

Article

Effect of *Dendrobium officinale* Leaf Extract on the Growth and Metabolism of *Streptococcus thermophilus*, a Most Commonly Used Bacterium in Yogurt Production

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Abstract

Dendrobium officinale leaf contains a variety of bioactive compounds, and has the potential to be used for functional yogurt production. However, the compounds in *D. officinale* leaf may affect the growth and metabolism of lactic acid bacteria. In this work, extracts from different dosages of *D. officinale* leaves were added into the media of *Streptococcus thermophilus* (one of the most commonly used lactic acid bacteria in yogurt production), and the biomass, lactic acid content, glucose uptake capacity, and cellular metabolic/reducing activity were analyzed. Simultaneously, the changes in biomass, lactic acid production capacity, and key enzyme activities were determined during the 24 h fermentation. Furthermore, transcriptomics analysis was performed to explore the molecular mechanisms. Results showed that *D. officinale* leaf extract can promote the growth, cellular metabolic/reducing activity, glucose uptake, lactic acid production, and β -galactosidase, glucokinase, pyruvate decarboxylase and lactic dehydrogenase activities of *S. thermophilus* at the appropriate dosage, and the extract from 4% (*w/v*) leaves is optimal. Further transcriptomic analysis revealed that *D. officinale* leaf extract enhances the growth and metabolism of *S. thermophilus* by up-regulating the expression of genes related to amino acid metabolism, translation, membrane transport, carbohydrate metabolism, energy metabolism, and nucleotide metabolism. These findings will provide a theoretical basis for the utilization of *D. officinale* leaves to fortify the health function of yogurt.

Keywords: *D. officinale* leaves; *S. thermophilus*; growth; metabolism; transcriptomics



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1. Introduction

Yogurt is a dairy product fermented by lactic acid bacteria (LAB), commonly *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Owing to its good flavor and health benefits, yogurt is consumed worldwide. To fortify its biological functions, researchers have made efforts to screen some specific strains with significant effects in preventing chronic metabolic diseases. Based on the α -glucosidase inhibition activity, Liu et al. screened *L. plantarum* YDA11, OD1, OD2, and YDB24 with hypoglycemic function from 36 strains of lactic acid bacteria [1]. Using angiotensin-converting enzyme inhibitory activity as an index, Wang et al. obtained a strain with blood-pressure-lowering function, *L. rhamnosus* HCS01-013 [2]. Chen et al. obtained several LABs with urate-reducing activity, including *L. helveticus* GQ1501, *L. paracasei* R2 and *L. plantarum* D1 [3]. In addition to the screen of functional LAB strains, incorporating edible plant extracts into the product is

another way to enhance the health function of yogurts [4]. The plant additives from fruits, vegetables, tea, and other edible and medicinal herbs are rich in bioactive ingredients such as polysaccharides, polyphenols, dietary fiber, flavonoids, alkaloids, carotenoids, saponins, steroids, etc., and show antioxidant, hypoglycemic, and other health-promoting properties, thereby increasing the health benefits of yogurt [4,5].

Dendrobium officinale is a precious medicinal and edible plant, and has been used as functional food for a long history in China, Korean, and other Asian countries. Nowadays, *D. officinale* has been widely cultivated in Zhejiang, Fujian, Yunnan, and other provinces of China, and has been approved to be used as food material by the National Health Commission of the People's Republic of China. Traditionally, the *D. officinale* stem is the main product, whereas the *D. officinale* leaf is the by-product [6]. Modern studies showed that *D. officinale* leaves contain a variety of bioactive compounds including polysaccharides, phenolics, alkaloids, and bibenzyl compounds, etc., with phenolics being the main constituents, and demonstrate multiple bioactivities including antioxidant, hypoglycemic, gut microbiota regulation, immune-modulation, anti-tumor, and anti-inflammatory effects [6–9]. Currently, a large amount of *D. officinale* leaves is produced annually; however, only a small amount has been processed into *D. officinale* leaf tea and other products, but a large number of them has not been effectively utilized [6,7].

To expand the utilization of *D. officinale* leaf in yogurt production, its extract was added into the media for *S. thermophilus* culture in this work, and the biomass, lactic acid content, enzymes (β -galactosidase, glucokinase, lactate dehydrogenase, pyruvate decarboxylase) activities, and cellular metabolic/reducing activity were analyzed. Furthermore, the transcriptome analysis was performed on the samples with and without *D. officinale* leaves extract. The aim of this work was to explore the effect of *D. officinale* leaf extract on the growth and metabolism of *S. thermophilus*, considering that *S. thermophilus* is the most commonly used lactic acid bacteria in yogurt production. The results from this work can provide a basis for the production of functional yogurt containing *D. officinale* leaf extract.

2. Materials and Methods

2.1. Materials and Reagents

S. thermophilus CGMCC 23415 was provided by Zhejiang Yiming Food Co. Ltd. (Wenzhou, China); *D. officinale* leaves (dried at 55 °C, pulverized and sieved through 40 mesh sieve) was obtained from Wenzhou Lvfang TiepiShihu Planting Cooperative (Wenzhou, China); glucokinase (GK) assay kit was purchased from Shanghai Saint-Bio Co. Ltd. (Shanghai, China); β -galactosidase (β -GAL), lactate dehydrogenase (LDH) and pyruvate decarboxylase (PDC) assay kits were purchased from Beijing Solarbio Technology Co., Ltd. (Beijing, China); glucose, lactose, beef extract, soya peptone, yeast powder, 3,5-dinitrosalicylic acid (DNS), 2,3,5-triphenyltetrazolium chloride (TTC), and other reagents were obtained from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Preparation of *D. officinale* Leaf Extract

D. officinale leaves were ultrasonically extracted using water at a ratio of 1:40 in an ultrasonic instrument (SB-4200DTD, Ningbo Scientz Biotechnology Co. Ltd., Ningbo, China) for 30 min at 480 W, 45 Hz and 50 °C. After that, the supernatant was collected by centrifugation, and used as the extract.

2.3. Determination of Reducing Sugar, Polysaccharide and Total Phenolics in *D. officinale* Leaf

Extraction was performed as described in Section 2.2. The reducing sugar, polysaccharide and total phenolic contents were determined using the DNS method [10], the phenol-sulfuric acid method [11], and the Folin–Ciocalteu reagent method [7] as described in our previous

works, respectively. Glucose (for reducing sugar and polysaccharide determination) and gallic acid (for total phenolics determination) were used as standards, respectively.

2.4. Cultivation of *S. thermophilus*

S. thermophilus was activated in MC media (soya peptone 5.0 g/L, beef extract 3.0 g/L, yeast powder 3.0 g/L, lactose 20.0 g/L, glucose 20.0 g/L, agar 20.0 g/L, pH 6.0) at 37 °C for 24 h. After that, the activated bacteria were inoculated into MC media without agar and incubated at 37 °C for 24 h to obtain the liquid inocula, which were directly used in the subsequent experiments at an inoculation amount of 1% (v/v). The cultures were performed at 37 °C in the media containing extract from 0, 0.5, 1, 2, 3, 4, and 5% (w/v) *D. officinale* leaves (designated as T0, T0.5, T1, T2, T3, T4, and T5), respectively.

2.5. Determination of Biomass and Lactic Acid Content

The biomass of *S. thermophilus* was collected by a filter using 0.22 µm membrane, dried to constant weight at 75 °C, and weighted. The lactic acid content was determined using the ferric chloride spectrophotometric method as described by Zhang et al. [12].

2.6. Determination of Glucose Uptake and Cellular Metabolic/Reducing Activity

The glucose uptake ability and cellular metabolic/reducing activity of *S. thermophilus* were determined using the DNS method and the TTC assay, respectively, as described by Chen et al. [13].

2.7. Determination of β -GAL, GK, LDH and PDC

After sampling, the *S. thermophilus* cells were collected by centrifugation, and ultrasonically disrupted in an ice bath at 200 W for 30 s with an interval of 10 s (repeat 30 times). The supernatant was collected by centrifugation at 4 °C and used for enzyme-activity analysis. The β -GAL, PDC, LDH, and GK were determined using an assay kit according to the description provided by the producer, respectively.

2.8. Transcriptome Analysis

Three independent biological replicates were prepared and used for the transcriptome analysis, which was performed by Majorbio Bio-pharm Biotechnology Co. Ltd. (Shanghai, China) on the Illumina NovaSeq X Plus platform (Illumina Inc., San Diego, CA, USA). After removing low-quality reads from the raw data, the reads were aligned to the reference genome of *S. thermophilus* CIRM 65 (GenBank accession: GCF_903886475.1). The transcript expression was normalized into transcript per million mapped reads (TPM) by feature counts. The genes with false discovery rate (FDR) < 0.01 and an absolute value of the $|\log_2FC| > 1$ were screened as differentially expressed genes (DEGs). Functional annotation, pathway analysis, and other bioinformatics analysis of these DEGs were all performed on the Majorbio cloud platform (<https://v.majorbio.com/project-center/overview>, accessed on 26 June 2026).

2.9. RNA-Seq Validation by RT-qPCR Analyses

Total RNA was extracted from the collected *S. thermophilus* samples using TRIzol reagent, and reverse-transcribed into cDNA using PrimerScript RT reagent kit (TARAKA, Dalian, China). To validate the gene expression results of RNA-seq, six DEGs (Table 1) were selected and quantified by real-time fluorescent quantitative PCR on the LightCycler 96 Real-Time PCR System (Roche Inc., Basel, Switzerland) using TB Green premix Ex Taq II (TARAKA, Dalian, China). The reaction was performed as follows: initial denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s, and 72 °C for 20 s. The

cycle threshold (Ct) values were obtained, and the relative mRNA expression level was calculated by the $2^{-\Delta\Delta C_t}$ method using RpoA as reference gene.

Table 1. Differentially expressed genes and their primers (5' → 3').

Gene	Forward Primer	Reverse Primer
H0506_RS02710	TCCCCTATCGCCCTTTACCA	GCTCTGGTGATAGTCCGACG
H0506_RS06920	TCAATCCGCACAGGCTGTAG	TGACATCCTCTCCTACCGCA
H0506_RS05900	ATGCTCAAGTGGAACGTCCA	ACCATAACCGTTACGCTCCA
H0506_RS03200	AGCCTGGAGCCTTTTGTTTT	GCCTCAAGTCCTTCAGCCAT
H0506_RS03745	ATACGGAGCTGGTTTTGGCA	ACCCTCGTTTAGCCTCATCAG
H0506_RS02245	TACCCCTGCCTCTCGATGAA	GACCATCCTCTTCAGCCACC
RpoA (reference)	ACTGTCATTGTTGCTTGAATG	AGCTGAGGTTACTGCTGGAGAT

2.10. Statistical Analysis

Cultivations and determinations were performed at least in triplicate. Statistical significance was determined by a one-way analysis of variance using SAS 8.1 software (SAS Institute Inc., Cary, NC, USA). Figure plotting was conducted using OriginPro 2021 software (Northampton, MA, USA).

3. Results

3.1. Effect of Different *D. officinale* Leaf Dosage

D. officinale leaves contain a series of compounds; among them, the contents of reducing sugar, polysaccharide and total phenolics were determined to be 58.92 ± 1.49 mg/g, 42.08 ± 0.22 mg/g, and 10.36 ± 0.25 mg/g, respectively. As shown in Figure 1, the biomass, lactic acid production, cellular metabolic/reducing activity and glucose uptake of *S. thermophilus* presented a trend of initial increase and then decreased with the increase in *D. officinale* leaves dosage, and the maximum values were determined at T4 group. Compared with that of control (T0), the biomass, lactic acid content, cellular metabolic/reducing activity, and glucose uptake ability of T4 were increased by 4.15, 1.75, 17.94, and 1.16 times, respectively. This indicated that the extract from a suitable dosage of *D. officinale* leaves can promote the growth and metabolism of *S. thermophilus*.

3.2. Changes in Fermentation Process of *S. thermophilus*

S. thermophilus was cultured in the media with and without the extract from 4% (*w/v*) *D. officinale* leaves for 24 h, respectively, and sampling was performed every 6 h. As shown in Figure 2, the biomass and lactic acid content of *S. thermophilus* were continuously increased with the fermentation progress, and the maximum values were all determined at 24 h in both control and *D. officinale* leaf extract group. The biomass and lactic acid content in *D. officinale* leaf extract group were significantly ($p < 0.05$) higher than those in the control group after 6 h of fermentation. The activities of β -GAL, GK, PDC, and LDH of *S. thermophilus* showed a trend of initial increase and then decreased with maximal values at 18 h, and the enzymes activities were all promoted by the addition of *D. officinale* leaf extract. Among them, significant ($p < 0.05$) differences were observed in β -GAL, GK, and PDC activities after 6 h, 12 h, 6 h of fermentation between the *D. officinale* leaf extract group and the control, respectively.

3.3. Transcriptomic Sequencing and DEGs Analysis

To further investigate the effect of *D. officinale* leaf extract on the gene express of *S. thermophilus*, transcriptomic analysis was performed and a total of 136,149,444 clean

reads were obtained from the three *D. officinale* leaf extract groups and the three control groups, with the maximum and minimum reads in sample being 25,157,372 and 19,767,948, respectively. The average percentages of Q20, Q30, and GC were 99.31%, 96.16%, and 41.21%, respectively. All clean reads were aligned to the reference genome of *S. thermophilus* CIRM 65, and the average genome mapped ratio was 92.48% for *D. officinale* leaf extract group and 90.84% for control group, respectively.

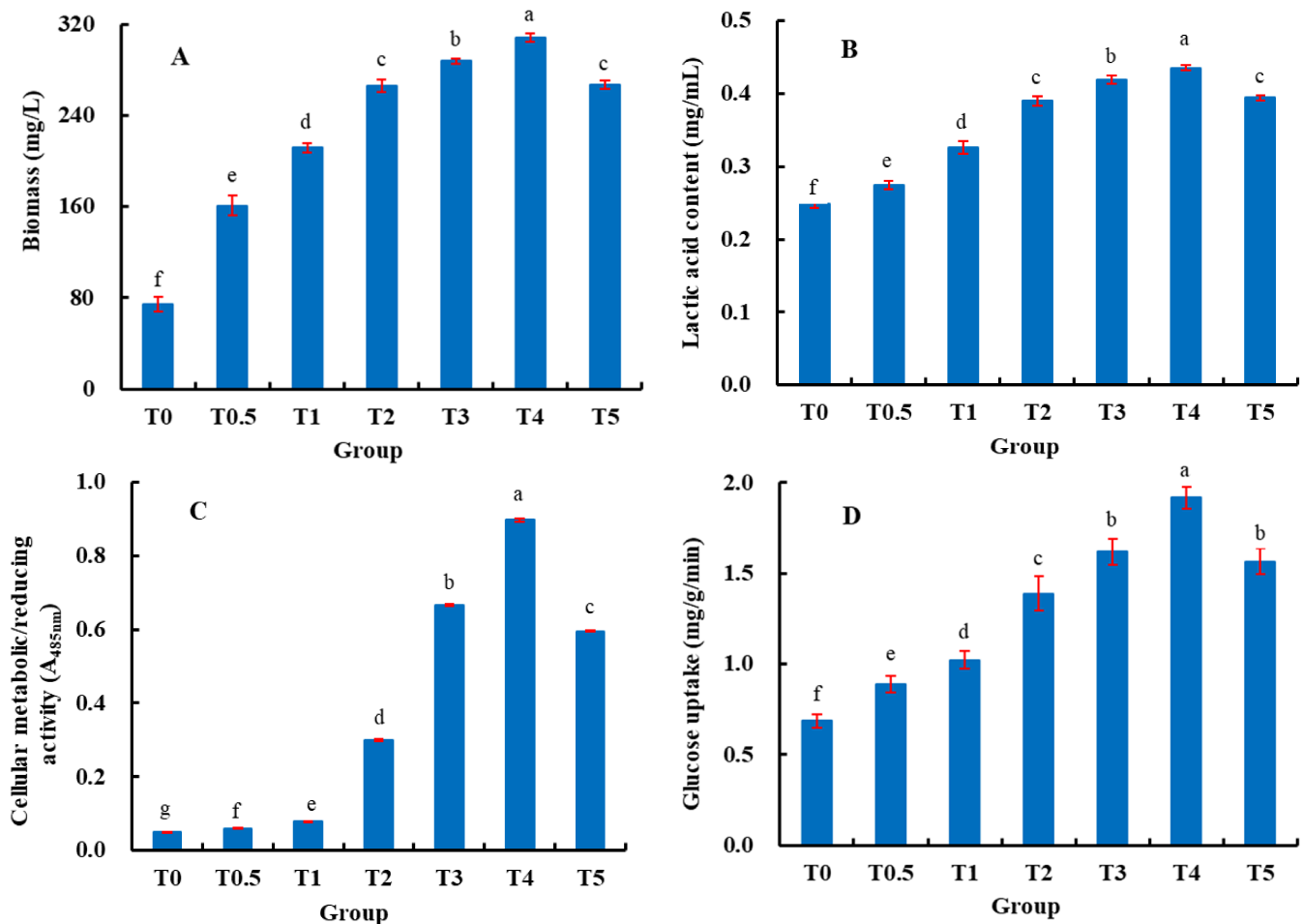


Figure 1. Effect of *D. officinale* leaf dosage on the growth and metabolism of *S. thermophilus*. Note: T0, T0.5, T1, T2, T3, T4, and T5 are the groups cultured with extract from 0, 0.5, 1, 2, 3, 4, and 5% (w/v) *D. officinale* leaves, respectively. (A) biomass; (B) lactic acid content; (C) cellular metabolic/reducing activity; (D) glucose uptake. Different letters indicate significant differences at $p < 0.05$.

The total expression genes (TPM > 1) were 4580 and 4599 genes in three control groups and three *D. officinale* leaf extract groups, respectively (Table S1). Principal component analysis (Figure 3A) showed the significant difference between the *D. officinale* leaf extract groups and control groups. In Figure 3A, *D. officinale* leaf extract groups and control groups were separated by principal component 1, with a variance contribution rate of 66.78%. Through the selection criteria ($FDR \leq 0.05$ and $\log_2FC > |1.0|$), 388 DEGs in the *S. thermophilus* cells were identified to be responsive to the addition of *D. officinale* leaf extract, with 246 genes being significantly up-regulated and 142 genes significantly down-regulated (Figure 3B). Hierarchical cluster analysis (Figure 3C) for the 388 DEGs also showed the significant differences between the control and *D. officinale* leaf extract groups.

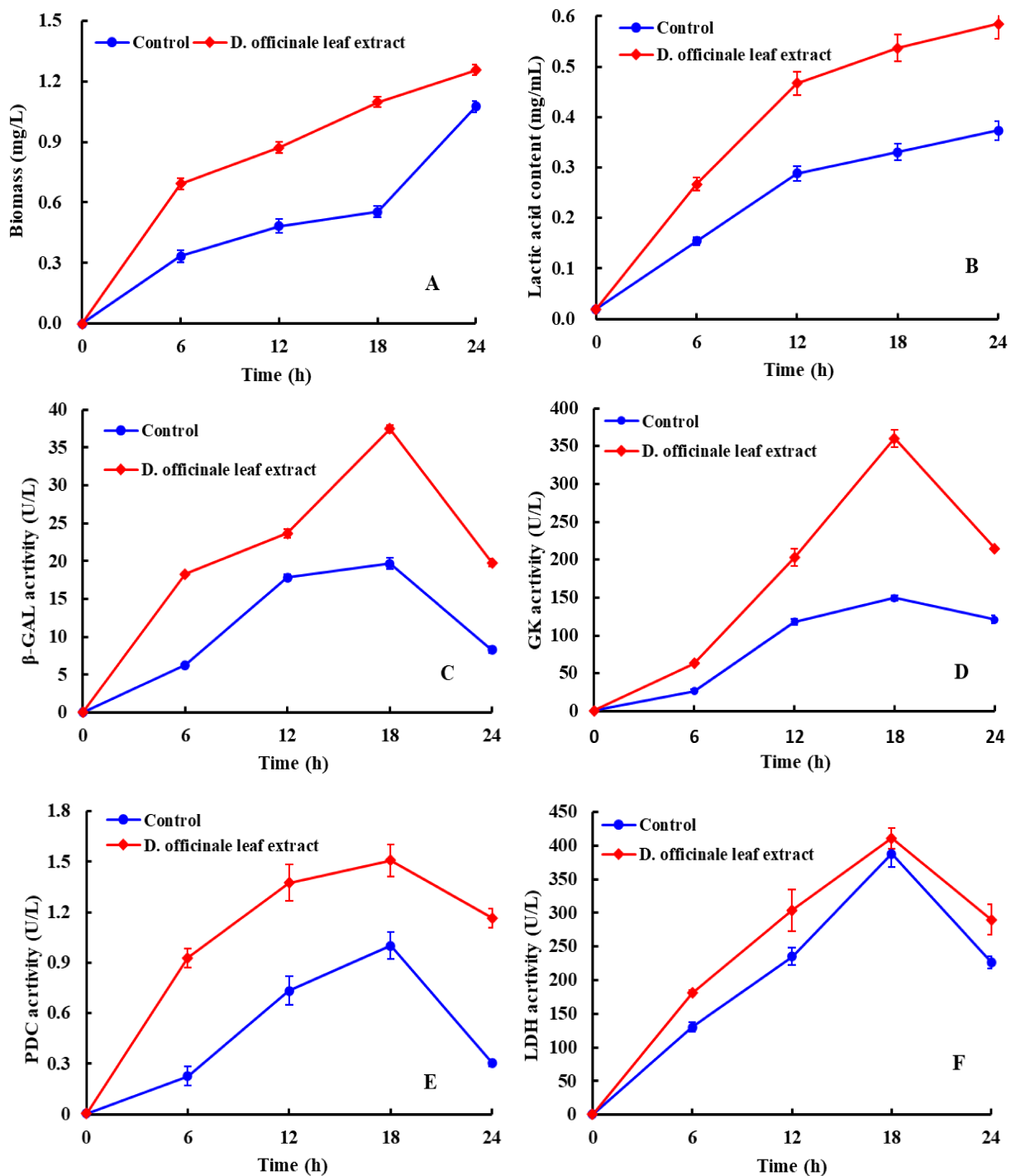


Figure 2. Changes in the growth (A), lactic acid production (B), and β -galactosidase (β -GAL, (C)); glucokinase (GK, (D)), pyruvate decarboxylase (PDC, (E)), and lactic dehydrogenase (LDH, (F)) activities of *S. thermophilus* during the culture in the media without (control) and with extract from 4% (w/v) *D. officinale* leaves.

3.4. Functional Annotation and Enrichment of DEGs

GO (Gene ontology) annotation analysis was carried out to classify the DEGs functions, and results (Figure 4) showed that the cellular process (GO: 0009987) and response to stimulus (GO: 0050896) were the most enriched terms in the biological process ontology, which indicated that the cellular metabolism of *S. thermophilus* was affected by *D. officinale* leaf extract. Cellular anatomical structure (GO: 0110165) and protein-containing complex (GO: 0032991) were the most enriched terms in the cellular component ontology, which

indicated that the cell structure and function protein of *S. thermophilus* were affected by the addition of *D. officinale* leaf extract. Binding (GO: 0005488) and catalytic activity (GO: 0003824) were the most enriched terms in the molecular function, which indicated that *S. thermophilus* may be resistant to *D. officinale* leaf compounds by regulating, catalyzing, and activating various enzymes.

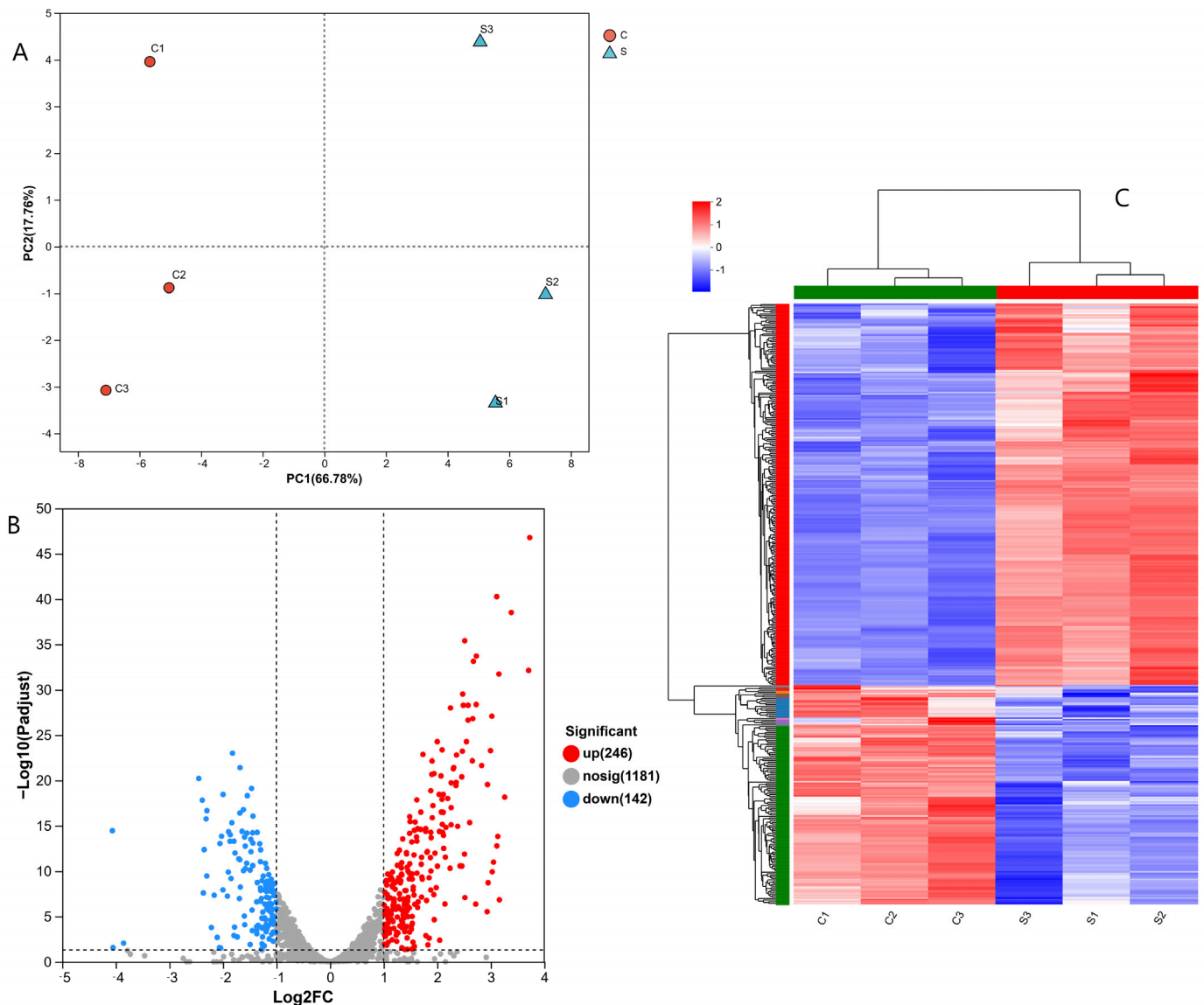


Figure 3. The differentially expressed genes (DEGs) of *S. thermophilus* affected by the addition of extract from 4% (*w/v*) *D. officinale* leaves. (A) The principal component analysis (PCA); (B) volcano plot of DEGs; and (C) hierarchical clustering analysis of all identified DEGs. C1, C2, and C3 are control groups (without *D. officinale* leaf extract); S1, S2, and S3 are *D. officinale* leaf extract groups.

The specific biological functions of the DEGs were elucidated by GO enrichment analysis, and results are shown in Figure 5A,B. The up-regulated DEGs were mainly enriched in the RNA binding, the ribonucleoprotein complex, the translation, the structural molecule activity, and the structural constituent of ribosome. Nevertheless, the down-regulated DEGs were mainly enriched in the racemase and epimerase activity, racemase and epimerase activity acting on carbohydrates and derivatives, the monosaccharide catabolic process, the ‘de novo’ IMP biosynthetic process, the chorismate biosynthetic process, and the protein modification process.

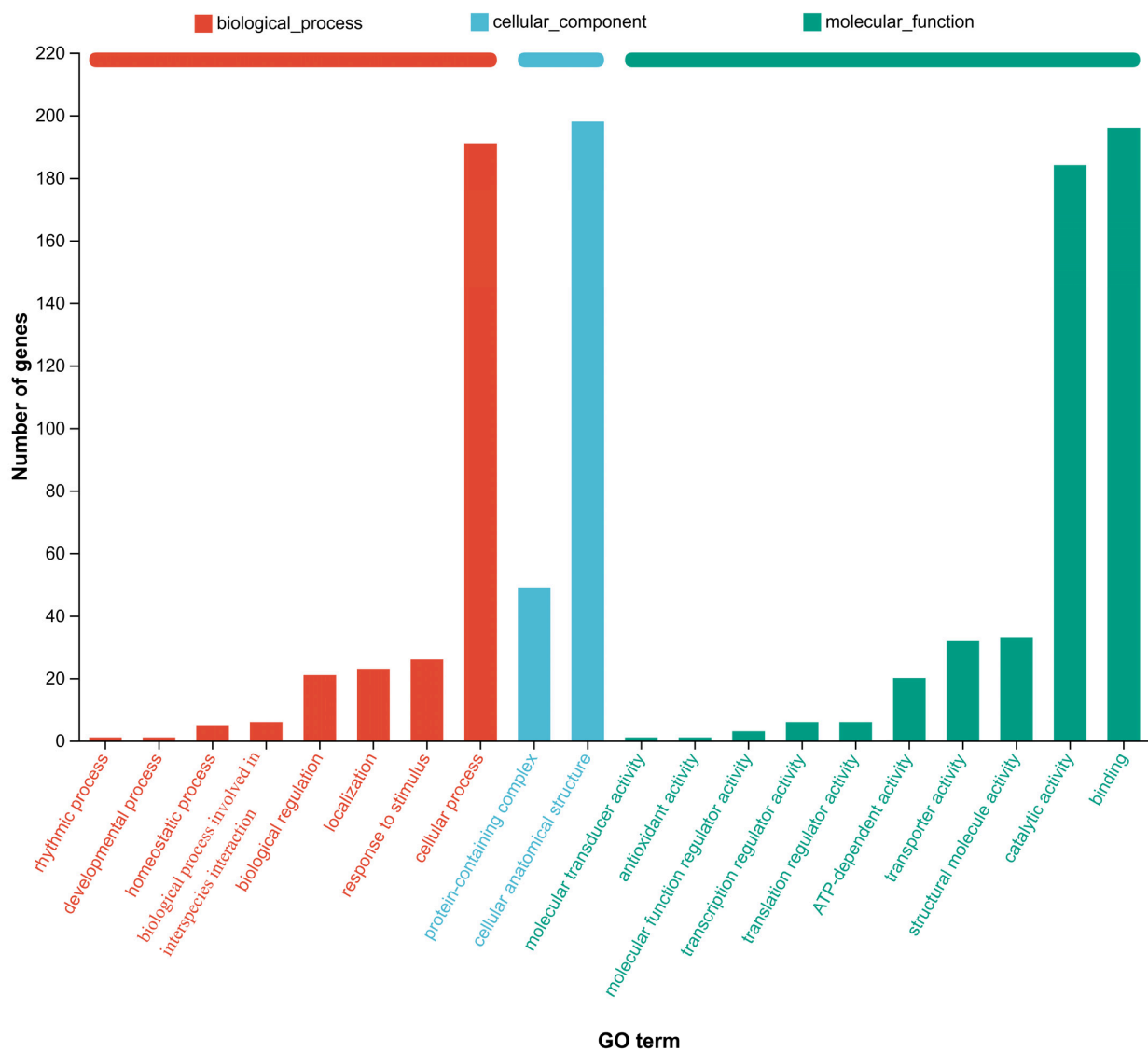


Figure 4. GO annotation analysis of DEGs in *S. thermophilus* affected by the addition of *D. officinale* leaf extract.

KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis for all DEGs was performed to confirm the biological pathways induced by the addition of *D. officinale* leaf extract. As shown in Figure 5C,D, the up-regulated DEGs were enriched in ribosome, ABC transporters, cysteine and methionine metabolism, metabolism of glycine, serine and threonine, other carbon fixation pathways, and propanoate metabolism, whereas the down-regulated DEGs were mainly enriched in purine metabolism, galactose metabolism, methane metabolism, and the biosynthesis of phenylalanine, tyrosine and tryptophan.

As shown in Table S2, the up-regulated DEGs related to amino acid metabolism, translation, membrane transport, carbohydrate metabolism, energy metabolism, nucleotide metabolism, and metabolism of cofactors and vitamins were 29, 36, 25, 15, 12, 6, and 10, respectively. It should be pointed out that some DEGs were simultaneously involved in different metabolic processes; among them, H0506_RS02080, H0506_RS06595, H0506_RS05815, H0506_RS05825, H0506_RS02075, and H0506_RS02070 were related to carbohydrate metabolism and energy metabolism; H0506_RS03765, H0506_RS01580, H0506_RS07295, and H0506_RS08560 were related to amino acid metabolism and metabolism of cofactors and vitamins; H0506_RS02820 and H0506_RS08750 were related to nucleotide metabolism and the metabolism of cofactors and vitamins.

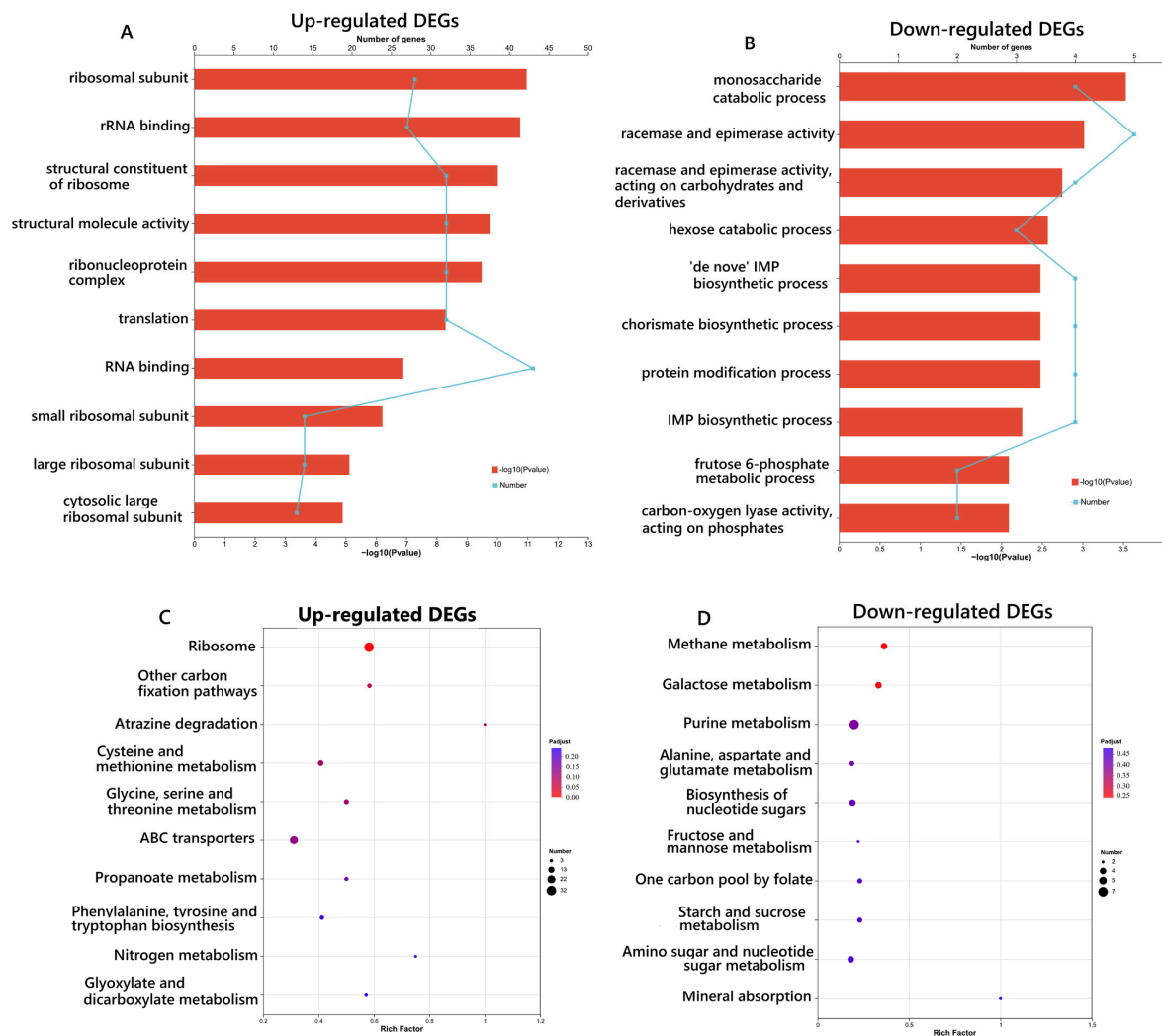


Figure 5. GO and KEGG enrichment analysis of DEGs in *S. thermophilus* affected by the addition of *D. officinale* leaf extract. (A,B) GO enrichment analysis and (C,D) KEGG pathways.

Moreover, GSEA (gene set enrichment analysis) was performed to demonstrate the gene regulation more comprehensively, and the results similar to GO and KEGG enrichment analysis were obtained (Figure 6). The up-regulated DEGs were related to cellular nitrogen compound biosynthetic process, protein metabolic process, organonitrogen compound metabolic process, cellular protein metabolic process, rRNA binding, peptide metabolic process, translation, ATPase activity, and transmembrane transporter activity, etc. Therefore, these pathways, amino acid metabolism, translation, membrane transport, carbohydrate metabolism, energy metabolism, and nucleotide metabolism should be connected by the addition of *D. officinale* leaf extract.

3.5. qRT-PCR Validation of DEGs Express

To validate the gene expression identified by RNA-seq, qRT-PCR was performed for H0506_RS03200, H0506_RS02245, H0506_RS02710, H0506_RS06920, H0506_RS03745, and H0506_RS05900, and these DEGs were related to amino acid metabolism, membrane transport, carbohydrate metabolism, energy metabolism, nucleotide metabolism, and metabolism of cofactors and vitamins, respectively. As shown in Figure 7, the expression levels of the six selected genes in *D. officinale* leaf extract group were higher than those in the control, indicating the accuracy of RNA-seq analysis.

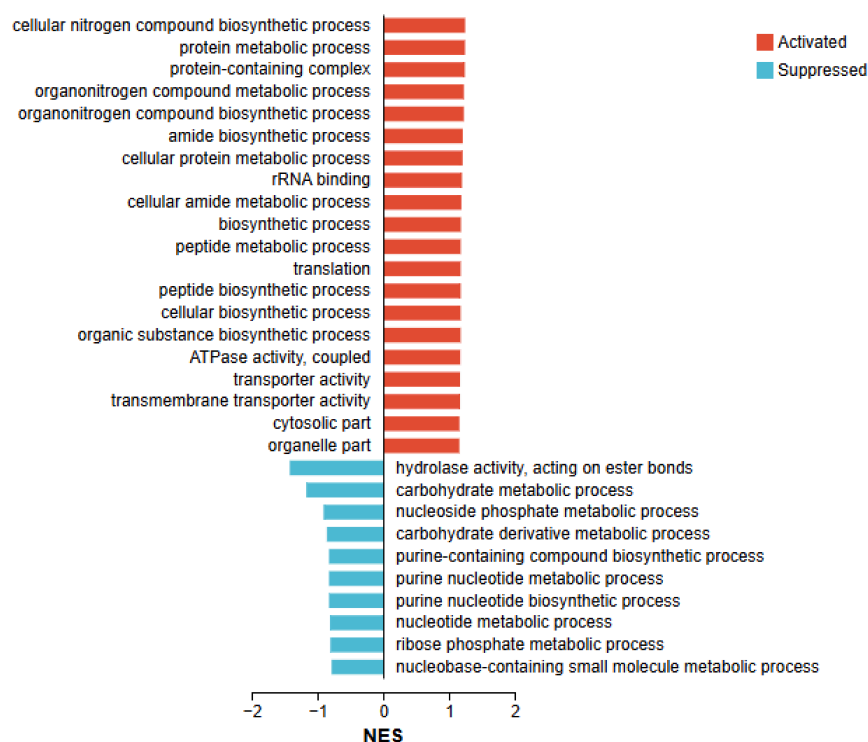


Figure 6. Gene set enrichment analysis (GSEA) of DEGs in *S. thermophilus* affected by the addition of *D. officinale* leaf extract.

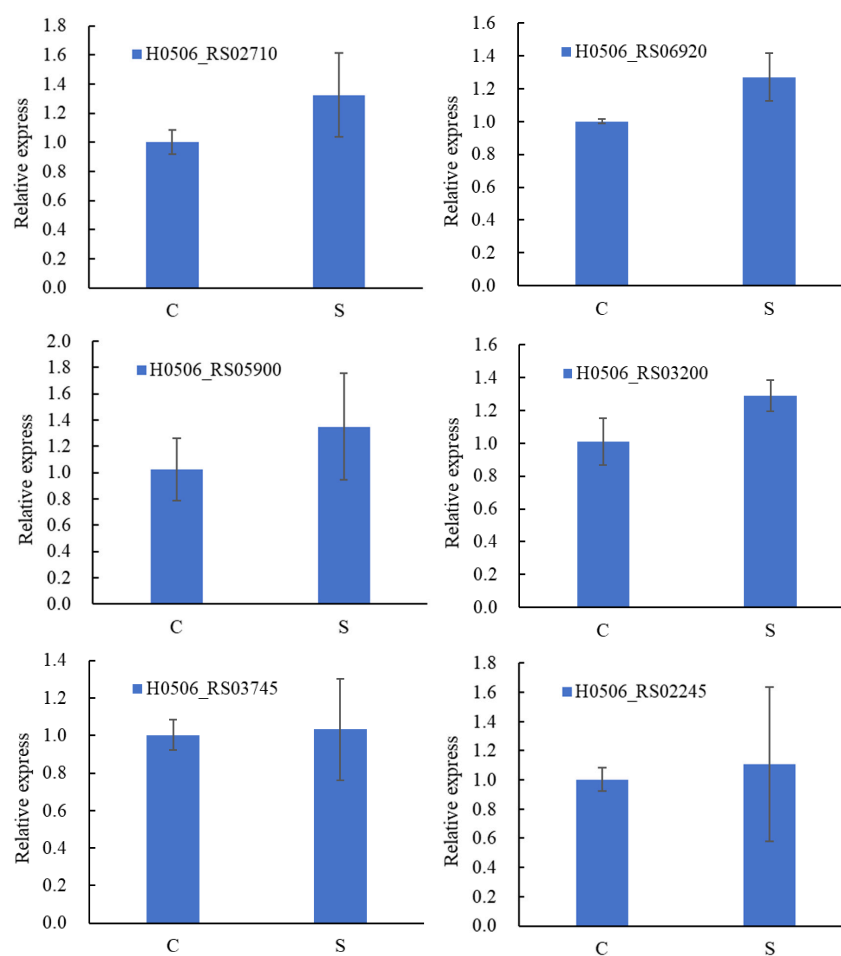


Figure 7. Validation of RNA-seq data by real-time PCR. Note: C and S are control and *D. officinale* leaf extract groups, respectively.

4. Discussion

Fruits, vegetables, and some edible and medicinal plants have been widely used to improve the flavor and health function of yogurt [4,5]. As reported previously, *D. officinale* stem has been used in yogurt to improve the physicochemical property and increase antioxidant activity [14]. *D. officinale* leaf also contains a number of bioactive compounds [7]; therefore, it has the potential to fortify the bioactivity of yogurt. However, previous studies showed that the ingredients in the plant extract may inhibit or promote the growth and metabolism of LABs during the fermentation. Tabasco et al. systematically studied the response of LABs to grape seed extract, and found that the growth of *S. thermophilus*, *L. vaginalis*, *L. acidophilus*, *L. fermentum*, and *B. lactis* BB12 are inhibited, but the growth of *L. plantarum*, *L. bulgaricus*, *L. casei*, *B. bifidum* HDD541, and *B. breve* 26M2 are enhanced [15]. Yang et al. found that phenolics enriched blackberry juice can significantly stimulate the growth of *L. casei*, *L. plantarum* and *L. rhamnosus* at 1–4 logs CFU/mL [16]. Zou et al. tested the effect of red ginseng extract on the growth of *L. rhamnosus* AS 1.2466T, and found that a 12.50% extract could increase the viable cells to 2.72×10^8 CFU/mL, which is ten times higher than that of the control [17]. Results from Ouyang et al. showed that Chinese yam polysaccharide can increase the viable count of *S. thermophilus* by 6.03–11.48% at a concentration of 0.1% [18]. Xu et al. found that polysaccharides from *Lycium barbarum* can shorten the lag phase of *S. thermophilus* G2 [19]. Nevertheless, results from Chiodelli et al. showed that the growth of *L. acidophilus* and *L. delbrueckii* ssp. *bulgaricus* is inhibited by the aloe extract [20]. The effect of tea leaf extract on the growth of LABs is closely related to its concentration [21]. *D. officinale* leaves contain polysaccharides, phenolics, and other compounds [6–9]. The results from this work showed that the regulating effect of *D. officinale* leaf extract on *S. thermophilus* was dosage-related, and the growth and lactic acid production of *S. thermophilus* were enhanced by the extract from 1 to 4% (*w/v*) *D. officinale* leaves, while inhibited with further increase in *D. officinale* leaf dosage (Figure 1).

S. thermophilus and *L. delbrueckii* ssp. *bulgaricus* are the most common LAB strains used in yogurt production, and glycolytic pathway is their major metabolic process with lactic acid as the main product [22]. The key enzymes in the metabolism include β -GAL, GK, PDC, LDH, etc., which may be influenced by the medium constituents. Li et al. reported that the LDH activity of *L. delbrueckii* ssp. *bulgaricus* was inhibited by algal carcass, thereby reducing the lactic acid production [23]. However, walnut soluble fiber could significantly increase the activities of β -GAL and LDH in *L. salivarius* [24]. Data from this work showed that *D. officinale* leaf extract could also enhance the activities of β -GAL, GK, PDC and LDH, resulting in the increase in lactic acid content (Figure 2).

Transcriptomics can interpret the changes in the expression levels of genes within an organism in response to the time phase and cell environment, primarily focusing on the functional components, molecular components, and biological processes [25], and has been widely used to investigate the gene expression processes of LABs [26–28]. In this work, 388 DEGs were obtained in *S. thermophilus* cultivated in the media containing the extract from 4% (*w/v*) *D. officinale* leaves (Figure 3). Through GO, KEGG, and GSEA, *D. officinale* leaf extract could up-regulate some genes involved in the amino acid metabolism, translation, membrane transport, carbohydrate metabolism, energy metabolism, nucleotide metabolism, and metabolism of cofactors and vitamins (Figure 4, Table S2), thereby enhancing the growth and metabolism of *S. thermophilus*.

Combining the activities of key enzymes in the glycolysis pathway with transcriptomic data of *S. thermophilus*, we screened six key genes involved in nutrient uptake: (H0506_RS02245), carbohydrate metabolism (H0506_RS02710), amino acid metabolism (H0506_RS03200), energy metabolism (H0506_RS06920), nucleotide metabolism (H0506_RS03745), and coenzyme synthesis (H0506_RS05900). Subsequent qRT-qPCR vali-

dated that the relative expression levels of these genes were all up-regulated by the addition of *D. officinale* leaf extract. During the growth and metabolism of *S. thermophilus*, multiple genes encode functional proteins that coordinately participate in key physiological activities such as nutrient uptake, central carbon metabolism, energy balance, cell-wall remodeling, stress response, and nucleotide synthesis. Among them, H0506_RS02245 encodes an ATP-binding protein of an ABC transporter, which efficiently transports oligopeptides and amino acids through ATP hydrolysis-driven conformational changes, serving as a core link for the bacterium to obtain nitrogen sources from the medium, maintain rapid growth and biomass accumulation, and also participate in metal ion homeostasis and high-temperature stress response [29]. In terms of carbon metabolism, H0506_RS02710 encodes glucosamine-6-phosphate deaminase (NagB), which converts GlcN6P to fructose-6-phosphate and releases ammonia—a key step in the breakdown of amino sugars entering glycolysis, ultimately producing lactate and ATP in *S. thermophilus* [30]. H0506_RS06920 encodes a glycerol-3-phosphate dehydrogenase (GPDH) family protein, which oxidizes NADH to regenerate NAD⁺ (maintaining glycolytic balance) and provides precursors for phospholipid synthesis, thereby affecting cell membrane fluidity and thermostability at high temperatures (40–45 °C) by regulating the interconversion of glycerol-3-phosphate and dihydroxyacetone phosphate [31–34]. In terms of nucleotide metabolism, H0506_RS03745 encodes thymidine kinase, catalyzing the phosphorylation of thymidine to dTMP and ultimately generating dTTP, providing essential components for DNA replication, thereby promoting the cell cycle and biomass accumulation [35,36]. H0506_RS05900 encodes pyridoxamine kinase (PdxK), responsible for phosphorylating pyridoxine, pyridoxal, and pyridoxamine to convert them into the coenzyme PLP, which is a cofactor for key enzymes such as transaminases in amino acid synthesis and catabolism, and is crucial for the growth of *S. thermophilus* in nutrient-limited environments such as milk [37–40]. Finally, H0506_RS03200 encodes glycerol kinase, the rate-limiting enzyme for the phosphorylation of glycerol to glycerol-3-phosphate, thereby introducing glycerol into glycolysis or glycerophospholipid synthesis pathways [41,42]. In summary, these gene products form a tightly coordinated network in nutrient uptake, energy metabolism, cell integrity, and environmental adaptation of *S. thermophilus*, collectively supporting its rapid growth and metabolic advantages in the milk-based high-temperature fermentation system.

5. Limitations

D. officinale has been widely artificially cultivated in Zhejiang, Fujian, Yunnan, Guizhou, and other provinces of China. In this work, *D. officinale* leaves were obtained from a planting cooperative located in Wenzhou, Zhejiang province, and results showed that the extract from 4% (*w/v*) *D. officinale* leaves was the optimal for the growth and metabolism of *S. thermophilus*. However, there may be differences in the components and/or their contents in *D. officinale* leaves from different sources; therefore, the dosage of *D. officinale* leaves from other sources should be verified before being used in LABs fermentation. In addition, the culture of *S. thermophilus* was conducted using MC medium, and this may limit the direct applicability of the findings from this work to actual yogurt production.

6. Conclusions

D. officinale leaf extract can promote the cellular metabolic/reducing activity, glucose uptake, and the activities of β -GAL, GK, PDC and LDH at suitable concentration, thereby increasing the growth and lactic acid production of *S. thermophilus*, and the optimal dosage is the extract from 4% (*w/v*) *D. officinale* leaves in MC media. Further transcriptomic analysis revealed that *D. officinale* leaf extract can up-regulate the expresses of some genes related to amino acid metabolism, translation, membrane transport, carbohydrate metabolism, energy

metabolism, and nucleotide metabolism, thereby enhancing the growth and metabolism of *S. thermophilus*. The results from this work will provide a basis for the resource utilization of *D. officinale* leaves and the development of functional yogurt products.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/beverages12100122/s1>, Table S1: The total expression genes in control (C) and *D. officinale* leaf extract (S) groups; Table S2: List of the DEGs involved in amino acids metabolism, translation, membrane transport, carbohydrate metabolism, energy metabolism, nucleotide metabolism, and metabolism of cofactors and vitamins.

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