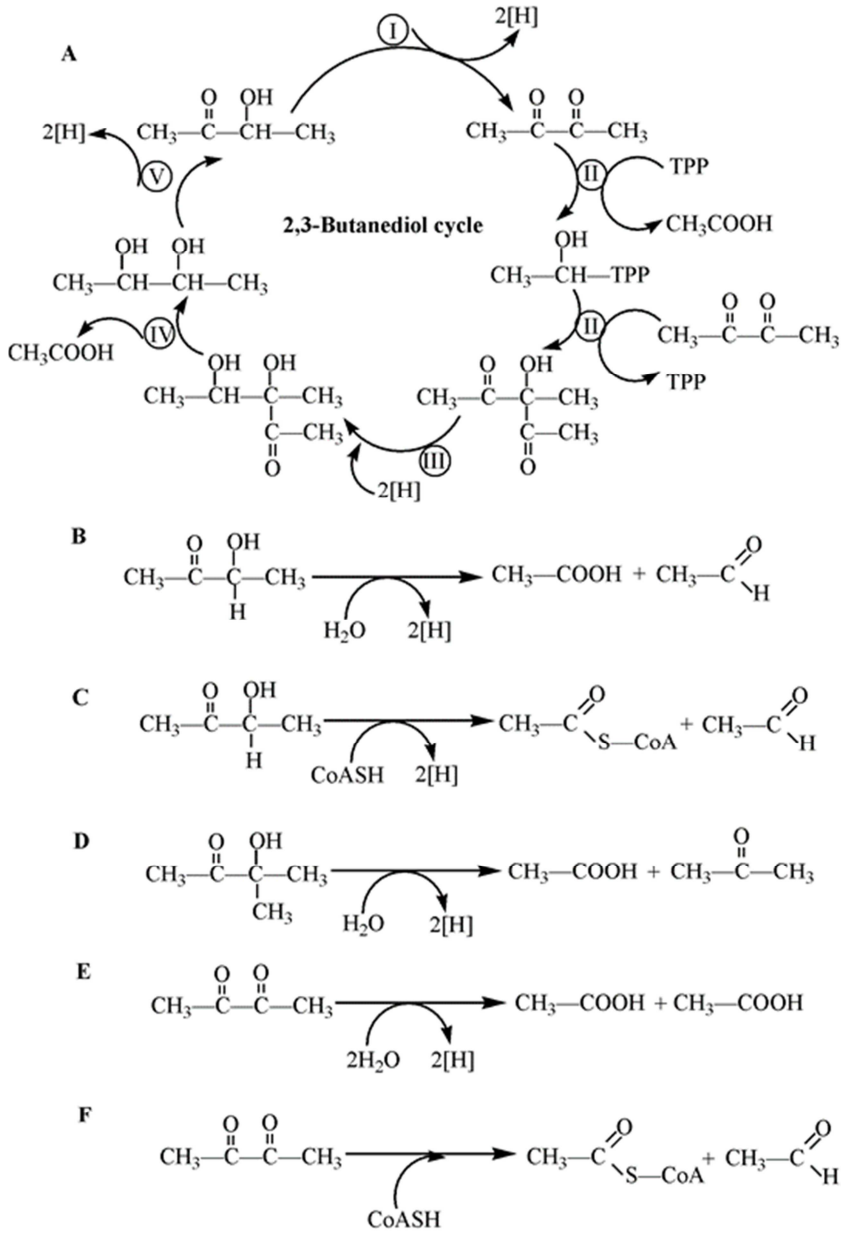

Figure 7. *Reaction process and Compounds involved in Catabolism of acetoin. Note: A: 2,3-butanediol cycle; (I) 2,3-butanediol dehydrogenase; (II) acetoin dehydrogenase; (III) acetoin synthase (IV) 3-acetyl-hydroxybutanone reductase; (V) 3-acetyl-butanediol hydrolase; B: Oxidative-hydrolytic cleavage (eg, Ao: DCPIP OR); C: Oxidative-thiolytic cleavage of acetoin; D: Oxidative hydrolysis of 3-methyl-hydroxybutanone (eg MeAo: DCPIP OR); E: Oxidative hydrolysis of diacetyl ketones (eg Di: DCPIP OR); F: thiocyanate cleavage of diacetyl; TPP: thiamine pyrophosphate; CoA: Coenzyme A; CoASH: reduced coenzyme A.*

3.6. Construction Strategy of Acetoin High-yield Strain

From the perspective of efficient accumulation of acetoin, the strain should be characterized by high activity of E1 and E2 and deletion or low level of E3 and E4. From a metabolic engineering perspective, it is also a very effective strategy to cut off branch metabolic pathways or reduce branch metabolic flux. Mallonee et al. [68] obtained a catabolic mutant of alpha-acetolactate-forming enzyme (E1) (AlsR-, phenotype) by eliminating anabolic alpha-acetolactate forming enzyme (E5). The yield of 2.3-butanediol was increased by a factor of three. Hikmet et al. [31] cloned the pUC8 plasmid encoding the *Vitreoscilla* (bacterial) hemoglobin (VHb) gene (vgb) into *Enterobacter aerogenes*. The expression of the hemoglobin (VHb) gene (vgb) increased the metabolic flux of the acetoin/butanediol metabolic pathway, increasing the yield of the strain fermentation products acetoin and butanediol by 83%. Zahler et al. [57] used the same screening procedure to obtain a B. subtilis mutant that began to produce acetoin in the early logarithmic growth phase. In order to obtain a high-yield acetoin producing strain, it is possible to genetically modify existing strains of high-yield butanedione or 2,3-butanediol, for example, knock out related genes encoding E3 and E4 genes such as *aco*A gene or reduce their gene transcription levels. On the other hand, strains can be produced by

naturally selecting acetoin having a high E1, E2 activity, and low E3, E4, and E5 enzyme levels. Using traditional mutagenesis combined with advanced breeding techniques such as genetic engineering and metabolic engineering to inhibit pyruvate and branched-chain amino acid metabolism, transform and construct high-yield acetoin production strains, and break through the microbial metabolic regulation mechanism. Therefore, improving the ability of the strain to biosynthesize acetoin is the key to the efficient accumulation of acetoin.

3.7. Acetoin Fermentation

There are few studies on the use of acetoin as a target product for fermentation, acetoin as a microbial metabolite is mostly mentioned as a flavor substance produced by fermentation of dairy products or as a by-product of fermentation of diacetyl and 2,3-butanediol. Han [23] obtained a high-yield acetoin mutant F-d31 by conventional mutagenesis treatment of *B. subtillis* SFs-4. The fermentation medium and fermentation conditions for producing acetoin in the mutant strain were optimized, with 130 g/L glucose as the main raw material, the fermentation yield of acetoin in shake flask fermentation reached 48.6 g/L. The fermentation conditions of mutant strain F-d31 acetoin were studied by 16 L and 50 L automatic fermenter, the rotation speed is 300 r/min, the pH value is about 6.0, the highest yield of acetoin is 55.7 g/L, and the conversion rate of sugar is 42.73%. Xiao et al. [20] optimized the *Bacillus subtilis* CICC 10025 medium by response surface test design. The effect of two components of molasses and bean cake hydrolysate on acetoin was investigated. In shake flask fermentation, the yield of acetoin was 37.9 g/L, and the optimal concentrations of molasses and bean cake hydrolysate were 22.0% (v/v) and 27.8% (v/v), respectively. The yield of acetoin was 56.4 hours in a 5 L fermentor and the yield was 35.4 g/L. Liu et al. [26] used *Bacillus subtilis* SFA-H31 (CGMCC1869) with glucose as the main raw material, in the 50 L fermenter, the conversion rate reached 48.26% in 52h, and the yield of acetoin reached 55.67 g/L. It was confirmed that the strain did not produce by-products diacetyl and 2,3-butanediol. Xu et al. [24] screened for a strain of *Bacillus pumilus* XH195 (DSM16187), the yield of acetoin was 63.0 g/L or 58.1 g/L after fermentation at 37°C for 60 h with glucose or sucrose as carbon source. In 2002, Teixeira et al. [27] reported that the experimental design was used to optimize the fermentation of acetoin in Candida sinensis (Hanseniaspora guilliermondii), its initial sugar concentration is 6.8%, temperature is 28°C, and pH 4-6, acetoin production can reach up to 0.36 g/L. Ley [69] studied the production of acetoin by lactic acid as the main substrate of several strains of acetic acid bacteria. The effects of substrate concentration and aeration on acetoin were investigated, the maximum conversion of the substrate reached 74% of the theoretical conversion rate, and a sample of acetoin was obtained. Dettwiler et al. [14] examined the possibility of continuously culturing B. subtilis to produce acetoin and butanediol using molasses as a carbon source. A simulation model for the separation and integration of continuous production of acetoin and butanediol by pervaporation method was established. Li et al. [70] studied the effects of intermediate metabolites and by-products on the anabolism of acetoin. It is believed that the fermentation process of acetoin simultaneously produces acetoin and mixed acid, glucose is the only carbon and energy source that is metabolized along two parallel pathways, oxidation and reduction. The target product and by-products and other associated end products are mainly organic acids such as butanediol, diacetyl, acetic acid and lactic acid. It was found that the use of sucrose and citric acid is conducive to the improvement of the overall fermentation level. Xu Hui and Liu Jianjun constructed a high-yield acetoin-producing *Bacillus subtilis* HB-32, which was fermented in a 50-liter fermenter for 72 h. The yield of acetoin was 63.55 g/L, and the fermentation rate was 0.883 g/L/h [1-4]. The residual sugar was 0.1 g/L, and the conversion rate of sugar consumption was 46.28%, reaching 95% of the theoretical conversion rate. The key to the industrialization of acetoin production by fermentation is the first breakthrough in upstream technology. The acetoin yield and performance of some strains in the above studies have potential for industrial application, especially the strain *Bacillus subtilis* HB-32 (CGMCC1869) which does not produce or accumulate small amounts of 2,3-butanediol and diacetyl. Combining traditional technology with modern biotechnology to further improve the yield of strains, strengthen the fermentation, extraction process and equipment supporting research, which is expected to be applied to industrial fermentation production of acetoin [57, 85-87].

4. Conclusion

As a kind of flavoring agent and an important platform compound, acetoin is in increasing demand, especially with the continuous improvement of people's living standards, which puts higher requirements on food safety. At present, there are mainly JM companies in the United States, BASF in Germany and Cinda in Japan producing acetoin. Domestically, it mainly includes Jiangsu Pannan Perfumery Factory, Henan Yumeng Group, Shanghai Taihe Chemical Co., Ltd., Shanghai Aipu Spice Co., Ltd. and Shanghai Kaixin Biotechnology Co., Ltd. The production of acetoin at home and abroad is still dominated by chemical synthesis processes, the scope of application of the product is limited. According to reports, at present, the domestic production of acetoin by bio-fermentation has been carried out by enterprises, and the products have obtained relevant certification which laid the foundation for the industrialization of acetoin production by fermentation [1-7].

Due to the pressures of population, resources and environment, the traditional chemical industry with fossil resources as raw materials is bound to be replaced by a new bio-chemical industry with renewable resources as raw materials and environmental friendliness. The research on the production of acetoin by microbial fermentation with environmentally friendly, abundant raw materials, mild conditions and pure natural products has

broad application prospects.

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References

[1] Xu, H., Jia, S. R., and Liu, J. J. (2011). Development of a mutant strain of *Bacillus subtilis* showing enhanced production of acetoin. African Journal of Biotechnology, 10 (5): 779-788.

[2] Tian, Y. J., Xu, H., Liu, J. J., Chen, W., Sun, W. T., and Chen, Y. Q. (2016). Construction of acetoin high-producing *Bacillus subtilis* strain. Biotechnology and Biotechnological Equipment, 30 (4): 700-705.

[3] Fan, Y. X., Tian, Y. J., Zhao, X. Y., Zhang, J. X., and Liu, J. J. (2013). Isolation of acetoin-producing bacillus strains from Japanese traditional food—natto. Preparative Biochemistry Biotechnology, 43 (6): 551–564.

[4] Zhang, J. J., Zhao, X. Y., Zhang, J. X., Zhao, C., Liu J. J., Tian, Y. J., and Yang, L. P. (2017). Effect of deletion of 2,3-butanediol dehydrogenase gene (bdhA) on acetoin production of *Bacillus subtilis*. Preparative Biochemistry and Biotechnology, 47 (8): 761-767.

[5] Yu, T. M., Wang, Y. H., and Yang, B. (2008). Study on the preparation of natural milk scent base by combination of microbial fermentation and enzymatic method. Food Fermentation Industry, 34 (10): 153-157.

[6] Zhang, X. Z., Zeng, C. Y., and Ren, X. G. (2001). Current status and prospects of synthetic study of acetyl couples. Jiangsu Chemical Industry, 4: 29-31.

[7] Ji, X. J., He, H., and Du, J. (2008). Progress in the synthesis and application of acetoin. Modern Chemical Industry, 28 (4): 81.

[8] Christen, P and López-Munguia, A (1994). Enzymes and food flavor: a review. Food Biotechnology, 8 (2): 167-190.

[9] Hummel, W., Kula, M. R., and Boermann, F. Microbiologically prepared diacetyl reductase. U. S. Patent 5,164,314, Nov 17, 1992.

[10] Defaveri, D., Torre, P., and Molinari, F. (2003). Carbon material balances and bioenergetics of 2,3-butanediol bio-oxidation by *Acetobacter hansenii*. Enzyme and Microbial Technology, 33 (5): 708-719.

[11] Xiao, Z. J., Liu, P. H., and Qin, J. Y. (2007). Statistical optimization of medium components for enhanced acetoin production from molasses and soybean meal hydrolysate. Applied Microbiology and Biotechnology, 74 (1): 61-68.

[12] Yu, E. K and Saddler J. N (1983). Fed-batch approach to production of 2,3-butanediol by *Klebsiella pneumoniae* grown on high substrate concentrations. Applied and Environmental Microbiology, 46: 630-635.

[13] Gupta, K. G., Yadav, N. K., and Dhawan, S. (1978). Laboratory-scale production of acetoin plus diacetyl by *Enterobacter cloacae* ATCC 27613. Biotechnology and Bioengineering, 20: 1895-1901.

[14] Dettwiler, B., Dunn, I. J., and Heinzle, E. (1993). A simulation model for the continuous production of acetoin and butanediol using *Bacillus subtilis* with integrated pervaporation separation. Biotechnology and Bioengineering, 41 (8): 791-800.

[15] Ui, S., Masuda, H., and Muraki, H. (1983). Laboratory-scale production of 2,3-butanediol isomers (D (−), L (+), and Meso) by bacterial fermentations. Journal of Fermentation Technology, 61: 253-259.

[16] Kaneko, T., Takahashi, M., and Suzuki, H. (1990). Acetoin fermentation by citrate-positive *Lactococcus lactis subsp. lactis* 3022 grown aerobically in the presence of hemin or Cu2+. Applied Environmental Microbiology, 56: 2644-2649.

[17] Zeng, A. P., Biebl, H., and Deckwer, W. D. (1991). Production of 2,3-butanediol in a membrane bioreactor with cell recycle. Applied Microbiology and Biotechnology, 34: 463-468.

[18] Boumerdassi, H., Monnet, C., and Desmazeaud, M. (1997). Isolation and properties of *Lactococcus lactis subsp. lactis* biovar diacetylactis CNRZ 483 mutants producing diacetyl and acetoin from glucose. Applied and Environmental Microbiology, 63: 2293-2299.

[19] Nakashimada, Y., Kanai, K., and Nishio, N. (1998). Optimization of dilution rate, pH and oxygen supply on optical purity of 2, 3-butanediol produced by *Paenibacillus polymyxa* in chemostat culture. Biotechnology Letters, 20: 1133-1138.

[20] Xiao, Z. J and Xu, P (2007). Acetoin metabolism in bacteria. Critical Reviews in Microbiology, 33: 127-140.

[21] Qin, J., Xiao, Z. J., and Ma, C. (2006). Production of 2,3-butanediol by *Klebsiella pneumoniae* using glucose and ammonium phosphate. Chinese Journal of Chemical Engineering, 14: 132-136.

[22] Afschar, A. S., Bellgardt, K. H., and VazRossell, C. E. (1991). The production of 2,3-butanediol by fermentation of high test molasses. Applied Microbiology and Biotechnology, 34 (5): 582-585.

[23] Han, L. (2007). Breeding and fermentation conditions of high-yield acetoin. Master thesis of Shandong Institute of Light Industry.

[24] Xu, P., Xiao, Z. J., and Du, Y. An acetoin high yield *Bacillus pumilus* strain. Chinese. Patent WO/2006/053480, May 26, 2006.

[25] Zhao, X. Y., Liu, J. J., and Zhang, J. X. A strain of *Bacillus subtilis* producing high purity 3-hydroxybutanone. Chinese. Patent ZL200710013402.5, Jan 28, 2009.

[26] Liu, J. J., Zhao, X. Y., and Tian, Y. J. Application of a strain of *Bacillus subtilis* in the preparation of 3-hydroxybutanone. Chinese. Patent ZL 200710013403. X, Nov 25, 2009.

[27] Teixeira, R. M., Cavalheiro, D., and Furigo, J. A. (2002). Optimization of acetoin production by *Hanseniaspora guilliermondii* using experimental design. Brazilian Journal of Chemical Engineering, 19 (2): 181-186.

[28] Olson, B. H and Johnson, M. J (1948). The production of 2,3-butylene glycol by *Aerobacter aerogenes*. Journal of Bacteriology, 55 (2): 209-222.

[29] Braneni, A. L and Keenan, T. W (1971). Diacetyl and acetoin production by *Lactobacillus casei*. Applied Microbiology, 22 (4): 517-521.

[30] Jeffrey, D., Hillman, S., and Andrews, W. (1987). Acetoin production by wild-type strains and a lactate dehydrogenase-deficient mutant of *Streptococcus* mutans. Infection and Immunity, 55 (6): 1399-1402.

[31] Hikmet, G., Zéev, B., and David, M. (2004). Enhanced production of acetoin and butanediol in recombinant *Enterobacter aerogenes* carrying Vitreoscilla hemoglobin gene. Bioprocess and Biosystems Engineering, 26 (5): 325-330.

[32] Hespell, R. B. (1996). Fermentation of xylan, corn fiber, or sugars to acetoin and butanediol by *Bacillus polymyxa* Strains. Current Microbiology, 32: 291-296.

[33] Juni, E. (1952). Mechanisms of formation of acetoin by bacteria. Journal of Biological Chemistry, 195 (2): 715-726.

[34] Harold, J. S and Sever, O (1954). Pyruvate oxidation system and acetoin formation. Journal of Biological Chemistry, 209: 313-326.

[35] Juni, E and Heym, G. A (1956). A cyclic pathway for the bacterial dissimilation of acetylmethylcarbinol and diacetyl. I. General aspects of the 2,3-butanediol cycle. Journal of Bacteriology, 71: 425-432.

[36] Juni, E and Heym, G. A (1956). A cyclic pathway for the bacterial dissimilation of 2,3-butanediol, acetylmethylcarbinol and diacetyl. II. The synthesis of diacetylmethylcarbinol from diacetyl, a new diphosphothiamin catalyzed reaction. Journal of Bacteriology, 72: 746-753.

[37] Juni, E and Heym, G. A (1957). A cyclic pathway for the bacterial dissimilation of 2,3-butanediol, acetylmethylcarbinol and diacetyl. III. A comparative study of 2,3-butanediol dehydrogenases from various microorganisms. Journal of Bacteriology, 74: 759-767.

[38] Ui, S., Hosaka, T., and Ohtsuki, T. (2002). Acetylacetoin synthase as a marker enzyme for detecting the 2,3-butanediol cycle. Journal of Bioscience and Biotechnology, 93: 248-251.

[39] López, J and Fortnagel, P (1972). The regulation of the butanediol cycle in *Bacillus subtilis*. Biochimical et biophysica acta, 279 (3): 554-560.

[40] López, J., Thoms, B., and Fortnagel, P. (1973). Mutants of *Bacillus subtilis* blocked in acetoin reductase. European Journal of Biochemistry, 40 (2): 479-483.

[41] López, J., Thoms, B., and Rehbein, H. (1975). Acetoin degradation in *Bacillus subtilis* by direct oxidative cleavage. European Journal of Biochemistry, 57: 425-430.

[42] Grundy, F. J., Turinsky, A. J., and Henkin, T. M. (1994). Catabolite regulation of *Bacillus subtilis* acetate and acetoin utilization genes by *Ccp*A. Journal of Bacteriology, 176 (15): 4527-4533.

[43] Huang, M., Oppermann, S., and Steinbüchel, A. (1999). Biochemical and molecular characterization of the *Bacillus subtilis* acetoin catabolic pathway. Journal of Bacteriology, 181 (12): 3837-3841.

[44] Priefert, H., Hein, S., and Krüger, N. (1991). Identification and molecular characterization of the *Alcaligenes eutrophus* H16 *aco* operon genes involved in acetoin catabolism. Journal of Bacteriology, 173 (13): 4056-4071.

[45] Huang, M., Oppermann, F. B., and Steinbüchel, A. (1994). Molecular characterization of the *Pseudomonas putida* 2,3-butanediol catabolic pathway. FEMS Microbiology Letters, 124: 141-150.

[46] Deng, W. L., Chang, H. Y., and Peng, H. L. (1994). Acetoin catabolic system of *Klebsiella pneumoniae* CG43: sequence, expression, and organization of the *aco* operon. Journal of Bacteriology, 176 (12): 3527-3535.

[47] Ali, N. O., Bigenon, J., and Rapoport, G. (2001). Regulation of the acetoin catabolic pathway is controlled by sigma L in *Bacillus subtilis*. Journal of Bacteriology, 183 (8): 2497-2504.

[48] Phalip, V., Christophe, M., and Philippe, S. (1994). Purification and properties of the α-acetolactate decarboxylase from *Lactococcus lactis subsp.* Lack NCDO 2118. FEBS Letters, 351: 95-99.

[49] Kruger, N., Oppermann, F. B., and Lorenzl, H. (1994). Biochemical and molecular characterization of the clostridium magnum acetoin dehydrogenase enzyme system. Journal of Bacteriology, 176 (1): 3614-3630.

[50] Carballo, J., Martin, R., and Bernardo, A. (1991). Purification, characterization and some properties of diacetyl (acetoin) reductase from *Enterobacter aerogene*. European Journal of Biochemistry, 18: 327-332.

[51] Isabel, V., Josefa, G., and Bernardo, A. (1988). Purification and characterization of diacetyl-reducing enzymes from *Staphylococcus aureus*. Biochemical Journal, 251: 461-466.

[52] Klaus, B., Øyvind, H., and Fredrik, C. S. (1971). The reduction of diacetyl and acetoin in *Aerobacter aerogenes* evidence for one enzyme catalyzing both reactions. European Journal of Biochemistry, 18: 116-119.

[53] Øyvind, H., Klaus, B., and Fredrik, C. S. (1971). Diacetyl (acetoin) reductase from *Aerobacter aerogenes* evidence for multiple forms of the enzyme. European Journal of Biochemistry, 20: 206-208.

[54] Liv, J., Sip, H. L., and Fredrik, C. S. (1973). Diacetyl (acetoin) reductase from *Aerobacter aerogenes* kinetic studies of the reduction of diacetyl to acetoin. European Journal of Biochemistry, 34: 97-99.

[55] Forlani, G., Mantelli, M., and Nielsen, E. (1999). Biochemical evidence for multiple acetoin-forming enzymes in cultured plant cells. Phytochemistry, 50: 255-262.

[56] Halpern, Y. S. (1967). Further evidence for two distinct acetolactate synthetases in *Aerobacter aerogenes*. Biochimica et Biophysica Acta, 139: 502-504.

[57] Zahler, S. A., Benjamin, L. G., Glatz, B. S. (1976). Genetic mapping of *als*A, *als*R, *thy*A, *kau*A, and *cit*D markers in *Bacillus subtilis*. In D. Schlessinger (ed.), Microbiology; American Society for Microbiology: Washington.

[58] Thomas, P. S and Jack, P (1952). Mechanism of acetoin synthesis by a-Carboxylase. Biochimica et Biophysica Acta, 9: 316-327.

[59] Holtzclaw, W. D and Chapman, L. F (1975). Degradative acetolactate synthase of *Bacillus subtilis*: purification and properties. Journal of Bacteriology, 121 (13): 917-922.

[60] Nakano, M. M., Dailly, Y. P., and Zuber, P. (1997). Characterization of anaerobic fermentative growth of *Bacillus subtilis*: identification of fermentation end products and genes required for growth. Journal of Bacteriology, 179 (21): 6749-6755.

[61] Williams, O. B and Morrow, M. B (1928). The bacterial destruction of acetyl-methyl-carbinol. Journal of Bacteriology, 16: 43-48.

[62] Ppermann, F. B., Schmidt, B., and Steinbüchel, A. (1991). Purification and characterization of acetoin: 2,6-dichlorophenolindophenol oxidoreductase, dihydrolipoamide dehydrogenase, and dihydrolipoamide acetyltransferase of the *Pelobacter carbinolicus* acetoin dehydrogenase enzyme system. Journal of Bacteriology, 173 (2): 757-767.

[63] Oppermann, F. B and Steinbüchel, A (1994). Identification and molecular characterization of the *aco* genes encoding the *Pelobacter carbinolicus* acetoin dehydrogenase enzyme system. Bacteriology, 176 (2): 469-485.

[64] Ui, S., Hosaka, T., and Mizutani, K. (1998). Purification and properties of acetylactoin synthase as from *Bacillus sp.* YUF-4. Journal of Bioscience Biotechnology and Biochemistry, 62: 795-797.

[65] Renna, M. C., Najimudin, N., and Wink, L. R. (1993). Regulation of the *Bacillus subtilis als*S, *als*D, and *als*R genes involved in post-exponential phase production of acetoin. Journal of Bacteriology, 175: 3863-3875.

[66] Lopez, J. M and Thoms, B (1976). Beziehungen zwischen katabolischer repression und sporulation bei *Bacillus subtilis*. Archives of Microbiology, 109 (1-2): 181-186.

[67] Grundy, F. J., Waters, D. A., and Takova, Y. T. (1993). Identification of genes involved in utilization of acetate and acetoin in *Bacillus subtilis*. Molecular Microbiology, 10 (2): 259-271.

[68] Mallonee, D. H and Speckman, R. A (1988). Development of a mutant strain of *Bacillus polvmvxa* showing enhanced production of 2,3-butanriol. Applied and Environmental Microbiology, 54: 168-171.

[69] Ley, J. D. (1959). On the formation of acetoin by acetobacter. Journal of general Microbiology, 21 (10): 852-865.

[70] Li, Y. F., Zhang, Z. B., and Du Y. (2008). Effects of intermediate metabolites and by-products on anabolic metabolism of acetoin. Proceedings of the 7th China Symposium on Flavor and Fragrance, 126-129.

[71] Ling, G. T. (1997). Food Additives Handbook. Chemical Industry Press, Beijing.

[72] Niels, K and Alexander, S (1992). Identification of *aco*R, a regulatory gene for the expression of genes essential for acetoin catabolism in *Alcaligenes eutrophus* H16. Journal of Bacteriology, 174 (13): 4391-4400.

[73] Priefert, H and Steinbuchel, A (1992). Identification and molecular characterization of the acetyl coenzyme A synthetase gene (*aco*E) of *Alcaligenes eutrophus*. Journal of Bacteriology, 174 (20): 6590-6599.

[74] Priefert, H., Kruger, N., and Jendrossek, D. (1992). Identification and molecular characterization of the gene coding for acetaldehyde dehydrogenase II (*aco*D) of *Alcaligenes eutrophus*. Journal of Bacteriology, 174 (3): 899-907.

[75] Thony, B., Anthamatten, D., and Hennecke, H. (1989). Dual control of the *Bradyrhizobium japonicum* symbiotic nitrogen fixation regulatory operon fix *Rnif*A: analysis of cis-and trans-acting elements. Journal of Bacteriology, 171 (8): 4162-4169.

[76] Saito, S., Inoue, H., and Ohno, K. Alpha-hydroxyketone derivatives, liquid crystal compositions containing said derivatives, and liquid crystal devices using said compositions. U. S. Patent 5,164,112, Nov 17, 1992.

[77] Oppermann, F. B., Schmidt, B., and Steinbuchel, A. (1991). Purification and characterization of acetoin: 2,6-dichlorophenolindophenol oxidoreductase, dihydrolipoamide dehydrogenase and dihydrolipoamide acetyltransferase of the *Pelobacter carbinolicus* acetoin dehydrogenase enzyme system. Journal of Bacteriology, 173 (2): 757-767.

[78] Oppermann, F. B., Steinbuchel, A., and Schlegel, H. G. (1989). Exidence for oxidative thiolytic cleavage of acetoin in *Pelobacter carbinolicus* analogous to aerobic oxidative decarboxylation of pyruvate. FMES Microbiology Letters, 60 (1): 113-118.

[79] Schink, B. (1984). Fermentation of 2,3-butanediol by *Pelobacter carbinolicus sp. nov.* and *Pelobacter propionicus sp. nov.,* and evidence for propionate formation from C2 compounds. Archives of Microbiology, 137 (1): 33-41.

[80] Claudia, F., Horst, P., and Alexander, S. (1989). Biochemical and genetic analyses of acetoin catabolism in *Alcaligenes eutrophus*. Journal of Bacteriology, 171 (2): 6539-6548.

[81] Varga, J., Stirewalt, V. L., and Melville, S. B. (2004). The *Ccp*A protein is necessary for efficient sporulation and enterotoxin Gene (*cpe*) regulation in *Clostridium perfringens*. Journal of Bacteriology, 186 (16): 5221–5229.

[82] Mizuno, W. G and Jezeski, J. J (1961). Starter metabolism. V. the mechanism of acetoin formation as determined with C14 labeled substrates. Journal of Dairy Science, 44 (4): 579-588.

[83] Susan, B. (1996). The Merck Index, Published by Merck Research Laboratorites.

[84] Opdyke, D. L. J. (1979). Monographs on fragrance raw meterials. Food and Cosmetics Toxicology, 17 (5): 509-511.

[85] Martin, S., Hans, U. B., and Martin, S. (1998). Hydrogenation of butane-2,3-dione with heterogeneous cinchona modified platinum catalysts: a combination of an enantioselective reaction and kinetic resolution. Chemical Communications, 9: 1053-1054.

[86] Hilmia Belgsira, E. M and Légera, J. M (1997). Electrocatalytic oxidation of aliphatic diols Part V. Electro-oxidation of butanediols on platinum based electrodes. Journal of Electroanalytical Chemistry, 435 (1-2): 69-75.

[87] Vanden, T. J., Reed, K. E., and Cronan, J. E. (1991). Lipoic acid metabolism in *Escherichia coli*: isolation of null mutants defective in lipoic acid biosynthesis, molecular cloning and characterization of the *E. coli* lip locus, and identification of the lipoylated protein of the glycine cleavage system. Journal of Bacteriology, 173 (20): 6411-6420.

[88] Giorno, L., Drioli, E., and Carvoli, G. (2001). Study of an enzyme membrane reactor with immobilized fumarase for production of L-malic acid. Biotechnologyand Bioengineering, 72 (1): 77-84.

[89] Lopez, J. M and Thoms, B (1977). Role of sugar uptake and metabolic intermediates on catabolite repression in *Bacillus subtilis*. Journal of Bacteriology, 129 (1): 217-224.

[90] Magee, R. K. N. (1987). The microbial production of 2,3-butanediol. Applied Microbiology, 32 (4): 89-161.

[91] Johansen, L., Bryn, K., and Stormer, F. C. (1975). Physiological and biochemical role of the butanediol pathway in *Aerobacter (Enterobacter) aerogenes*. Journal of Bacteriology, 123 (3): 1124-1130.

[92] Tsau, J. L., Guffanti, A. A., and Montville, T. J. (1992). Conversion of pyruvate to acetoin helps to maintain pH homeostasis in *Lactobacillus plantarum*. Applied and Environmental Microbiology, 58 (3): 891-894.

[93] Mayer, D., Schlensog, V., and Bock, A. (1995). Identification of the transcriptional activator controlling the butanediol fermentation pathway in *Klebsiella terrigena*. Journal of Bacteriology, 177 (18): 5261-5269.

[94] Elena, L., Teru, O., and Simon, M. C. (1997). Characterization of the *fts*H gene of *Bacillus subtilis*. Microbiology, 143 (3): 971-978.

[95] Stragier, P and Losick, R (1996). Molecular genetics of sporulation in *Bacillus subtilis*. Annual Review of Genetics, 30: 279-341.

[96] Mansour, S., Bailly, J., and Bonnarme, P. (2009). Investigation of associations of *Yarrowia lipolytica*, *Staphylococcus xylosus*, and *Lactococcus lactis* in culture as a first step in microbial interaction analysis. Applied and Environmental Microbiology, 75 (20): 6422-6430.

[97] Ui, S., Mimura, A., and Okuma, M. (1998). The production of D-acetoin by transgenic *Escherichia. Coli*. Soc. Applied Microbiology, 26 (4): 275-278.

[98] Royon D., Daz M., Ellenrieder G. (2007). Enzymatic production of biodiesel from cotton seed oil using t-butanol as a solvent. Bioresource Technology, 98 (3): 648-653.