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Genomic and Transcriptional Characteristics of Strain *Rum-meliibacillus* sp. TYF-LIM-RU47 with an Aptitude of Directly Producing Acetoin from Lignocellulose

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Abstract: *Rummeliibacillus* sp. TYF-LIM-RU47, isolated from the fermentation substrate of grain vinegar, could produce acetoin using a variety of carbon sources, including pentose, hexose and lignocellulose. The draft genome of TYF-LIM-RU47 was constructed and the genomic information revealed that TYF-LIM-RU47 contains genes related to starch and sucrose metabolism, pyruvate metabolism, the oxidative phosphorylation metabolic pathway and lignocellulosic metabolism. The acetoin anabolic pathway of TYF-LIM-RU47 has been deduced from the sequencing results, and acetoin is produced from α-acetolactate via decarboxylation and diacetyl reductase catalytic steps. The results of quantitative real-time PCR tests showed that the synthesis and degradation of acetoin had a dynamic balance in acetoin metabolism, and the transcription of the α-acetolactate synthase gene might exist to the extent of feedback regulation. This study can help researchers to better understand the bioinformation of thermophilic-lignocellulosic bacteria and the mechanisms of the acetoin biosynthesis pathway.

Keywords: lignocellulose; acetoin; genome; quantitative real-time PCR

Citation: Feng, G.; Fan, X.; Liang, Y.; Li, C.; Xing, J.; He, Y. Genomic and Transcriptional Characteristics of Strain *Rum-meliibacillus* sp. TYF-LIM-RU47 with an Aptitude of Directly Producing Acetoin from Lignocellulose. *Fermentation* **2022**, *8*, 414. https://doi.org/10.3390/fermentation8080414

Academic Editor: Konstantina Kourmentza

Received: 9 June 2022
Accepted: 17 August 2022
Published: 22 August 2022

1. Introduction

Lignocellulose is a macromolecular complex comprising lignin, cellulose and hemicellulose. Lignocellulose is considered a sustainable and potential renewable resource substitute for fossil fuels [1]. In the past few years, research into developing innovative methods to exploit lignocellulosic biomass for fuels, energy and valuable chemicals has been increasing because lignocellulose can be harvested from various sources, such as agricultural waste, wood and forest residues [2,3]. At present, the most effective methods to utilize lignocellulosic biomass are mainly the thermal-chemical method and the biological method, of which the biological method is more promising due to mild reaction conditions, low costs and no secondary pollution, etc. [4]. Anaerobic digestion, ethanol fermentation and aerobic composting are the most commonly used biological methods to utilize lignocellulosic biomass. However, due to the complex biochemical structure, the biodegradation rate of lignocellulosic biomass by microorganisms is low, which is considered an essential limiting step of biological methods [5]. In addition, products obtained by biological methods do not maximize the economic benefits of lignocellulosic biomass. Therefore, the development of more efficient microorganisms and more valuable products from lignocellulosic biomass will bring significant economic and environmental benefits [6].

Acetoin (3-hydroxy-2-butanone or acetyl methyl carbinol) is one of the 30 platform chemicals that have been prioritized by the US. It has been widely used in food manufacture, pharmaceutical and chemical productions [7,8]. For instance, with a pleasant yogurt odor and a creamy, buttery taste, acetoin can be used as a flavoring agent to improve the

taste of food. Acetoin is also a good precursor of many chemical substances in chemical synthesis, especially in the synthesis of heterocyclic compounds. To date, three methods have been developed to produce acetoin, namely chemical synthesis, enzymatic catalysis and microbial fermentation [9]. Of these, chemical synthesis is the one that has been employed the most extensively, using butanedione or 2,3-butanediol as the raw materials [10]. However, the further development and application of the conventionally chemical methods are overwhelmingly restricted due to their disadvantages of harsh reaction conditions, complex processes, raw materials shortage and high levels of environmental pollution. All the above issues have prompted the search for new and alternative production methods that are sustainable using renewable materials. With the development of microbial fermentation technology and synthetic biology, more valuable chemicals can be produced with microorganisms. Compared with chemosynthesis, microbial fermentation has the advantages of cheaper starting materials, less dangerous production processes and, more importantly, is safe for food adjunction [11,12], etc.

To date, acetoin has already been produced by several strains, such as *Engineerbacillus subtilis* [12], *Lactococcus lactis* [13], *Enterobacter aerogenes* [14], *Serratia marcescens* [15], *Bacillus amyloliquefaciens* [16] and the lactic acid-producing bacteria *Paenibacillus polymyxa* and *Enterobacteriaceae* [17], etc. Of these, Bacillus strains have excellent fermentation ability to produce acetoin. For example, *Bacillus amyloliquefaciens* E-11 [16] and *Bacillus subtilis* TH-49 [18] can produce acetoin with glucose as substrate, and the yield can reach 71.5 g/L and 56.9 g/L, respectively. Because most acetoin bioproduction strains use glucose and sucrose as fermentation substrates, the costs are relatively high and the conversion efficiency is expected to be improved, thereby restricting the large-scale bio-production of acetoin. Considerable efforts have been taken to improve the bioproduction efficiency of acetoin, including screening strains with high acetoin yield [1], metabolic engineering breeding [2] and fermentation optimization. Strains producing acetoin with a cheap carbon source are expected to be screened and studied in detail.

This study reports a thermophilic-lignocellulolytic bacterium *Rummeliibacillus* sp. TYF-LIM-RU47, which was isolated from the fermentation substrate of grain vinegar. The substrate is rich in lignocellulosic and acetic acid. TYF-LIM-RU47 can produce acetoin using several carbon sources, such as arabinose, xylose, glucose, mannose, galactose, xylan, starch, microcrystalline cellulose and corn straw. The growth characteristics and the draft genome of *Rummeliibacillus* sp. TYF-LIM-RU47 were further studied, and the functional genes involved in acetoin synthesis are summarized based on the genomic information. Moreover, the expression levels of critical genes in the acetoin biosynthesis pathway were analyzed by quantitative real-time PCR (qPCR) tests, aiming to explore the acetoin synthesis characteristic of TYF-LIM-RU47 from a transcriptional perspective.

2. Materials and Methods

2.1. Isolation and Identification of Strains

Strain TYF-LIM-RU47 was isolated from fermentation substrate of grain vinegar with Man, Rogosa and Sharpe (MRS) solid medium and was stored as primary cultures in 25% glycerin (0.5 mL 50% glycerin and 0.5 mL medium) at −80 °C and MRS agar-inclined medium at 4 °C. For the seed culture, test tubes containing 5 mL MRS medium were incubated in a rotary shaker at 45 °C and 200 rpm overnight. Then the seed culture was transferred to 250 mL Erlenmeyer flasks containing 50 mL MRS medium to expand cultivation. The effects of carbon sources on acetoin production were further performed at 45 °C. The culture mediums consisted of yeast extract (5 g/L), $(NH_4)_2SO_4$ (5 g/L), $CaCl_2$ (3 g/L) and a carbon source (30 g/L), namely arabinose, xylose, glucose, mannose, galactose, xylan, starch, microcrystalline cellulose and corn straw, respectively.

The genome of strain TYF-LIM-RU47 was extracted by Genome Extraction Kit (TIANGEN Biotech (Beijing) Co., Ltd., Beijing, China). Universal primers 27F and 1492R were used to amplify the 16S rRNA gene fragment (Table 1). PCR products were sequenced (Sangon Biotech Co. LTD., Shanghai, China) and compared with 16S rRNA gene sequences

of related groups obtained from NCBI GenBank (https://www.ncbi.nlm.nih.gov/ accessed on 25 February 2020).

Table 1. Primers for the amplification of 16S rRNA and the relative enzymes.

Gene	Gene Description	Primer Sequence (5′-3′) Forward/Reverse	Product (bp)
16S rRNA	16S rRNA	AGAGTTTGATCCTGGCTCAG GGTTACCTTGTTACGACTT	1500
GAPDH	glyceraldehyde-3 phosphate dehydrogenase	GTCGTATCGGCCGTATGGTT CAAAGCTGCTTTGCTACGCT	293
lccA	laccase	GGCTGGCAAGGGACGATTAA TGGTAATGTGAGCGCTTGGT	280
axe	acetylxylan esterase	ACCAACGCTATTCTCGCTCC TGGCCATCTGCGTACACTTT	206
alsS	α-acetolactate synthase	ACAGGTGCTGCTAATGCCAT GCTGTTCTCTTGGCAGGAGT	299
butB	diacetyl reductase	GGCAGGCATTTGTGGAAGTG TCCACCGTCTCCATGCAATC	271
acuABC	acetoin utilization protein *acuABC*	TGCTTCAGGTGTCGATGCTT TCCGTTGCAGCAACCTCTAG	166
ytrABCDEF	operon related to acetoin transport *ytrABCDEF*	TAGGGGTCTTAGGGACGGTG TAGTTGCCTTTCGAGCTGGG	225

The obtained 16S rRNA sequence of the *Rummeliibacillus* sp. TYF-LIM-RU47 was registered to the GenBank database with accession number ON326626.

2.2. Metabolite Analysis

Headspace solid-phase microextraction (HS-SPME) combined with gas chromatography–mass spectrometry (GC-MS) was used to detect metabolites in the culture medium. A volume of 8 mL of fermentation liquid was added to a 20 mL headspace sample bottle, and then 3 g of sodium chloride was added to the bottle. The solution was stirred at 300 r/min with a magnetic stirrer for 15 min. Then, the extraction head was inserted into the sample bottle to adsorb the metabolites for 40 min, and it was subsequently desorbed at the gas chromatography inlet (230 °C) for 5 min. The oven temperature was set as follows: (1) 40 °C for 2 min; (2) from 40 to 100 °C at a heating rate of 4 °C/min, (3) from 100 to 200 °C at a heating rate of 10 °C/min, (4) from 200 to 230 °C at a heating rate of 8 °C/min; (5) the capillary column temperature was maintained at 230 °C for about 10 min. The flow rate of carrier gas helium was 1 mL/min. The mass spectrometry (MS) source was kept at 230 °C and the electron impact mass spectra (EIMS) was taken at 70 eV of energy. The mass range used for the mass selective detector was 30–500 m/z.

The concentration of acetoin in the fermentation culture of TYF-LIM-RU47 was analyzed by high-performance liquid chromatography (HPLC) (Waters, Milford, MA, USA). After centrifugation, the fermentation liquid was diluted to an appropriate concentration with deionized water and was filtered with a 0.22 μm filter membrane before sampling. The injection volume was 10 μL and the subsequent analysis was performed with a Shodex C18-120-54E column (250 × 4.6 mm) (Acchrom Tech, Beijing, China) and a UV-detector at 297 nm. A mixed solution of acetonitrile:water (3:7, v/v) was used as the mobile phase at 45 °C with a flow rate of 0.8 mL/min.

The reducing sugar concentration was measured with the 3,5-dinitrosalicylic acid (DNS) method [19].

2.3. Genome Sequencing, Assembly and Annotation

Genomic DNA was extracted with the SDS method [20] using a DNA extraction kit (TIANGEN Biotech (Beijing) Co., Ltd., China). The harvested DNA was detected by the agarose gel electrophoresis and quantified by Qubit® 2.0 fluorometer (Thermo Scientific, Shanghai, China). Whole-genome sequencing was performed on the Illumina HiSeq PE150 platform. A-tailed fragmented DNA, ligated to paired-end adaptors and PCR amplified with a 350 bp insert was used for library construction at Beijing Novogene Bioinformatics Technology Co., Ltd. (Beijing, China). Illumina PCR adapter reads and low-quality reads, such as those with more than 10% N, with 25 bases of low quality (≤Q20) and duplication contamination, were filtered by a quality control step using readfq (vision 10) (Anaconda, Austin, TX, USA). All good quality paired reads were assembled into scaffolds using SOAPdenovo [21,22]. Finally, the filter reads were handled by the next step of the gap-closing.

The functions of critical genes were predicted using databases of GO (Gene Ontology) [23], KEGG (Kyoto Encyclopedia of Genes and Genomes) [24,25], COG (Clusters of Orthologous Groups) [26], NR (Non-Redundant Protein Database databases) [27], TCDB (Transporter Classification Database) [28] and Swiss-Prot [29].

The whole genome sequence has been deposited at GenBank under accession no.VVIR00000000.

2.4. Total RNA Extraction, Reverse Transcription and Real-Time PCR

To analyze the transcriptional characteristic of critical genes in the acetoin biosynthesis of strain TYF-LIM-RU47, the total RNA samples of TYF-LIM-RU47 were isolated from different culture stages using RNAkey™ Reagent (Seven Bio, Beijing, China). RNA concentrations were determined using a NanoVue Plus ultramicro spectrophotometer (Biochrom, Cambridge, UK). The first strand of cDNA was synthesized using the All-in-One First-Strand cDNA Synthesis Kit II for qPCR (Seven Bio, China). The GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene was used as an internal reference gene. The primers of critical genes in acetoin biosynthesis and GAPDH gene were designed according to the genome sequencing results (Table 1).

SYBR Green I, containing a ROX passive reference dye, was used for all qPCR assays. The reactions were performed on Applied Biosystems StepOne™ Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) in a 20 μL reaction mixture volume. Each reaction mixture contained 10 μL of 2× SYBR Green qPCR MasterMix (ROX) (Seven Bio, China), 200 nM of each primer, 1 μL of cDNA template and sterilized ultrapure water. All samples were measured in triplicate, with a control excluding templates for each run.

The PCR cycling program for all the genes consisted of 5 min at 95 °C, followed by 45 cycles of 20 s at 95 °C, 20 s at 60 °C and 30 s at 72 °C. An additional step was used (heating at a rate of 0.05 °C/s over a temperature range of 55–95 °C) for dissociation curve analysis. For qPCR, Ct (Cycle Threshold) values were used to calculate mRNA levels with the formula $2^{-\Delta\Delta Ct} = 2^{-[\Delta Ct - \Delta Ct\ control]}$ [30].

3. Results and Discussion

3.1. Identification of Strains

The 16S rRNA gene sequence of strain TYF-LIM-RU47 shares 99% similarity with the 16S rRNA gene sequence of *Rummeliibacillus stabekisii* 128 on the NCBI website (Figure 1). The genus *Rummeliibacillus* belongs to the Bacillaceae (family), Bacillales (order), Bacilli (class) and Firmicutes (phylum).

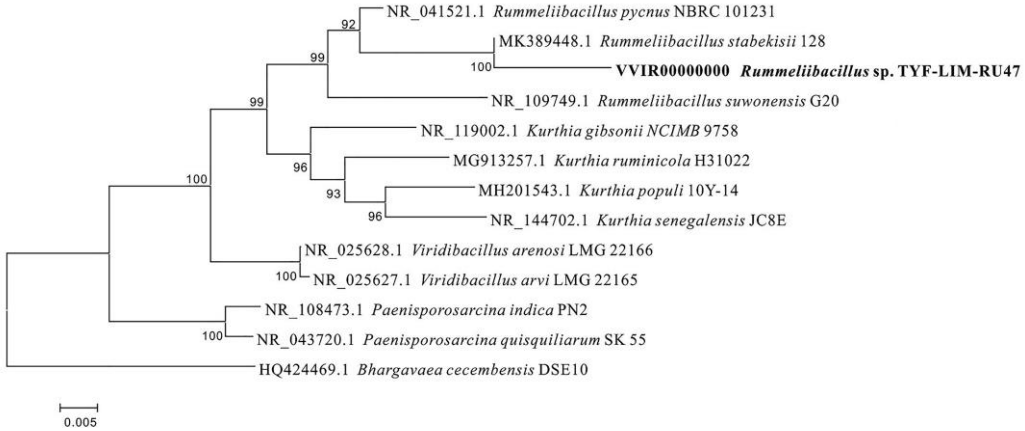

Figure 1. Phylogenetic tree of *Rummeliibacillus* sp. TYF-LIM-RU47 based on its 16S rRNA gene sequences.

3.2. Metabolites Analysis

Acetoin was the dominant fermentation product in the culture of TYF-LIM-RU47 using corn straw as the main carbon source, with a relative content of 53.88%, followed by 2,3-butadione with a relative content of 18.12%. The specific products from the culture of TYF-LIM-RU47, fermented with corn straw, are shown in Table 2.

Table 2. Metabolites of *Rummeliibacillus* sp. TYF-LIM-RU47 GC-MS analysis results.

Substrate	Keep Time (min)	Product Name	Relative Content %
Corn straw	6.815	Ethanol	4.41
	7.538	Acetone	10.88
	10.146	2,3-butanedione	18.12
	10.45	Butanone	2.93
	12.341	Valeraldehyde	3.38
	12.627	Ethyl allyl ether	1.71
	15.524	Acetoin	53.88
	16.077	Propane	1.23
	22.071	Propionaldehyde	0.92
	25.19	Benzaldehyde	1.51

Strain TYF-LIM-RU47 could produce acetoin from various carbon sources, such as arabinose, xylose, glucose, mannose, galactose, xylan, starch, microcrystalline cellulose and corn straw (Figure 2a). The yield of acetoin reached the highest (11.0 g/L) with corn straw as the main carbon source. When corn straw was used as the main carbon source, the acetoin concentration and reducing sugar concentration in the culture medium were monitored (Figure 2b). The results showed that the strain could grow well in the culture and the reducing sugar concentration increased at the initial stage of culture, indicating that TYF-LIM-RU47 could exploit lignocellulose. TYF-LIM-RU47 also showed the ability to utilize hemicellulose, with acetoin yields of 9.8 g/L and 9.2 g/L with xylose and xylan as the main carbon sources, respectively.

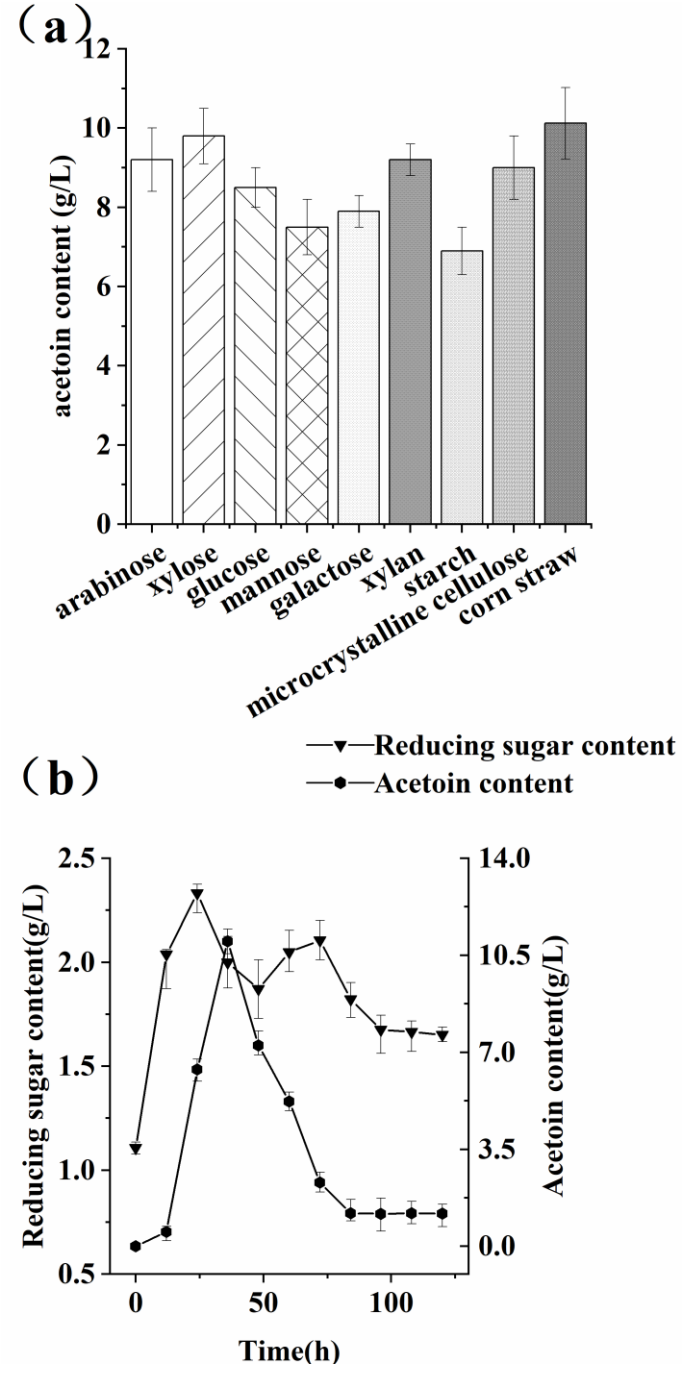

Figure 2. Acetoin production of TYF-LIM-RU47 on different substrates (**a**), changes of acetoin concentration and reducing sugar concentration during fermentation with corn straw as carbon source (**b**). The experiment was repeated three times, and the error bars corresponded to the standard deviation (SD).

3.3. Genome Assembly and Annotation

TYF-LIM-RU47 has a genome size of 3,298,449 bp with a GC content of 38.37%, and it comprises 14 scaffolds with 3418 genes, 11 rRNAs and 86 tRNAs, predictably. More detailed sequencing statistics of TYF-LIM-RU47 are shown in Table 3.

Table 3. Genome statistics of *Rummeliibacillus* sp. TYF-LIM-RU47.

Attribute	Value
GC content (%)	38.37
Scaffolds (>500 bp)	14
Scaffold N50 (bp)	2,231,473
Scaffold N90 (bp)	188,997
Total sequence length (bp)	3,298,449
Gene length (bp)	2,834,418
Total number of genes	3418

The amino acid sequences of TYF-LIM-RU47 have been compared in the COG database to obtain corresponding gene function annotations and the results (Figure 3a). Genes involved in transcription, amino acid transport, metabolism, translation, ribosomal structure and biogenesis have been annotated. Moreover, 187 genes with unknown functions have been annotated and need further study.

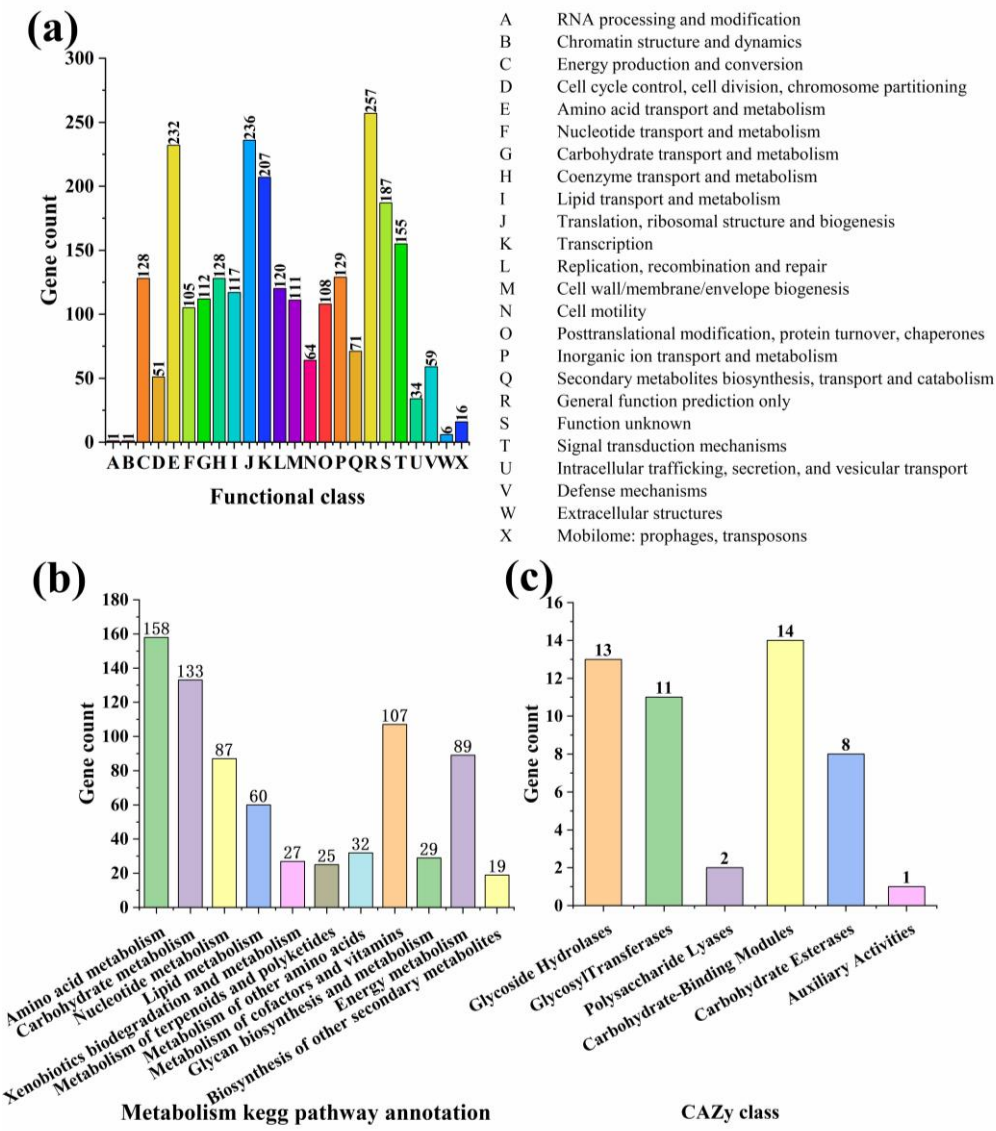

Figure 3. COG functional categories of strain *Rummeliibacillus* sp. TYF-LIM-RU47 (**a**), metabolism KEGG pathway annotation (**b**) and CAZy function classification (**c**).

A total of 3163 genes have been annotated in the genome sequencing results of strain TYF-LIM-RU47 in the NR database, among which 2864 genes are annotated as belonging to *Rummeliibacillus stabekisii*. The above results also confirm the identification result of TYF-LIM-RU47. According to the KEGG data, 158 gene-coding enzymes are annotated as related to amino acid metabolism, followed by enzymes involved in carbohydrate metabolism (133) (Figure 3b). Some annotated genes are related to carbohydrate metabolism, including starch and sucrose metabolism, pyruvate metabolism and oxidative phosphorylation metabolic pathways, in which abundant oxidative phosphorylation enzymes enable microorganisms to oxidize further and decompose the carbohydrates.

Moreover, according to the GO database, 1305 genes and 1254 genes from TYF-LIM-RU47 are highly correlated with cellular processes and metabolic processes, respectively.

Diamond software has been used to compare the amino acid sequence of TYF-LIM-RU47 with the CAZy database. Carbohydrate binding modules (CBMs) and glycoside hydrolases (GHs) are the main components from the CAZy database, followed by glycosyl transferases (GTs), carbohydrate esterases (CEs), polysaccharide lyases (PLs) and auxiliary activities (AA), which are involved in carbohydrate utilization (Figure 3c). Furthermore, acetyl xylan esterase (EC: 3.1.1.72) and feruloyl esterase (EC: 3.1.1.73) are annotated in the CAZy database, which are involved in hemicellulose degradation. The presence of laccase (EC: 1.10.3.2) indicates that strain TYF-LIM-RU47 may have the potential to decompose lignin [31].

3.4. Acetoin Bioproduction Pathway of TYF-LIM-RU47

The genome sequencing results of TYF-LIM-RU47 indicate it has a complete acetoin production pathway, and the functional genes in this pathway have been identified, including acetolactate synthase (EC: 2.2.1.6), diacetyl reductase (EC: 1.1.1.76), Embden–Meyerhof and pentose phosphate pathway enzymes.

Based on the genomic annotation information from the above databases, the acetoin metabolic pathway of TYF-LIM-RU47 was proposed (Figure 4). Glucose is degraded to pyruvate by the glycolytic pathway, and other hexoses can also be catabolized via r the glycolytic pathway by their respective enzymes. Pentoses, such as arabinose and xylose, enter the non-oxidative pentose phosphate pathway (PPP) via an isomerase pathway to form glyceraldehyde-3-P, which subsequently enters the Embden–Meyerhof–Parnas pathway (EMP). Pyruvate, the product of glycolysis, is first converted to α-acetolactate by α-acetolactate synthase. Then α-acetolactate spontaneously forms diacetyl, and eventually, the diacetyl is reduced to acetoin by diacetyl reductase. Subsequently, acetoin can be reduced to 2,3-butanediol by diacetyl reductase, and 2,3-butanediol can be converted back to acetoin by 2,3-butanediol dehydrogenase.

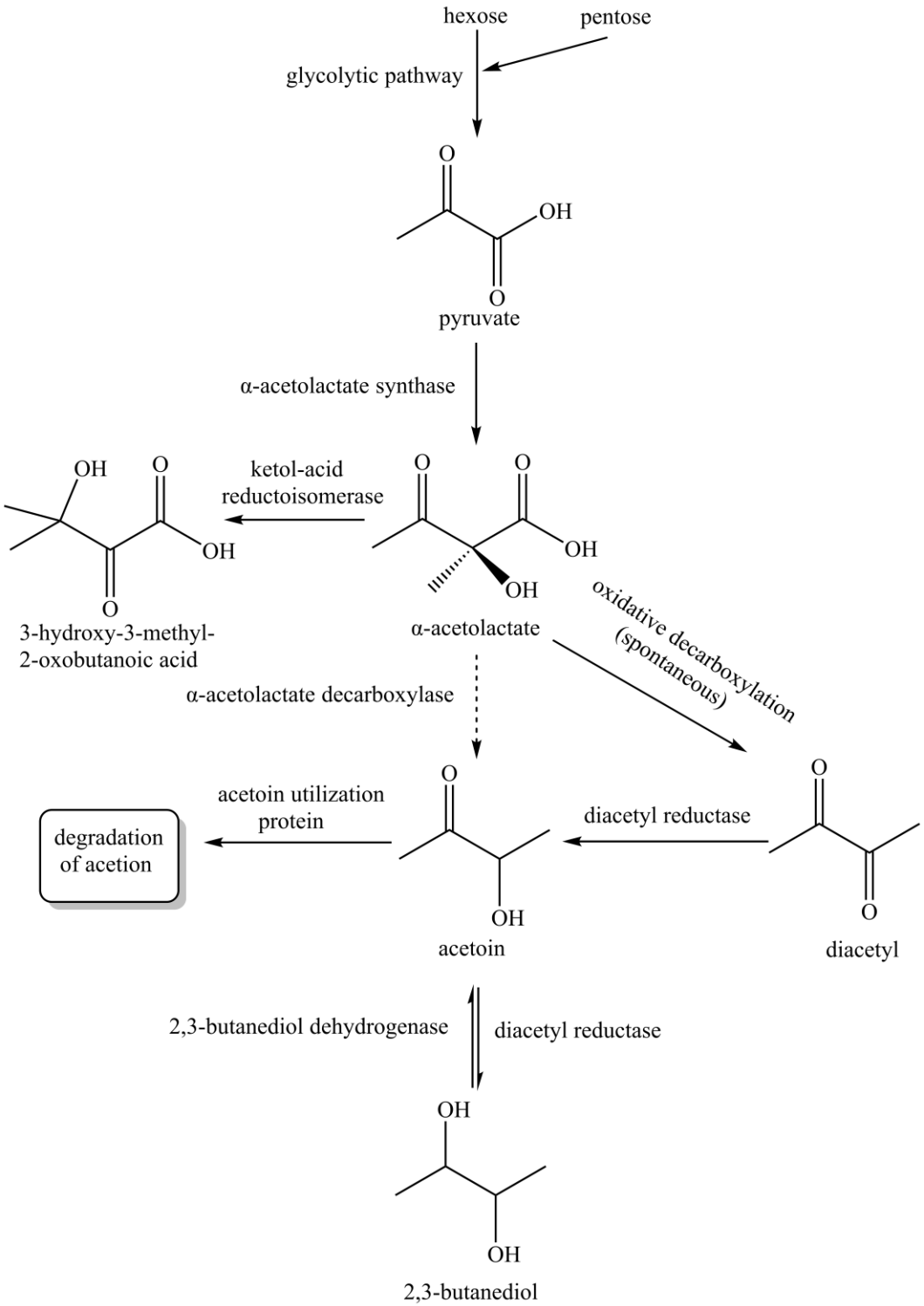

Figure 4. The metabolic pathway of acetoin in *Rummeliibacillus* sp. TYF-LIM-RU47.

Most of the reported separated natural strains producing acetoin are *Bacillus subtilis.* They produce acetoin mainly by converting α-acetolactate decarboxylation with the catalysis of acetolactate decarboxylase. However, the gene of acetolactate decarboxylase was not found in the genome of TYF-LIM-RU47. Notably, acetolactate synthase and diacetyl reductase are the critical enzymes in the acetoin biosynthesis pathway of the strain. According to the BLAST results on the NCBI website, the gene sequence of acetolactate synthase of TYF-LIM-RU47 has an identity of 97.7% with the corresponding gene of *Rummeliibacillus*

stabekisii strain PP9 (Sequence ID: CP014806.1), and the gene of diacetyl reductase shares an identity of 86.51% with the butanediol dehydrogenase of strain *Lysinibacillus* sp. YS11 (Sequence ID: CP026007.1).

In addition, acetoin utilization proteins and *ytrABCDEF*, an operon related to acetoin transport, have also been annotated in the genome of TYF-LIM-RU47. The gene cluster for acetoin utilization proteins consists of three genes (*acuA*, *acuB*, *acuC*), named *acuABC*. As an external carbon storage compound, acetoin can be further utilized as a carbon source for bacterial growth or spore formation by *acuABC* encoding enzymes [32]. An ATP-binding cassette (ABC) transport system was encoded by *ytrABCDEF*. It can accelerate the trans-membrane transport of acetoin and thus speed up the utilization of acetoin [33] (Figure 5).

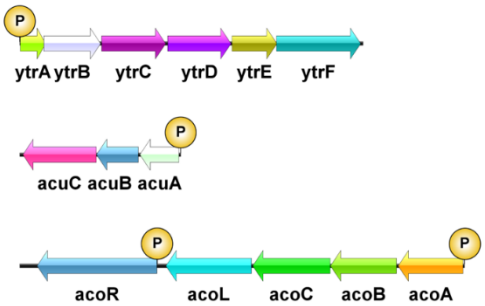

Figure 5. Gene clusters related to acetoin transport and degradation. The long arrowheads indicated the orientation and location of the ORFs, P: Transcription start site.

3.5. Transcriptional Level of Genes Related to Acetoin Metabolite

Since TYF-LIM-RU47 can efficiently produce acetoin from corn straw, quantitative real-time PCR (qPCR) was performed to characterize the transcriptional level of genes related to acetoin metabolite. As the growth of TYF-LIM-RU47 and the yield of acetoin were in a logarithmic phase from 12 h to 36 h of the culture, and the concentration of reducing sugar increased the highest at 24 h (Figure 3b), four nodes (10 h, 24 h, 48 h, 96 h) were selected. Genes of laccase (*lccA*) and acetyl xylan esterase (*axe*) (involved in the degradation of lignin and hemicellulose), α-acetolactate synthase (*alsS*) and diacetyl reductase (*butB*) (involved in the synthesis of acetoin), *acuABC* (qPCR primers were set on *acuB* gene) (involved in the degradation of acetoin) and *ytrABCDEF* (qPCR primers were selected on *ytrA* gene) had been screened to perform the qPCR tests.

The results showed that the *axe* gene had a higher transcription level at 24 h and 48 h, and the *lccA* gene had a higher transcription level at 48 h (Figure 6). Thus, combined with analysis in Figure 3b, it is improved that corn straw can be used as a carbon source for bacterial growth and acetoin production. The transcription levels of *alsS*, *butB*, operon *acuABC* and operon *ytrABCDEF* were higher at 24 h, 48 h, 96 h and 24 h, respectively, indicating that the synthesis and degradation of acetoin existed simultaneously. When the synthesis rate of acetoin was higher than its degradation rate, the content of acetoin increased, otherwise, the content of acetoin decreased. After 84 h, the content of acetoin maintained unchanged, suggesting that the synthesis and degradation of acetoin reached dynamic equilibrium. The expression of the *alsS* gene decreased after 24 h, indicating that α-acetolactate synthase may be the rate-limiting enzyme of acetoin biosynthesis, and its transcription may be regulated by feedback mechanisms.

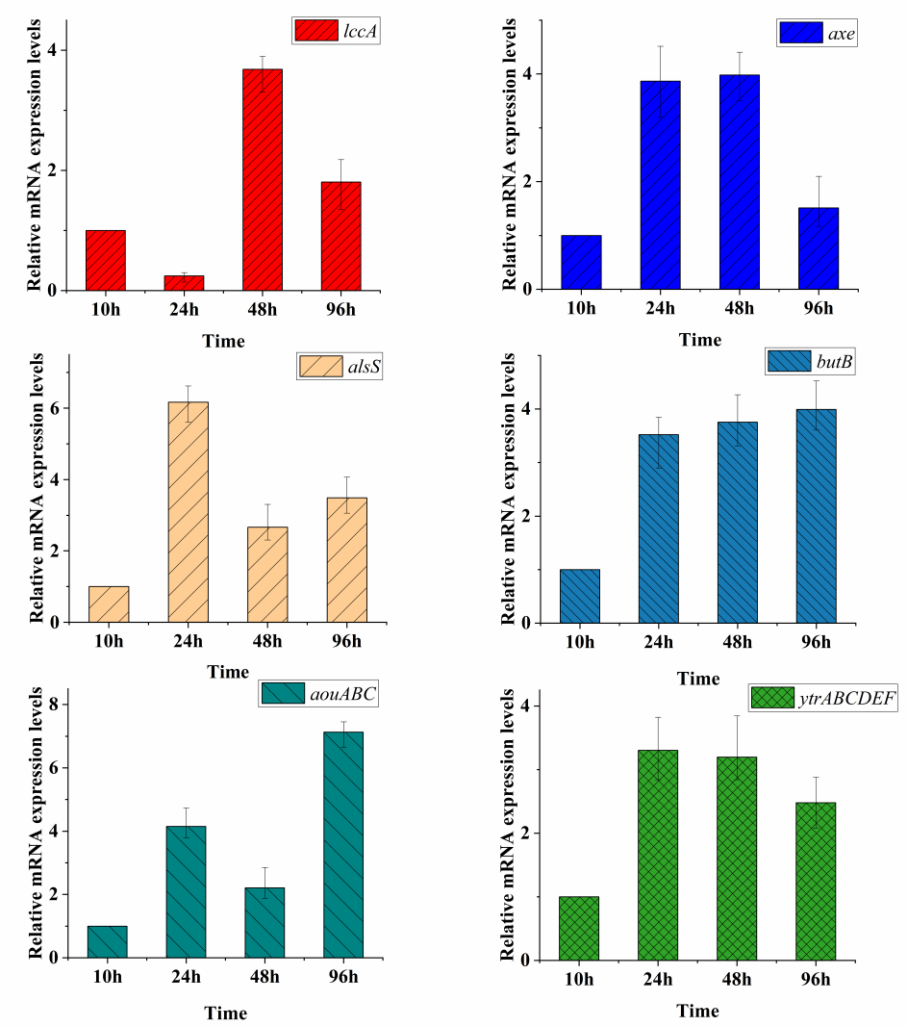

Figure 6. Relative mRNA expression levels of genes in the acetoin metabolite pathway. *lccA*: laccase; *axe:* acetyl xylan esterase; *alsS*: α-acetolactate synthase; *butB*: diacetyl reductase; *acuABC*: acetoin utilization protein; *ytrABCDEF*: ATP-binding cassette (ABC) transport system. The experiment was repeated three times, and the error bars corresponded to the standard deviation (SD).

4. Conclusions

In this paper, an acetoin-producing strain TYF-LIM-RU47 with lignocellulosic exploitation capacity was screened from the fermentation substrate of grain vinegar. TYF-LIM-RU47 can produce acetoin using a variety of carbon sources, including pentose, hexose and lignocellulose. The results of genome sequencing indicate that TYF-LIM-RU47 may express a series of enzymes related to lignocellulosic degradation and a complete pathway for acetoin biosynthesis. The transcriptional evidence of genes associated with lignocellulosic degradation and rate-limiting enzymes of acetone biosynthesis was identified by qPCR tests. This study provides a critical reference for recognizing the mechanism of acetoin biosynthesis in lignocellulosic microbes. In the future, industrial production can be realized through further research on the fermentation characteristics of strain tyf-lim-ru47, which is of great significance to the production of acetoin and the treatment of corn straw.

Author Contributions: Conceptualization, G.F. and X.F.; methodology, G.F. and X.F.; formal analysis, J.X., C.L., Y.H. and X.F.; data curation, G.F.; writing—original draft preparation, G.F.; writing—review and editing, X.F.; project administration, X.F. and Y.L.; funding acquisition, X.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by Science and Technology Project for Overseas Students in 2019 in Shanxi Province (grant number 201946) provided by Department of Human Resource and Social Security of Shanxi Province, Shanxi-China Overseas Students Park Project (grant number 20180008) provided by Shanxi Transformation and Comprehensive Reform Demonstration Zone Management Committee, and Key Research and Development Program of Shanxi Province (grant number 201703D121044) provided by Science and Technology Department of Shanxi Province.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tu, W.C.; Hallett, J.P. Recent advances in the pretreatment of lignocellulosic biomass. *Curr. Opin. Green Sustain. Chem.* **2019**, *20*, 11–17. [CrossRef]
2. Kucharska, K.; Hołowacz, I.; Konopacka-Łyskawa, D.; Rybarczyk, P.; Kamiński, M. Key issues in modeling and optimization of lignocellulosic biomass fermentative conversion to gaseous biofuels. *Renew. Energy* **2018**, *129*, 384–408. [CrossRef]
3. Shi, F.; Yu, H.; Zhang, N.; Wang, S.; Li, P.; Yu, Q.; Liu, J.; Pei, Z. Microbial succession of lignocellulose degrading bacteria during composting of corn stalk. *Bioengineered* **2021**, *12*, 12372–12382. [CrossRef] [PubMed]
4. Ma, Y.Q.; Shen, Y.Q.; Liu, Y. State of the art of straw treatment technology: Challenges and solutions forward. *Bioresour. Technol.* **2020**, *313*, 123656. [CrossRef] [PubMed]
5. Zhao, L.; Wu, K.K.; Chen, C.; Ren, H.Y.; Wang, Z.H.; Nan, J.; Ren, N.Q. Role of residue cornstalk derived biochar for the enhanced bio-hydrogen production via simultaneous saccharification and fermentation of cornstalk. *Bioresour. Technol.* **2021**, *330*, 125006. [CrossRef]
6. Zhao, L.; Wang, Z.; Ren, H.Y.; Chen, C.; Nan, J.; Cao, G.L.; Yang, S.S.; Ren, N.Q. Residue cornstalk derived biochar promotes direct bio-hydrogen production from anaerobic fermentation of cornstalk. *Bioresour. Technol.* **2021**, *320*, 124338. [CrossRef]
7. Xiao, Z.; Lu, J.R. Strategies for enhancing fermentative production of acetoin: A review. *Biotechnol. Adv.* **2014**, *32*, 492–503. [CrossRef]
8. Zhang, B.; Li, X.L.; Fu, J.; Li, N.; Wang, Z.; Tang, Y.-J.; Chen, T. Production of acetoin through simultaneous utilization of glucose, xylose, and arabinose by engineered *Bacillus subtilis*. *PLoS ONE* **2016**, *11*, e0159298. [CrossRef]
9. Zhang, X.; Zhang, R.Z.; Bao, T.; Yang, T.W.; Xu, M.J.; Li, H.Z.; Xu, Z.H.; Rao, Z.M. Moderate expression of the transcriptional regulator ALsR enhances acetoin production by *Bacillus subtilis*. *J. Ind. Microbiol. Biotechnol.* **2013**, *40*, 1067–1076. [CrossRef]
10. Qiu, M.; Chen, C.F.; Zhang, A.Z.; Zhou, X.J. Partial Reduction of α-Diketones by Sodium Hydrogen Selenide. *Synth. Commun.* **1992**, *22*, 1529–1534. [CrossRef]
11. Roncal, T.; Caballero, S.; Guereñu, M.D.; Rincón, I.; Prieto-Fernández, S.; Ochoa-Gómez, J.R. Efficient production of acetoin by fermentation using the newly isolated mutant strain *Lactococcus lactis* subsp. *lactis CML B4*. *Process Biochem.* **2017**, *58*, 35–41. [CrossRef]
12. Zhang, X.; Zhang, R.Z.; Bao, T.; Rao, Z.M.; Yang, T.W.; Xu, M.J.; Xu, Z.H.; Li, H.Z.; Yang, S.T. The rebalanced pathway significantly enhances acetoin production by disruption of acetoin reductase gene and moderate-expression of a new water-forming NADH oxidase in *Bacillus subtilis*. *Metab. Eng.* **2014**, *23*, 34–41. [CrossRef]
13. Passerini, D.; Laroute, V.; Coddeville, M.; Le Bourgeois, P.; Loubière, P.; Ritzenthaler, P.; Cocaign-Bousquet, M.; Daveran-Mingot, M.L. New insights into *Lactococcus lactis* diacetyl-and acetoin-producing strains isolated from diverse origins. *Int. J. Food Microbiol.* **2013**, *160*, 329–336. [CrossRef] [PubMed]
14. Geckil, H.; Barak, Z.E.; Chipman, D.M.; Erenler, S.O.; Webster, D.A.; Stark, B.C. Enhanced production of acetoin and butanediol in recombinant *Enterobacter aerogenes* carrying *Vitreoscilla* hemoglobin gene. *Bioprocess Biosyst. Eng.* **2004**, *26*, 325–330. [CrossRef] [PubMed]
15. Sun, J.N.; Zhang, L.Y.; Rao, B.; Han, Y.B.; Chu, J.; Zhu, J.W.; Shen, Y.L.; Wei, D.Z. Enhanced acetoin production by *Serratia marcescens* H32 using statistical optimization and a two-stage agitation speed control strategy. *Biotechnol. Bioprocess Eng.* **2012**, *17*, 598–605. [CrossRef]
16. Luo, Q.L.; Wu, J.; Wu, M.C. Enhanced acetoin production by *Bacillus amyloliquefaciens* through improved acetoin tolerance. *Process Biochem.* **2014**, *49*, 1223–1230. [CrossRef]
17. Jung, M.Y.; Jung, H.M.; Lee, J.; Oh, M.K. Alleviation of carbon catabolite repression in *Enterobacter aerogenes* for efficient utilization of sugarcane molasses for 2,3-butanediol production. *Biotechnol. Biofuels* **2015**, *8*, 106. [CrossRef]
18. Xu, H.; Jia, S.R.; Liu, J.J. Production of acetoin by *Bacillus subtilis* TH-49. In Proceedings of the 2011 International Conference on Consumer Electronics, Communications and Networks (CECNet), Xianning, China, 16–18 April 2011; pp. 1524–1527. [CrossRef]
19. Breuil, C.; Saddler, J.N. Comparison of the 3, 5-dinitrosalicylic acid and Nelson-Somogyi methods of assaying for reducing sugars and determining cellulase activity. *Enzym. Microb. Technol.* **1985**, *7*, 327–332. [CrossRef]

20. Lim, H.J.; Lee, E.H.; Yoon, Y.; Chua, B.; Son, A. Portable lysis apparatus for rapid single-step DNA extraction of *Bacillus subtilis*. *J. Appl. Microbiol.* **2016**, *120*, 379–387. [CrossRef]
21. Li, R.Q.; Li, Y.R.; Kristiansen, K.; Wang, J. SOAP: Short oligonucleotide alignment program. *Bioinformatics* **2008**, *24*, 713–714. [CrossRef]
22. Li, R.; Zhu, H.; Ruan, J.; Qian, W.; Fang, X.; Shi, Z.; Li, Y.; Li, S.; Shan, G.; Kristiansen, K.; et al. De novo assembly of human genomes with massively parallel short read sequencing. *Genome Res.* **2010**, *20*, 265–272. [CrossRef] [PubMed]
23. Gardner, P.P.; Daub, J.; Tate, J.G.; Nawrocki, E.P.; Kolbe, D.L.; Lindgreen, S.; Wilkinson, A.C.; Finn, R.D.; Griffiths-Jones, S.; Eddy, S.R.; et al. Rfam: Updates to the RNA families database. *Nucleic Acids Res.* **2009**, *37*, D136–D140. [CrossRef] [PubMed]
24. Hsiao, W.; Wan, I.; Jones, S.J.; Brinkman, F.S. IslandPath: Aiding detection of genomic islands in prokaryotes. *Bioinformatics* **2003**, *19*, 418–420. [CrossRef] [PubMed]
25. Nawrocki, E.P.; Kolbe, D.L.; Eddy, S.R. Infernal 1.0: Inference of RNA alignments. *Bioinformatics* **2009**, *25*, 1335–1337. [CrossRef]
26. Zhou, Y.; Liang, Y.; Lynch, K.H.; Dennis, J.J.; Wishart, D.S. PHAST: A fast phage search tool. *Nucleic Acids Res.* **2011**, *39*, W347–W352. [CrossRef]
27. Grissa, I.; Vergnaud, G.; Pourcel, C. CRISPRFinder: A web tool to identify clustered regularly interspaced short palindromic repeats. *Nucleic Acids Res.* **2007**, *35*, W52–W57. [CrossRef]
28. Ashburner, M.; Ball, C.A.; Blake, J.A.; Botstein, D.; Butler, H.; Cherry, J.M.; Davis, A.P.; Dolinski, K.; Dwight, S.S.; Eppig, J.T.; et al. Gene ontology: Tool for the unification of biology. *Nat. Genet.* **2000**, *25*, 25–29. [CrossRef]
29. Kanehisa, M.; Goto, S.; Kawashima, S.; Okuno, Y.; Hattori, M. The KEGG resource for deciphering the genome. *Nucleic Acids Res.* **2004**, *32*, D277–D280. [CrossRef]
30. Schmittgen, T.D.; Livak, K.J. Analyzing real-time PCR data by the comparative CT method. *Nat. Protoc.* **2008**, *3*, 1101–1108. [CrossRef]
31. Andlar, M.; Rezić, T.; Marđetko, N.; Kracher, D.; Ludwig, R.; Šantek, B. Lignocellulose degradation: An overview of fungi and fungal enzymes involved in lignocellulose degradation. *Eng. Life Sci.* **2018**, *18*, 768–778. [CrossRef]
32. Grundy, F.J.; Waters, D.A.; Takova, T.Y.; Henkin, T.M. Identification of genes involved in utilization of acetate and acetoin in *Bacillus subtilis*. *Mol. Microbiol.* **1993**, *10*, 259–271. [CrossRef] [PubMed]
33. Yoshida, K.I.; Fujita, Y.; Ehrlich, S.D. An operon for a putative ATP-binding cassette transport system involved in acetoin utilization of *Bacillus subtilis*. *J. Bacteriol.* **2000**, *182*, 5454–5461. [CrossRef] [PubMed]