Effects of Corn Steep Liquor on ß-poly(L-malic Acid) Production in Aureobasidium Melanogenum

Genan Wang
Tianjin university of science and technology https://orcid.org/0000-0001-9501-869X
Pan Zhang
Tianjin University of Science and Technology
Bingyi Shi
Tianjin University of Science and Technology
Tingbin Zhao
Tianjin Huizhi Biostrans bioengineering Co.,Ltd
Haisong Yin
Tianjin modern vocational technology college
Changsheng Qiao (qiaochangsheng@163.com)
https://orcid.org/0000-0003-2058-3867

Original article

Keywords: ß-poly(L-malic acid) (PMLA), Corn steep liquor, Aureobasidium melanogenum, metabolomics

Posted Date: July 14th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-38237/v1

Version of Record: A version of this preprint was published on December 1st, 2020. See the published version at https://doi.org/10.1186/s13568-020-01147-8.

Abstract

ß-poly(L-malic acid) (PMLA) is a water-soluble biopolymer used in medicine and other industries. However, the concentration of PMLA produced by microorganisms was too low for large-scale production. In this experiment, corn steep liquor (CSL) was selected due to its high nutritional value and low-cost growth factor to increase the production capacity of PMLA in the strain *Aureobasidium melanogenum*, and the strain's CSL-influenced metabolic change was investigated. The PMLA production, cell growth, and yield ($Y_{p/x}$) of *A. melanogenum* increased by 32.76%, 41.82%, and 47.43%, respectively, with the addition of 3 g/L CSL. Metabolomics analysis showed that the intracellular metabolites of *A. melanogenum*, such as amino acids, organic acids, and key intermediates in the TCA cycle, increased after the addition of CSL. Meanwhile, the data found that tyrosine may play a key role in the PMLA biosynthesis. These results demonstrated that the addition of CSL is an efficient approach for improving the production of PMLA.

Key Points:

- CSL can benefit the ß-poly(L-malic acid) production
- CSL influences the amino acids metabolism
- CSL could be used as an economic nitrogen source for PMLA production

Introduction

Polymalic acid (PMLA) is a polyester of L-malic acid with a wide range of applications in the medical, food, and environmental industries due to its excellent biochemical properties, including biocompatibility, biodegradability, and chemical modifiability(Zeng et al. 2019). Several chemical synthesis routes are available in the production of PMLA(Kajiyama et al. 2004; Portilla-Arias et al. 2008; Vert 1998), but these methods are costly, polluted, and difficult to scale up for commercial applications. Microorganisms, such as *Aureobasidium melanogenum*, can also produce PMLA from sugar during fermentation at high production rates, optical purity, and high molecular weights suitable in many applications(Zou et al. 2019).

Regardless of the microorganism used in PMLA production, L-malic acid is the only precursor in PMLA biosynthesis(Zeng et al. 2019). The three major metabolic pathways in PMLA biosynthesis are the Tricarboxylic acid cycle(TCA), Reductive TCA (rTCA), and Glyoxylate pathway(Chi et al. 2016). In recent years, Several factors were tested to increase PMLA production, including the screening of mutant strains, optimizing fermentation conditions, selecting suitable carbon sources, and adding growth factors(Cao et al. 2019a; Cao et al. 2019b). Moreover, the researcher speculated that the metabolic pathway of PMLA may vary in different strains. The research found that PMLA production is considerably associated with the glyoxylate pathway in *Physarum polycephalum* with the addition of intermediates and inhibitors(Lee et al. 1999), and it can also be catalyzed by nonbiotin-dependent carboxylation, which is related to the rTCA pathway in *A. pullulan*(Cao et al. 2014). Due to the varied biosynthesis pathways for PMLA

production, it is challenging to construct the genetically engineered strains. As a result, selecting a suitable growth factor becomes an easy way to improve PMLA production.

Corn steep liquor (CSL) is a by-product of the corn wet-milling industry that contains nutrients essential for microbial growth. It has been proposed as a potentially effective substrate for many target metabolites produced by microorganisms(Amado et al. 2017). Researchers found that CSL increases the production of citric acid and calcium malate in *Yarrowia lipolytica*(Cavallo et al. 2020) and *Penicillium viticola*(Khan et al. 2014), respectively. However, in recent years research of PMLA biosynthesis, little attention has been paid to investigate the mechanism from a metabolomics aspect.

Gas chromatography–mass spectrometry (GC–MS) is a widely used analytical technique with high separation efficiency and sensitivity detection in resolving complex biological mixtures(He et al. 2018). Moreover, the intracellular metabolites produced by microorganisms influenced by the growth factor during fermentation could be detected via GC–MS. The researcher investigated the various effects of deregulating enzymes on the metabolites between engineered l-lysine-producing *Corynebacterium glutamicum* and the wild-type strain was investigated through intracellular metabolite profiles(da Luz et al. 2017). Some other researchers combined intracellular metabolites with proteomics to identify the difference between marine sediment(Beale et al. 2017) and trace pollution(Beale et al. 2018). This approach could easily detect the main difference between microorganisms under different conditions or growth factors.

In this study, we aimed to explore the effect of CSL on the enhancement of cell growth and PMLA production in *A. melanogenum*. Metabolomics technology was used to gain insight into the working mechanism and to analyze the change in the key intracellular metabolites of *A. melanogenum* after CSL addition. The results will help to determine an efficient approach to improve PMLA production.

Materials And Methods

Microorganism and Medium

Aureobasium. melanogenum CGMCC18996 was isolated in our laboratory and then preserved in the China General Microbiological Culture Collection Center (Beijing, China No. CGMCC18996). The strain was stored in potato dextrose agar (PDA) slants at 4 °C and subcultured every 2 weeks. The seed medium contained 60 g/L sucrose, 3 g/L yeast extract, 2 g/L succinic acid, 1 g/L ammonium sulfate, 0.4 g/L K_2CO_3, 0.1 g/L KH_2PO_4, 0.1 g/L $MgSO_4$, 0.05 g/L $ZnSO_4$, and 0.1% CSL (V/V). The fermentation medium contained 100 g/L sucrose, 35 g/L peptone, 0.1 g/L KH_2PO_4, 2 g/L $NaNO_3$, 0.3 g/L $MgSO_4$, 0.5 g/L KCl, 0.05 g/L $MnSO_4$, and 20% $CaCO_3$ (V/V). Both seed and fermentation media were sterilized at 121 °C for 20 min before use.

The amino acids and vitamins in CSL were determined via the methods of Culea(Culea et al. 2015) and Klejdus (Klejdus et al. 2004).

Fermentation conditions

The primary seed culture of *Aureobasidium. melanogenum* CGMCC18996 was prepared by inoculating cells grown on solid medium into 500 mL Erlenmeyer flasks containing 100 mL seed culture medium and then cultured at 25 °C for approximately 40 h in a rotary shaker (IS-RDS3, Crystal Technology and Industries, Inc., USA). CSL at 1, 3, 5, 7, and 9 g/L was placed into 500 mL Erlenmeyer flasks containing 100 mL fermentation medium with primary seed culture (10%, v/v), and fermentation cultivation was conducted at 25 °C for 144 h in a rotary shaker at 200 rpm. Fed-batch fermentation kinetics was investigated in a 5 L stirred tank fermenter (GRJB-5D, Zhenjiang Gree Co., Ltd., China) containing 3 L fermentation medium inoculated with 300 mL seed culture, and the fermentation medium was operated at 25 °C for 156 h with an agitation speed and aeration rate of 500 rpm and 1.3 vvm, respectively.

Assay of PMLA production

Fermentation broth (10 mL) was collected at different time points and centrifuged at 15,000 r/min. The resulting supernatant (5 mL) was mixed with 5 mL 2 M H_2SO_4 and then incubated at 110 °C for 11 h. After neutralization, the sample was analyzed with HPLC (L-2000, Hitachi Ltd., Japan) by using a PrevailC18 organic acid column at 25 °C eluted with 25 mM KH_2PO_4 at a rate of 1.0 mL/min. The PMLA concentration was determined by comparing the difference in L-malic acid concentrations before and after hydrolysis.

Assay of fermentation parameters

Cell density was determined via the method of dry cell weight (DCW) in three steps. Prior to measurement, HCl (3 M) was added to 10 mL of fermentation broth to eliminate the excess concentration of $CaCO_3$. The fermentation broth (10 mL) was centrifuged at 5,000 rpm for 10 min, and the resulting precipitate was washed twice with phosphate buffer saline (PBS) buffer. After recentrifugation, the precipitates were dried overnight at 80 °C and then weighed.

The residual sugar was analyzed with the 3,5-dinitrosalicylic acid assay(Miller 1959).

Specific growth rate, PMLA productivity, and PMLA yield (Yp/x) calculation

The specific growth rate was measured using the increased biomass versus interval time(Liu et al. 2005), and $Y_{p/x}$ was measured by determining the ratio of increased PMLA to the increased cell biomass concentration over the interval time(Yin et al. 2019).

Extraction of intracellular metabolites and metabolomics analysis

In the extraction of intracellular metabolites, three independent fermentation broth samples (50 mL) at the fermentation times of 72, 96, 120, and 144 h were collected from the 5 L stirred tank fermenter. The excess concentration of $CaCO_3$ that was not consumed by *A. melanogenum* was removed by centrifuging the samples at 5,000 rpm for 30 s. The resulting supernatant was centrifuged at 15,000 rpm for 5 min. The precipitate was collected, washed twice with normal saline at -4 °C, and ground in liquid nitrogen for 25 min. The cell fragment (200 mg) of liquid nitrogen grind was collected and mixed with 1 mL of precooling methanol (60%). The resulting mixture was then centrifuged at 10,000 r/min for 5 min. After the derivatization process, the resulted mixture was subjected to refrigerated centrifugation at 10,000 rpm for 5 min. The resulting supernatant was prepared for GC–MS analysis after storage for 2 h at 25 °C.

Data processing and analysis

GC-MS files(.MS) were converted to AIA(.CDF) format for XCMS online(Gowda et al. 2014) analysis. Peak detection and alignment were measured by default centwave method for GC Single Quadruple(Agilent 7980A/5975C, GC-MSD), and the METLIN database was used for Metabolites identification.

GC-MS pre-processing data were wrote as .csv files and imported to the MetaboAnalyst(Chong et al. 2019) for data normalization. The peak intensity was represented by the relative concentration, and the downstream analysis was performed by R studio using package BiocManager version 1.30.10.

Results

Effect of CSL addition on PMLA production of *A. melanogenum*

In this study, the effects of different CSL concentrations on PMLA production of *A. melanogenum* cultured in a rotary shaker were evaluated. The accumulation of biomass increased with increasing CSL concentration after 144 h of fermentation. However, after the addition of 3 g/L CSL, PMLA production reached the maximum of 69.8 g/L, which was 36.8% higher than the control (Fig. 1). The effect of the addition of 3 g/L CSL on the PMLA production of *A. melanogenum* was further verified by culturing *A. melanogenum* in a 5 L fermenter (Fig. 2). The addition of 3 g/L CSL facilitated the growth and PMLA production of *A. melanogenum.* After 156 h of fermentation, PMLA production reached 73.72 g/L, as shown in Fig. 2[a], which was 32.76% higher than that of the control group. The biomass reached 62.83 g/L, as shown in Fig. 2[b], which was 41.82% higher than that of the control group. Meanwhile, after 24 h of fermentation, the highest specific growth rate reached 0.19 h^{-1}, as shown in Fig. 2[c], which was 37.72% higher than that of the control group. However, a rapid decline in the specific growth rate was

detected from 0.19 h^{-1} to 0.021 h^{-1} after 24 h when no distinct difference existed between the control and CSL groups. The PMLA yield ($Y_{p/x}$) in CSL showed a significant increase in the late stage of fermentation after the addition of 3 g/L CSL, as shown in Fig. 2[d]. After 120 h, the highest $Y_{p/x}$ reached 1.82 g/g, which was 47.43% higher than that of the control group. Moreover, the rate of residual sugar (Fig. 2[e]) was consumed rapidly after the addition of 3 g/L CSL, and the end time was 12 h earlier than that of the control.

Metabolomics analysis of *A. melanogenum* cultivated with the addition of 3 g/L CSL

The PLS-DA scores plot (Fig. 4) showed a clear variation in the metabolite profiles under both groups, and the metabolomics data revealed a total of thirty-six metabolites of *A. melanogenum* that were detected via GC–MS at 72, 96, 120, and 144 h time points (Fig. 3). Among them, most of the metabolite concentrations in the CSL group were increased compared to the control, especially at 120 h (Fig. 3). The enrichment analysis (Fig S1) indicated that the concentrations of metabolite related to PMLA biosynthesis increased obviously, which were mainly deoxyinosine, homogentisate, fumarate acid, and 5-aminolevulinic. These metabolites were involved in the Purine metabolism($P < 0.05$), Tyrosine metabolism($P < 0.05$), TCA cycle($P < 0.05$), and Glycine and serine metabolism.

Metabolomics analysis of PMLA metabolic pathway

The metabolic pathway related to PMLA biosynthesis and the relative concentration changes were depicted(Fig. 5). The results showed that, compared to the control, nearly all of the metabolite concentrations increased and peaked at 120 h. Among them, the concentrations of six metabolites related to the Tryptophan metabolism were improved, which were serotonin, 5-Methoxytryptamine, indole-3-acetonitrile, 2-aminomuconate semialde hyde, L-Kynurenine, and Kynurenate. In addition, the CSL increased the concentration of 5-aminolevulinate involved in the Glycine and serine metabolism. The concentrations of homogentisate and R-reticuline, which related to the Tyrosine metabolism, and D-lactaldehyde, which would flow to the pyruvate, were increased respectively, and that of fumarate and oxalosuccinate, which involved in the TCA cycle, also showed an increasing effect. Meanwhile, the results indicated that a decreasing concentration was observed on the metabolites, L-valine and hydroxypruvate.

Assay of the amino acids on PMLA production

The nutritional substances of CSL were tested. Seventeen kinds of amino acids and three types of vitamins were detected. Table 1 showed the ratio of each amino acid quality to the CSL quality. Among them, four amino acids (tyrosine, serine, glycine, and tryptophan) were selected based on the PMLA pathway of the metabolomics data(Fig. 5) and added to the fermentation broth(without peptone)

separately to evaluate the PMLA production. The result demonstrated that the PMLA production increased by 29.5% after tyrosine adding, 21.9% after serine adding, 9.3% after tryptophan adding, 7.6% after glycine adding, and 39.47% after the CSL adding(Fig. 6). Moreover, significant changes were observed between tyrosine and the control ($P < 0.01$) and the CSL and the control ($P < 0.01$).

Table 1
Nutritional substances contained in the corn steep liquor

Amino acids					
Name	Unit	Test result	Name	Unit	Test result
Aspartic acid	%	2.22	Alanine	%	3.84
Threonine	%	1.20	Valine	%	1.98
Serine	%	1.28	Methionine	%	0.72
Glutamate	%	5.41	Isoleucine	%	1.21
Glycine	%	2.05	Leucine	%	3.40
Tyrosine	%	0.62	Phenylalanine	%	1.38
Lysine	%	1.16	Histidine	%	1.16
Arginine	%	1.51	Proline	%	3.20
tryptophan	%	0.17			
Vitamins					
Name	Unit	Test result	Name	Unit	Test result
Vitamin B_2	mg/kg	11.40	Choline	mg/100 g	344
Inositol	mg/100 g	226			

Discussion

The use of the growth factor is an economical way to improve PMLA production(Cao et al. 2019b). However, less study on the metabolomics changes under the different growth factor has limited the better understanding of the mechanism for PMLA biosynthesis from the metabolic aspect.

In this study, the addition of 3 g/L CSL facilitates cell growth and PMLA production in *A. melanogenum*. The maximal PMLA production and biomass increased by 32.76% and 41.82%, respectively. Furthermore, the specific growth rate revealed a rapid increase during the early phase of fermentation, and $Y_{p/x}$ in the CSL group was higher than that of the control in the late stage. Moreover, CSL contained various amino acids and vitamins (Table 1) that could function as an effective stimulatory agent of cell growth and PMLA biosynthesis. It was found that the rich nutrients of CSL became a source of nitrogen, essential

minerals, and cofactors required for *Pichia pastoris* cell growth(Zheng et al. 2012). Moreover, the addition of CSL increased the biomass of *Trametes versicolor* in the initial 12 h of fermentation, and an accelerated growth rate was observed(Wang et al. 2014). The addition of 3 g/L CSL provided various amino acids and vitamins (Table 1), indicating that the CSL could be an effective stimulatory agent of cell growth in *A. melanogenum*. The high cell growth of *A. melanogenum* led to a high level of PMLA production. Therefore, we speculated that various nutritional substances provided by CSL benefited cell growth, which in turn benefited PMLA production.

With the help of metabolomics technology, the working mechanism underlying the effect of the addition of 3 g/L CSL on the metabolism of *A. melanogenum* was analyzed. The metabolomics data showed that the relative concentrations of metabolites involved in amino and organic acid metabolism were changed during fermentation after the addition of 3 g/L CSL (Fig. 5). And the PLS-DA showed a clear separation between 2 groups. In addition, the Tyrosine metabolism, Glycine and serine metabolism, and TCA cycle were up-regulated after the 3 g/L CSL addition. Among them, for the tyrosine metabolism, tyrosine is first converted to 4-hydroxyphenylpyruvate then to homogentisate that is further converted to fumarlacetoacetate, and fumarlacetoacetate is finally transferred to fumarate and enter the TCA cycle(Wang et al. 2019). The metabolomics data showed that the relative concentration of homogentisate increased by 1.44-fold at 144 h compared to the control, and that of fumarate increased by 1.52-fold. Moreover, the concentration of 5-aminolevulinate, which related to the Glycine and serine metabolism, increased by 1.2-fold under the CSL. And a 1.2-fold change on 2-aminomuconate semialde hyde concentration resulted in the Tryptophan up-regulated metabolism that leads tryptophan into the glutaric acid pathway and then the TCA cycle(Fukuwatari et al. 2001). The result also proved that deoxyinosine, involved in purine metabolism, increased by 1.2-fold, and this metabolism is related to the cell's nitrogen absorption, which can provide molecules that are essential to DNA and RNA biosynthesis, energy metabolism and signal transduction(Jessica and James 2017). Combined with the fermentation data(Fig. 2), the result showed that PMLA production sustained and PMLA yield decreased after 120 h. it was consistent with the metabolomics data that the metabolites related to the PMLA biosynthesis reached the highest at 120 h then it began to decrease.

Metabolomics data can express the metabolic changes under different conditions. However, after the peak alignment and the data normalization, some of the target-metabolites may lose in the data. Therefore, we speculated that the concentrations of tyrosine, glycine, serine, and tryptophan, which related to the up-regulated Tyrosine pathway, Glycine and serine pathway, and Tryptophan pathway, were improved in the CSL group. These pathways may further cause the PMLA production increased. Consequently, we tested the nutritional substances of CSL and found seventeen kinds of amino acids and three types of vitamins (Table 1). Among them, serine is 1.28%, tyrosine is 0.62%, tryptophan is 0.17%, and glycine is 2.05% of the total CSL weight.

In order to figure out which amino acid most benefiting PMLA production in the CSL, different amino acids from the up-regulated pathway (Fig. 5) were added to the fermentation broth based on the ratio of their quality to the total CSL quality to evaluate the effect on PMLA production. The result demonstrated

that all these four amino acids increased PMLA production by 29.5%, 21.9%, 9.3%, and 7.6%, respectively. Apart from that, a significant change between tyrosine and the control($P < 0.01$) was observed, and this result is consistent with the enrichment analysis, indicating that the CSL significantly influenced the Tyrosine metabolism($P < 0.05$). The data obtained from the PMLA-related pathway (Fig. 5) suggested that tyrosine flows to the TCA cycle by converted to fumarate, which is further converted to malic acid and increases the production of PMLA. Meanwhile, it also illustrated that all of the four amino acids, tyrosine, glycine, serine, and tryptophan, eventually flows to the TCA cycle(Fig. 5). However, due to the relative concentration changes of the metabolites (pyruvate and fumarate), we can speculate that the tyrosine probably plays a crucial role in increasing PMLA production under the CSL.

The results, as mentioned above, showed that the TCA cycle was up-regulated after the addition of 3 g/L CSL and led to an increase in malic acid production, which would further increase PMLA production. Therefore, we can conclude that the up-regulated TCA cycle is the key metabolic pathway under the 3 g/L CSL for the increase of PMLA production among three speculated metabolic pathways which are the glyoxylate acid pathway, the reductive TCA pathway, and the TCA pathway.(Zou et al. 2019). Meanwhile, the energy provided by the up-regulated purine metabolism may accelerate this process.

The improvement in amino acid and organic acid metabolism in *A. melanogenum* from the addition of 3 g/L CSL generated various amino acids and organic acids to improve cell growth. The conversions of the metabolites related to the TCA cycle were enhanced after the addition of 3 g/L CSL. Therefore, we speculated that CSL is an effective stimulatory agent for cell growth and PMLA biosynthesis in *A. melanogenum*. Meanwhile, CSL could be used as an economic nitrogen source due to its high nutrition and low cost. As a potential replacement of peptone and yeast extract in PMLA production, CSL has satisfactory use prospects in the PMLA industry.

Declarations

Ethics approval and consent to participate

not applicable

Consent for publication

not applicable

Availability of data and material

All data generated or analysed during this study are included in this paper.

Competing interests

the authors declare that they have no competing interests

Funding:

This study was funded by the Tianjin Municipal Science and Technology Commission(No. 17PTGCCX00190, No. 17PTSYJC00080, No. 17YFCZZC00310 and No. 16YFXTSF00460) and the Tianjin Engineering Research Center of Microbial Metabolism and Fermentation Process Control (No. ZXKF20180301).

Authors' Contributions:

CQ and HY conceived and designed research, BS and PZ conducted experiments. TZ contributed new reagents or analytical tools. GW analyzed data and wrote the manuscript. All authors read and approved the manuscript.

Acknowledgements

Not applicable.

References

1. Amado IR, Vázquez JA, Pastrana L, Teixeira JA (2017) Microbial production of hyaluronic acid from agro-industrial by-products: Molasses and corn steep liquor. Biochem Eng J 117:181-187 doi:https://doi.org/10.1016/j.bej.2016.09.017
2. Beale D, Crosswell J, Karpe A, Metcalfe SS, Morrison P, Staley C, Ahmed W, Sadowsky M, Palombo E, Steven A (2018) Seasonal metabolic analysis of marine sediments collected from Moreton Bay in South East Queensland, Australia, using a multi-omics-based approach. Sci Total Environ 631:1328-1341 doi:https://doi.org/10.1016/j.scitotenv.2018.03.106
3. Beale DJ, Crosswell J, Karpe AV, Ahmed W, Williams M, Morrison PD, Metcalfe S, Staley C, Sadowsky MJ, Palombo EA, Steven ADL (2017) A multi-omics based ecological analysis of coastal marine sediments from Gladstone, in Australia's Central Queensland, and Heron Island, a nearby fringing platform reef. Sci Total Environ 609:842-853 doi:https://doi.org/10.1016/j.scitotenv.2017.07.184
4. Cao W, Cao W, Shen F, Luo J, Yin J, Qiao C, Yinhua W (2019a) Membrane-assisted β-poly(L-malic acid) production from bagasse hydrolysates by *Aureobasidium pullulans* ipe-1. Bioresour Technol 295:122260 doi:https://doi.org/10.1016/j.biortech.2019.122260
5. Cao W, Luo J, Qi B, Zhao J, Qiao C, Ding L, Su Y, Wan Y (2014) β-poly(l-malic acid) production by fed-batch culture of *Aureobasidium pullulans* ipe-1 with mixed sugars. Eng Life Sci 14(2):180-189 doi:https://10.1002/elsc.201200189

6. Cao W, Wang Y, Shen F, Luo J, Yin J, Qiao C, Wan Y (2019b) Efficient β-poly(l-malic acid) production from Jerusalem artichoke by *Aureobasidium pullulans* ipe-1 immobilized in luffa sponge matrices. Bioresour Technol 288:121497 doi:https://doi.org/10.1016/j.biortech.2019.121497
7. Cavallo E, Nobile M, Cerrutti P, Foresti ML (2020) Exploring the production of citric acid with *Yarrowia lipolytica* using corn wet milling products as alternative low-cost fermentation media. Biochem Eng J 155:107463 doi:https://doi.org/10.1016/j.bej.2019.107463
8. Chi Z, Liu G-L, Liu C-G, Chi Z-M (2016) Poly(beta-L-malic acid) (PMLA) from *Aureobasidium* spp. and its current proceedings. Appl Microbiol Biotechnol 100(9):3841-3851 doi:https://doi.org/10.1007/s00253-016-7404-0
9. Chong J, Wishart DS, Xia J (2019) Using MetaboAnalyst 4.0 for Comprehensive and Integrative Metabolomics Data Analysis. Curr Protoc Bioinformatics 68(1):e86 doi:https://doi.org/10.1002/cpbi.86
10. Culea M, Scrob S, Suvar S, Podea P, Haş I, Muste S (2015) Determination of Amino Acids in Corn Seed by Gas Chromatography–Mass Spectrometry. Anal Lett 48 doi:https://doi.org/10.1080/00032719.2014.930869
11. da Luz JA, Hans E, Frank D, Zeng A-P (2017) Analysis of intracellular metabolites of *Corynebacterium glutamicum* at high cell density with automated sampling and filtration and assessment of engineered enzymes for effective l-lysine production. Eng Life Sci 17(5):512-522 doi:https://doi.org/10.1002/elsc.201600163
12. Fukuwatari T, Morikawa Y, Hayakawa F, Sugimoto E, Shibata K (2001) Influence of Adenine-induced Renal Failure on Tryptophan-niacin Metabolism in Rats. Biosci, Biotechnol, Biochem 65(10):2154-2161 doi:https://doi.org/10.1271/bbb.65.2154
13. Gowda H, Ivanisevic J, Johnson CH, Kurczy ME, Benton HP, Rinehart D, Nguyen T, Ray J, Kuehl J, Arevalo B, Westenskow PD, Wang J, Arkin AP, Deutschbauer AM, Patti GJ, Siuzdak G (2014) Interactive XCMS Online: Simplifying Advanced Metabolomic Data Processing and Subsequent Statistical Analyses. Anal Chem 86(14):6931-6939 doi:https://doi.org/10.1021/ac500734c
14. He Y, Zhang Z, Ma P, Ji H, Lu H (2018) GC-MS Profiling of Leukemia Cells: An Optimized Extraction Protocol for Intracellular Metabolome. Anal Methods-UK 10 doi:https://doi.org/10.1039/C7AY02578E
15. Jessica C, James F (2017) Purine Acquisition and Synthesis by Human Fungal Pathogens. Microorganisms 5(2):33- doi:https://doi.org/10.3390/microorganisms5020033
16. Kajiyama T, Kobayashi H, Taguchi T, Saito H, Kamatsu Y, Kataoka K, Tanaka J (2004) Synthesis of activated poly(α,β-malic acid) using N-hydroxysuccinimide and its gelation with collagen as biomaterials. Mat Sci and Eng: C 24:815-819 doi:https://doi.org/10.1016/j.msec.2004.08.023
17. Khan I, Nazir K, Wang Z-P, Liu G-L, Chi Z-M (2014) Calcium malate overproduction by *Penicillium viticola* 152 using the medium containing corn steep liquor. Appl Microbiol Biotechnol 98(4):1539-1546 doi:https://doi.org/10.1007/s00253-013-5326-7
18. Klejdus B, Petrlova J, Potesil D, Adam V, Mikelová R, Vacek J, Kizek R, Kubáň V (2004) Simultaneous determination of water- and fat-soluble vitamins in pharmaceutical preparations by high-

performance liquid chromatography coupled with diode array detection. Anal Chim Acta 520:57-67 doi:https://doi.org/10.1016/j.aca.2004.02.027

19. Lee BS, Maurer T, Kalbitzer HR, Holler E (1999) beta-Poly(L-malate) production by *Physarum polycephalum* - C-13 Nuclear magnetic resonance stwdies. Appl Microbiol Biotechnol 52(3):415-420 doi:https://doi.org/10.1007/s002530051540
20. Liu Y, Liu Q-S, Tay J-H (2005) Initial conditions-dependent growth kinetics in microbial batch culture. Process Biochem 40(1):155-160 doi:https://doi.org/10.1016/j.procbio.2003.11.052
21. Miller GAIL (1959) Use of Dinitrosalicylic Acid Reagent for Detection of Reducing Sugars. ANAL CHEM 31 doi:https://doi.org/10.1021/ac60147a030
22. Portilla-Arias J, García-Alvarez M, Martinez de Ilarduya A, Holler E, Muñoz S, Muñoz-Guerra S (2008) Synthesis, Degradability, and Drug Releasing Properties of Methyl Esters of Fungal Poly(β,L-malic acid). Macromol Biosci 8:540-50 doi:https://doi.org/10.1002/mabi.200700248
23. Vert M (1998) Chemical routes to poly(β-malic acid) and potential applications of this water-soluble bioresorbable poly(β-hydroxy alkanoate). Polym Degrad and Stabil 59(1):169-175 doi:https://doi.org/10.1016/S0141-3910(97)00158-4
24. Wang F, Hu J-H, Guo C, Liu C-Z (2014) Enhanced laccase production by *Trametes versicolor* using corn steep liquor as both nitrogen source and inducer. Bioresour Technol 166:602-605 doi:https://doi.org/10.1016/j.biortech.2014.05.068
25. Wang M, Toda K, Block A, Maeda H (2019) TAT1 and TAT2 tyrosine aminotransferases have both distinct and shared functions in tyrosine metabolism and degradation in *Arabidopsis thaliana*. J Biol Chem 294:jbc.RA118.006539 doi:https://doi.org/10.1074/jbc.RA118.006539
26. Yin H, Gao C, Ye K, Zhao T, Sun A, Qiao C (2019) Evaluation of surfactant effect on β-poly(L-malic acid) production by *Aureobasidium pullulans*. Biotechnol Biotec Eq 33(1):954-966 doi:https://doi.org/10.1080/13102818.2019.1631718
27. Zeng W, Zhang B, Liu Q, Chen G, Liang Z (2019) Analysis of the L-malate biosynthesis pathway involved in poly(-L-malic acid) production in *Aureobasidium melanogenum* GXZ-6 by addition of metabolic intermediates and inhibitors. J Microbiol 57(4):281-287 doi:https://doi.org/10.1007/s12275-019-8424-0
28. Zheng J, Zhao W, Guo N, Lin F, Tian J, Wu L, Zhou H (2012) Development of an industrial medium and a novel fed-batch strategy for high-level expression of recombinant β-mananase by *Pichia pastoris*. Bioresour Technol 118:257-264 doi:https://doi.org/10.1016/j.biortech.2012.05.065
29. Zou X, Cheng C, Feng J, Song X, Lin M, Yang S-T (2019) Biosynthesis of polymalic acid in fermentation: advances and prospects for industrial application. Crit Rev Biotechnol 39(3):408-421 doi:https://doi.org/10.1080/07388551.2019.1571008

Figures

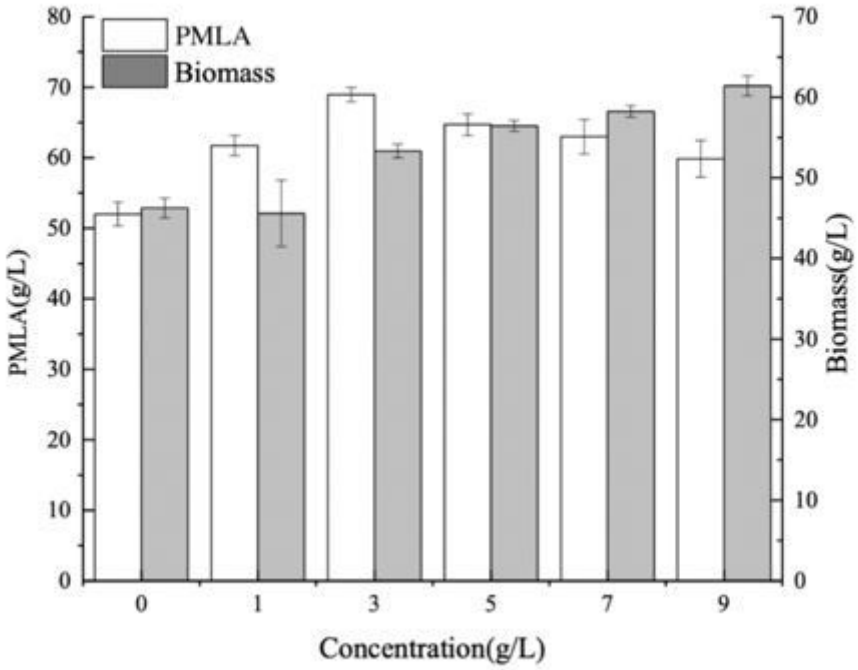

Figure 1

Different concentrations of CSL on PMLA production and biomass

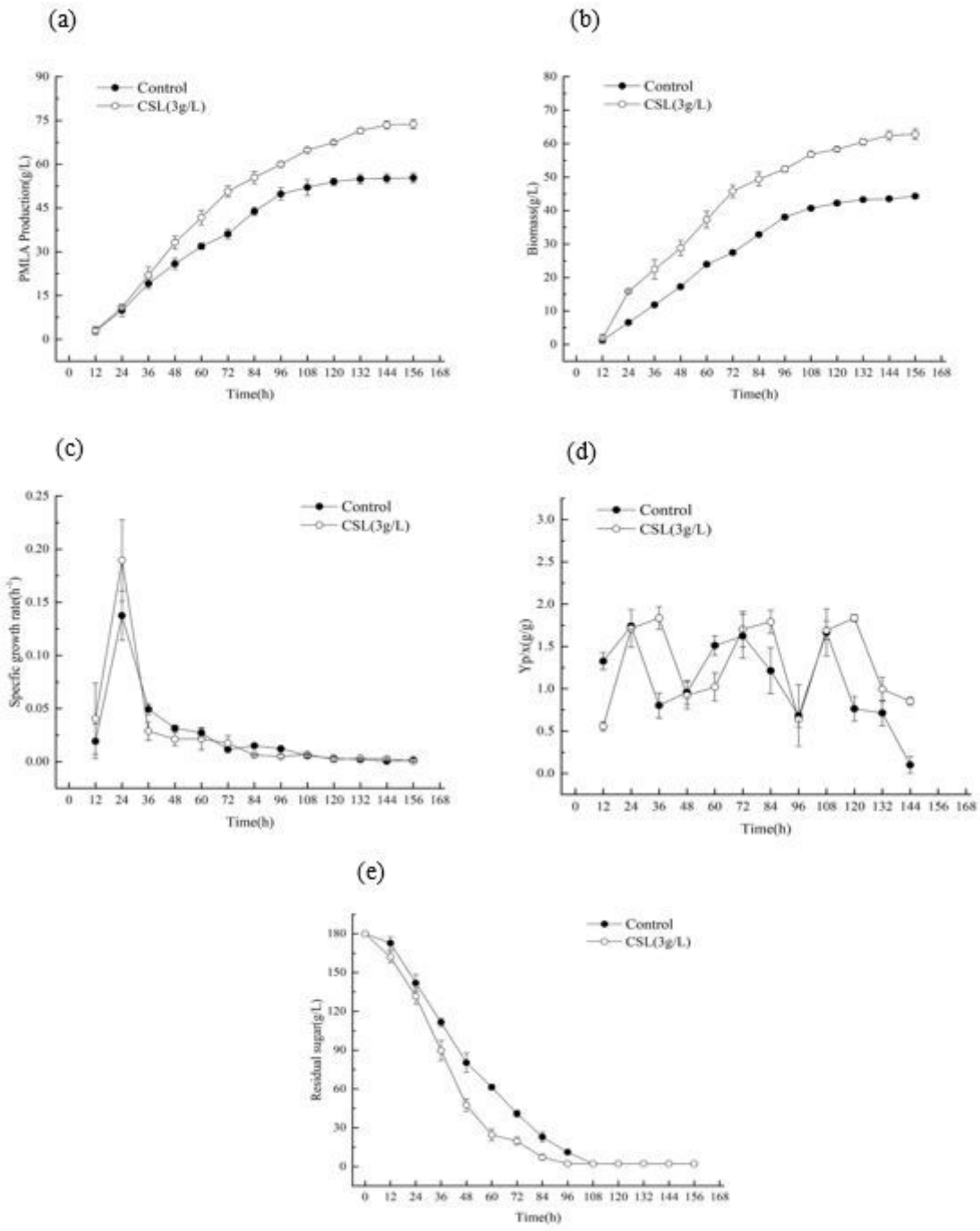

Figure 2

PMLA production versus (a) biomass, (b) specific productivity rate, (c) specific growth rate, (d) PMLA yield, and (e) residual sugar in the 5 L fermenter

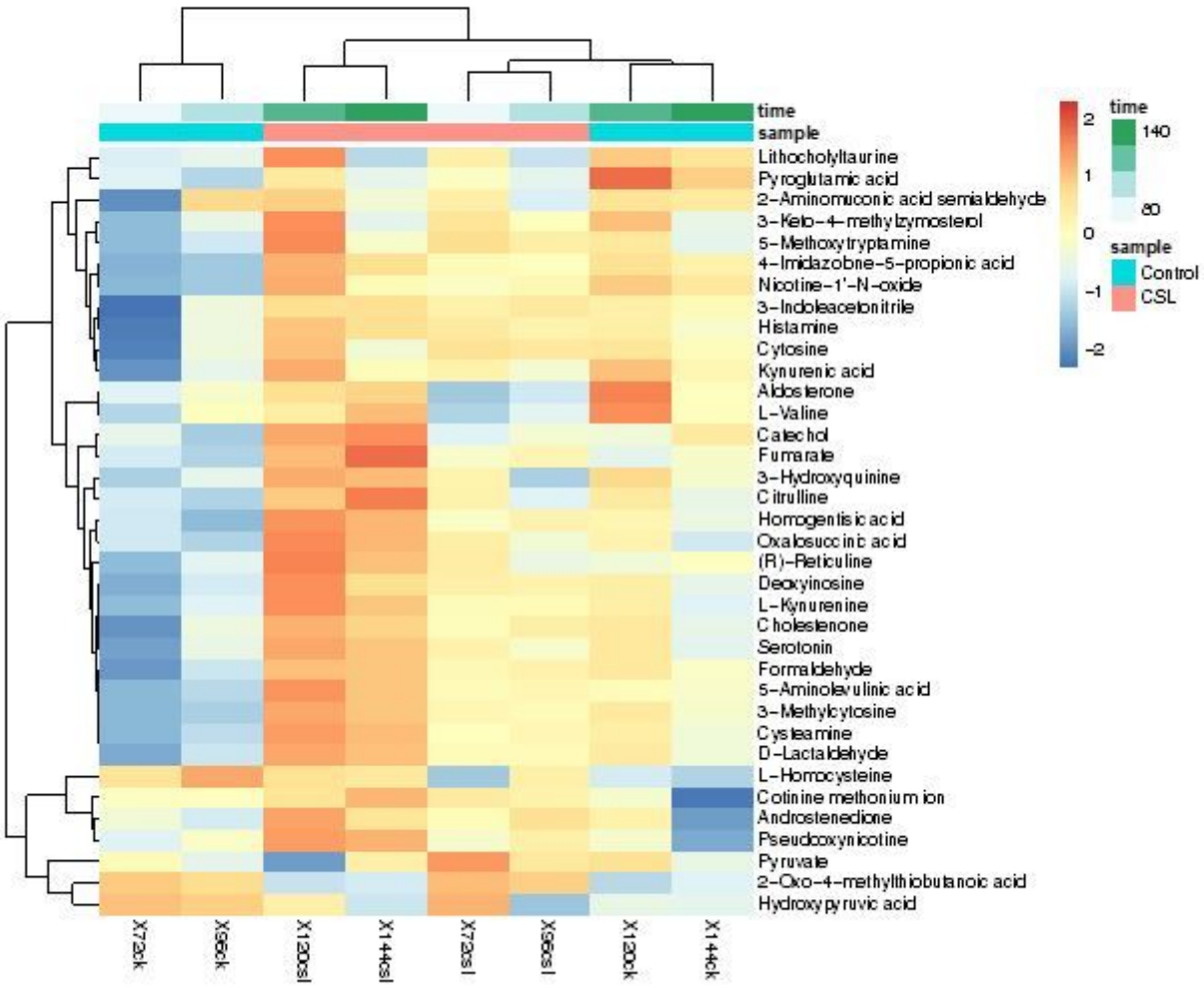

Figure 3

Heat map of the relative concentrations of metabolites in the Control and CSL

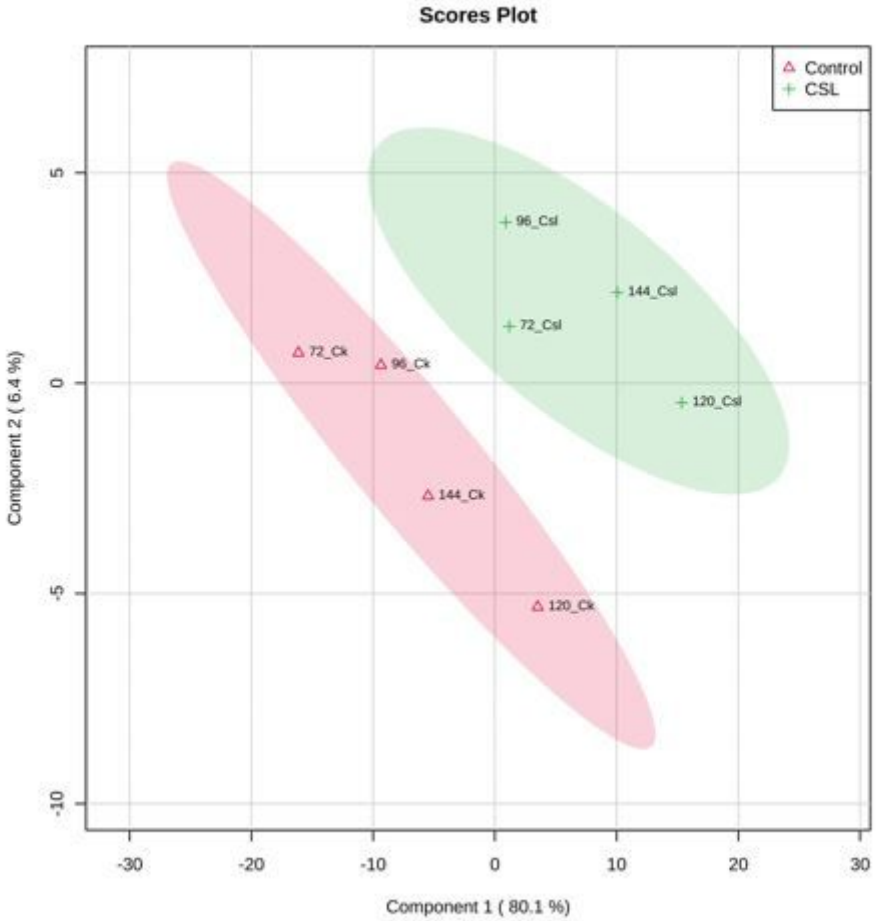

Figure 4

PLS-DA score plot of the CSL and Control

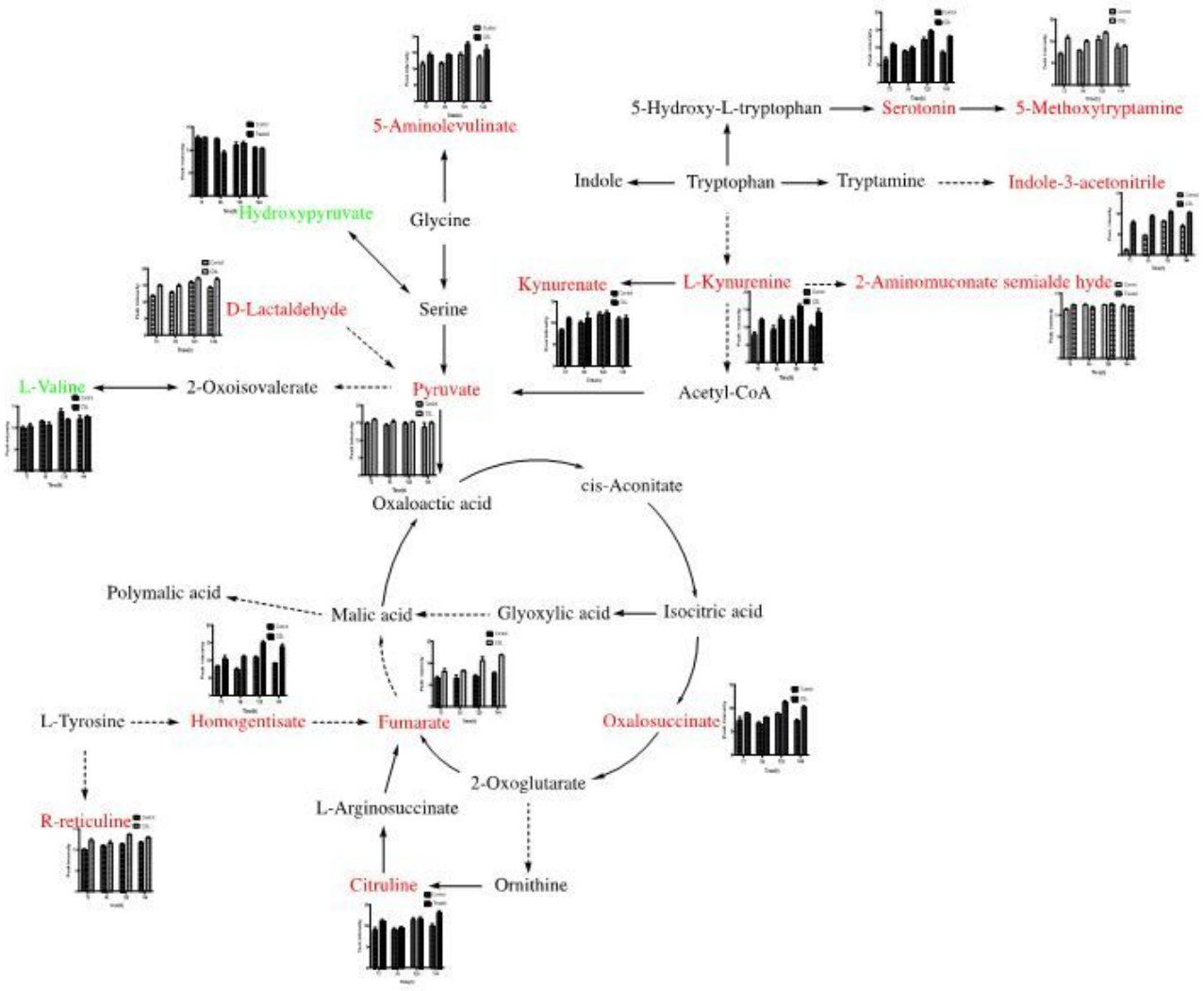

Figure 5

Metabolic pathway related to the polymalic acid biosynthesis based on the metabolomics data(color in red and green represent up-regulated and down-regulated metabolites)

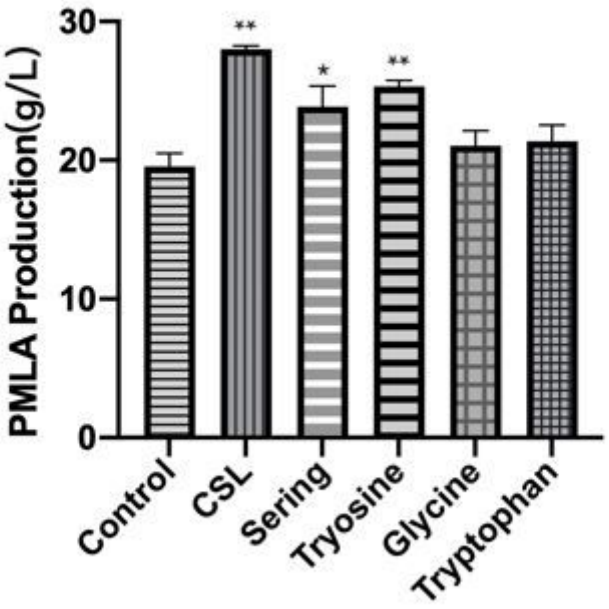

Figure 6

Different amino acids on PMLA production (*P□0.05, **P□0.01 versus the control group by the LSD analysis)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementarymaterial.pdf