Co-production of biopolymers and quinone via no-sugar fermentation—a case by *Methylobacterium* sp. XJLM

Peiwu Cui

Zhejiang University of Technology Chaohui Campus: Zhejiang University of Technology

Yunhai Shao

Zhejiang University of Technology Chaohui Campus: Zhejiang University of Technology

Yanxin Wang

Zhejiang University of Technology Chaohui Campus: Zhejiang University of Technology

Rui Zhao

Zhejiang University of Technology Chaohui Campus: Zhejiang University of Technology

Huihui Zhan

Hunan University of Chinese Medicine

Weihong Zhong (✉ whzhong@zjut.edu.cn)

Zhejiang University of Technology Chaohui Campus: Zhejiang University of Technology

<https://orcid.org/0000-0002-5673-2222>

Research Article

Keywords: *Methylobacterium* sp. XJLW, metabolic pathway mining, methanol-based process, PHB, CoQ10, Fed-batch fermentation

Posted Date: March 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-254045/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Purpose To explore a competitive PHB producing fermentation process, this study evaluated the potential for *Methylobacterium* sp. XJLW to produce simultaneously PHB and coenzyme Q 10 (CoQ 10) using cheap and abundant methanol as sole carbon and energy source. **Methods** The metabolic pathways of PHB and CoQ 10 biosynthesis in XJLW strain were first mined based on the genomic and comparative transcriptomics information. Then, Real-time fluorescence quantitative PCR (RT-qPCR) was employed for comparing the expression level of important genes involved in PHB and CoQ10 synthesis pathways response to methanol and glucose. Transmission electron microscope (TEM), gas chromatography/mass spectrometry (GC-MS), nuclear magnetic resonance (NMR), Fourier transformation infrared spectrum (FT-IR), and liquid chromatography/mass spectrometry (LC-MS) methods, were used to elucidate the yield and structure of PHB and CoQ 10 , respectively. PHB and CoQ 10 productivity of XJLW strain were evaluated in flasks for medium optimization, and in a 5-L bioreactor for methanol fed-batch strategy according to dissolved oxygen (DO) and pH control. **Results** Comparative genomics and transcriptomics analysis showed that the PHB and CoQ 10 biosynthesis pathways coexist in XJLW strain, and the transcription level of key genes in both pathways response to methanol was significantly higher than that response to glucose. Correspondingly, strain XJLW can produce PHB and CoQ 10 simultaneously with higher yield using cheap and abundant methanol than using glucose as sole carbon and energy source. The isolated products showed the structure characteristics same to that of standard PHB and CoQ 10 . The optimal medium and cultural conditions for PHB and CoQ 10 co-production by XJLW strain was in M3 medium containing 1% (v/v) of methanol, 0.5 g/L of ammonium sulfate, 0.1% (v/v) of Tween 80, and 1.0 g/L of sodium chloride, under 30°C and pH 7.0. In a 5-L bioreactor coupled with methanol fed-batch process, a maximum DCW value (46.31 g/L) with the highest yields of PHB and CoQ 10 , reaching 6.94 g/L and 22.28 mg/L, respectively. **Conclusion** *Methylobacterium* sp. XJLW is potential for efficiently co-producing PHB and CoQ 10 employing methanol as sole carbon and energy source. However, it is still necessary to further optimize fermentation process, and genetically modify strain pathway, for enhanced production of PHB and CoQ 10 simultaneously by XJLW. It also suggests a potential strategy to develop efficiently co-producing other high value metabolites using methanol-based bio-process.

Introduction

Nowadays, along with the increasing demands for polymer plastics, which can be widely used from product packing and daily tools to equipment parts and construction sectors, the growing serious petroleum-based plastic pollution has drawn more attractive attentions due to its less biodegradation property (Cardoso et al. 2020; Mostafa et al. 2020). In order to solve this global circumstance, many scientists have put great efforts on biodegradable polymers production. For showing similar thermoplastic, elastomeric and other physical-chemical properties to conventional plastics, polyhydroxyalkanoates (PHAs) are regarded as the most potential substituent, which can be completely degraded to CO₂ and H₂O (Sukruansuwan 2018; Mostafa et al. 2020). However, the high cost of PHAs production from costly substrates has seriously limited the utilization of PHAs in commercial fields,

which forces scientists to explore alternative approaches to produce it at a lower price (Parveez et al. 2015). The production costs of PHAs depend on many factors including strains, substrates, cultivation conditions, extraction and purification processes (Gamez-Perez et al. 2020). Carbon source is regarded as the major factor that accounts for 70-80% of the total expenses of PHAs (Mohandas et al. 2017), because PHAs are usually synthesized under a specific condition of limitation of nutrients and excess of carbon source (Cardoso et al. 2020). Thus, development of a PHAs producing process with a cheap and renewable substrate is still necessary. As one of the common industrial by-products and a cheaper and renewable chemical feedstock, methanol has been widely used as carbon and energy source in methylotrophs fermentation processes for value-added chemicals production (Zaldivar Carrillo et al. 2018; Zhang et al. 2019). Hence, methanol-based fermentation for PHAs production is still a highly promising process without sugar consumption and carbon emissions.

Among all PHAs, polyhydroxybutyrate (PHB) is considered as the most competitive biopolymer because of its good biocompatibility, biodegradability, and similar properties to polypropylene (Parveez et al. 2015; Sharma 2019). Meanwhile, coenzyme Q₁₀ (CoQ₁₀) is the most valuable product among all natural quinone metabolites, and it is a good clinic biological drug for removing free radicals in the body, keeping biological membrane stable, anti-lipid peroxidation and strengthening the nonspecific immune (Qiu et al. 2012; Lu et al. 2013). Thus, PHB and CoQ₁₀ were selected as representatives of biopolymers and quinone metabolites, respectively, to evaluate the potential for their co-production via methanol-based process.

In our previous work, a new formaldehyde degradable methylotrophic bacterium was isolated and identified as *Methylobacterium* sp. XJLW (Qiu et al. 2014; Shao et al. 2019). Its completed genome has been sequenced (Shao et al. 2019). Comparative genomic analysis exhibited XJLW contains both pathways of CoQ₁₀ and PHB biosynthesis (Fig.1), suggesting the possibility to develop a new fermentation process to realize co-production of PHB and CoQ₁₀ with the abundant methanol as sole carbon source at the same time, which will provide a more economic process for PHB production.

In the present research, we aims to 1) verify the potential of PHB and CoQ₁₀ co-production by XJLW with different carbon sources, glucose and methanol; 2) elucidate the expression difference of the key genes in both pathways of PHB and CoQ₁₀ biosynthesis in XJLW response to methanol and glucose; 3) evaluate the effects of medium composition and cultivation conditions on PHB and CoQ₁₀ co-production in Erlenmeyer flasks and in a 5-L stirred bioreactor employing methanol fed-batch strategy. This study will provide a new reference of strategy for improving value added products productivity with methanol-based fermentation process employing methylotrophs.

Materials And Methods

Chemicals

PHB (purity above 95%, CAS No: 26063-00-3) and CoQ₁₀ (purity above 99.9%, CAS No: 303-98-0) were purchased from Sigma-Aldrich. Alcohol (HPLC grade, purity above 99.5%) was purchased from Tjshield fine chemicals Co., Ltd. (Tianjin, China). Other chemicals were analytical reagents purchased from local company.

Strain and Medium

Methylobacterium sp. XJLW was isolated from Huangyan Sewage Treatment Plant, Zhejiang Province, China. Now it has been deposited at China Center for Type Culture Collection (CCTCC) under the accession number CCTCC M2012065.

Medium M3 (Bourque et al. 1995) contained (g L⁻¹) (NH₄)₂SO₄ 0.5, KH₂PO₄ 1.305, Na₂HPO₄·7H₂O 4.02, MgSO₄·7H₂O 0.45, CaCl₂·2H₂O 0.02, FeSO₄·H₂O 0.02, and 1 mL L⁻¹ trace elements solution. The trace elements solution contained (g L⁻¹) MnSO₄·H₂O 4.9, ZnSO₄·7H₂O 2.6, CuSO₄·5H₂O 0.8, Na₂MoO₄·2H₂O 0.8, CoCl₂·6H₂O 0.8, and H₃BO₃ 0.6.

Mineral salt medium (MSM) (Qiu et al. 2014) contained (g L⁻¹) KH₂PO₄ 0.7, K₂HPO₄ 0.85, (NH₄)₂SO₄ 1.2, MgSO₄·7H₂O 0.1, CaCl₂ 0.01, FeSO₄·7H₂O 0.001, and 1 mL L⁻¹ trace elements solution. The trace elements solution contained (g L⁻¹) H₃BO₃ 6, CoCl₂·6H₂O 4, ZnSO₄·7H₂O 2, MnCl₂·4H₂O 0.6, Na₂MoO₄·2H₂O 0.6, NiCl₂·4H₂O 0.4, and CuCl₂·2H₂O 0.2.

Initial pH of the above media was adjusted to 7.0 with 1 M NaOH. 1%(v/v) methanol was added to the two media used as sole carbon source after being autoclaved at 115°C for 30 minutes.

Culture Conditions

Fifty microliters suspension of frozen stock XJLW was inoculated into a 250 mL Erlenmeyer flask containing 50 mL medium M3, and incubated for 96 hours. Then 2 mL culture was inoculated into 250 mL Erlenmeyer flasks containing 50 mL fermentation medium and incubated under 180 rpm and 30°C for 5 days.

Cell morphology observation via transmission electron microscope

Cells in 1 mL culture broth was harvested by centrifuged at 8000 × g for 10 min at 4°C, and then were suspending in 4%(v/v) pre-cooled glutaraldehyde and immobilized for 1 h at 4°C. The ultrathin section of immobilized cell was observed under transmission electron microscope (HITACHI H-7650, Japan).

Physiology characteristics analysis combined with RNA-seq and RT-qPCR

The cell growth and simultaneously production ability of PHB and CoQ₁₀ was detected in M3 medium supplemented with glucose and methanol respectively. Meanwhile, the cells were harvested for RNA-seq and RT-qPCR.

RNA-Seq data analysis

After culture in M3 containing methanol and glucose as carbon source, respectively, at 30°C to log phase (OD_{600} 0.8), *Methylobacterium* sp. XJLW cells was harvested via centrifugation at 4000 rpm for 5 min under 4°C. Then, cells pellets were immediately mixed with RNA protect Bacteria Reagent (QIAGEN China Co. Ltd), and then stored at -80°C for RNA extraction. A total amount of 1 µg qualified RNA sample was used as input material for the library preparation. Library concentration was measured using Qubit® RNA Assay Kit in Qubit® 3.0 to preliminary quantify. Insert size was assessed using the Agilent Bioanalyzer 2100 system, after the insert size consistent with expectations, qualified fragment were accurately quantified using qPCR by Step One Plus Real-Time PCR system (ABI, USA). The raw reads were filtered by removing reads containing adaptors, ploy-N (i.e., unrecognized bases, reads with a recall ratio less than 5%) and low quality reads (the number of bases ≤ 10 and occupied less than 50% of the entire read) for subsequent analysis. Firstly, we used Tophat2 (Kim et al. 2013) to evaluate the sequencing data by comparison with the genomic sequences of reference strains. Based on the Tophat2 alignment results, Cufflinks-2.2.1 was used to perform quantitative gene expression analysis. Gene expression is calculated as follows: FPKM (expected number of Fragments PerKilobase of transcript sequence per Millions of sequenced base pairs). In general, the screening criteria for significantly differentially expressed genes are: $|\log_2 \text{fold-change}| \geq 1$ and $p\text{-value} \leq 0.05$. Scatter plot and volcano map are used to present the overall profile of gene expression differences.

RNA extraction and quantitative RT-qPCR

The cells in the early exponential stage, cultured in M3 medium supplemented with glucose and methanol respectively, were centrifuged at $10000 \times g$ for 5 min at 4 °C. The total RNA was extracted by using RNA isolator (Vazyme Biotech Co., Ltd., Nanjing). And then, HiScript II Q RT SuperMix qPCR kit (Vazyme Biotech Co., Ltd., Nanjing) was used to develop reverse transcription reactions. The reaction buffer system of RT-qPCR was prepared with ChamQ SYBR qPCR Master Mix, and performing the quantitative PCR with Bio-Rad CFX real-time PCR system. The expression level of the 16S rRNA gene was used as internal references. Each reaction was repeated at least three times. The primers used for RT-qPCR are listed in Table 1.

Optimization of XJLW strain fermentation in shake flask level

Firstly, to choose a better initial medium, the cell growth and biosynthesis of target products of XJLW strain cultivated in M3 and MSM were evaluated. A one-factor-at-a-time design was employed to analyze the effects of methanol concentration, ammonium sulfate concentration, fermentation temperature, initial pH of medium, different types of oxygen carriers and osmotic pressure regulated by adding different concentration of sodium chloride on XJLW strain growing and target metabolites biosynthesis.

Scale-up of XJLW strain fermentation system with fed-batch strategy

After optimization of fermentation conditions in flask, a fed-batch fermentation was carried out in a 5 L stirred tank reactor (Biostat-Bplus-5L, B.Braun Germany) with a working volume of 3.0 L, at 30°C, 400 rpm and pH 5.5 (controlled using aqueous NH_4OH solution), and with a dissolved oxygen concentration above 20% of air saturation. Firstly, the basal salts of optimal medium were dissolved in 2670 mL ddH₂O and were autoclaved in the bioreactor. To start the fermentation, 30 mL methanol and 300 mL inoculum suspension were added to the bioreactor by peristaltic pump. Filter-sterilized air was the source of oxygen and was supplied at a flow rate of 3 vvm. After initial added methanol was completely exhausted implied by the dissolved oxygen level rising up to 100%, additional methanol (mixed with 1% trace elements solution) was pulse fed into the reactor regulated by the dissolved oxygen monitor to further increase the cell density. At the same time pH was also adjusted at a stable level of 5.7 by adding NH_4OH solution which could supply nitrogen source simultaneously. If needed, increasing stirred speed strategy was also employed to increase dissolved oxygen level. The whole fermentation period was about 5 to 7 days.

Separation of CoQ₁₀ and PHB

After fermentation, cell biomass was separated by centrifugation at 8000 × g, 4°C for 10 min (Biofuge Stratos Sorvall, Thermo, Germany), then 20 mL alcohol was added to the pellets for suspending cells. Subsequently, the cell suspension was subjected to sonication in an ultrasonicator at 500 W for 12 minutes with a pulse of 15 seconds on and 10 seconds off. After cell disruption, the suspension was centrifuged at 8000 × g, 4°C for 10 min, and then the supernatant was sampled for CoQ₁₀ analysis, while the precipitation was sampled and kept in a 45°C oven to a constant weight before PHB extraction.

For PHB extraction, a certain amount of chloroform was added to the dry disruption cell for 1 h extraction at 60°C. Then, the PHB extract was separated by vacuum filtration and air dried as the crude PHB, which was further purified by adding acetone-methanol mixed liquor (volume ratio 7:2) and washing for twice to remove the pigment. The purified PHB was obtained after drying at 45°C.

Assays methods

Methanol was analyzed by gas chromatography (GC; Shimadzu-2010, Japan) equipped with flame ionization detector (FID) and elastic quartz capillary column (AT-FFAP). Chromatographic condition: injection temperature 200°C, detector temperature 250°C, temperature programming: keeping at 70°C for 4 min, then heating to 150°C at the speed of 50°C per min and keeping for 1 min. The carried gas is nitrogen and column flow was 3.0 mL/min, split ratio 10/L and a sampling quantity of 1 µL.

Cell biomass was measured by analyzing the optical density at 600 nm. Firstly 1 mL culture samples were centrifuged at 8000 × g for 10 min at 4°C; the cells were washed twice in distilled water, centrifuged at the same condition, and finally were diluted by adding distilled water to the linear concentration range according to the standard curve describing the fitting relation between dry cell weigh (DCW) and absorbance at 600 nm (OD_{600}), OD_{600} was tested and DCW would be calculated according to a standard

curve of the relationship between optical density of cells and DCW of XJLW. Each sample was in triplicate.

PHB content analysis was according to Pal A's method (Pal et al. 2009). Firstly 10 mg PHB sample was turned into crotonic acid by treatment with 10 mL concentrated H_2SO_4 in the boiling water bath for 30 min, then the tube was naturally cooled to room temperature, and the absorbance was tested under 235 nm by the UV-1800 spectrophotometer (Shimadzu, Japan) with concentrated H_2SO_4 as the blank. The standard curve was drawn by the same method. The chemical structure of PHB was identified by gas chromatography-mass spectrometric (GC-MS), nuclear magnetic resonance (NMR) spectroscopy and Fourier transform infrared (FT-IR) spectroscopy, respectively.

To find the polymer composition, the purified PHB was dissolved in chloroform (5 mg PHB mL^{-1}), one microliter of which was injected into a GC-MS instrument (Agilent Technologies 7890A GC System, America; Bruker esquire 6000 MS instrument, German). The column and temperature profile used for GC analysis were as follows: capillary column (HP5MS), $30\text{m} \times 0.25\text{mm}$, film thickness $0.25\mu\text{m}$; injection temperature 250°C , ion source temperature 200°C , and transfer line temperature 275°C ; oven temperature programming: initially at 60°C , then heating to 250°C at the speed of 20°C per min and keeping for 15.5 min. The carried gas is helium and column flow was 40 cm/s .

Proton (^1H) and carbon (^{13}C) NMR spectra were recorded by using a Anance III spectrometer (Bruker, Switzerland) at 400 MHz and 100 MHz, respectively, at the following experimental conditions: 0.5% (w/v) polymer sample was dissolved in spectrochem grade deuteriochloroform (CDCl_3) and tetramethylsilane (TMS) was used as an internal reference. The chemical shift scale was in parts per million (ppm).

For FT-IR analysis, 2 mg polymer sample was thoroughly mixed with 100 mg spectroscopic grade KBr with the help of mortar and pestle. From this mixture 15 mg was used for making KBr pellets. The pellets were kept in an oven at 100°C for 4h to remove atmospheric moisture from the sample. The IR spectrum of the polymer sample was recorded with a Nicolet 6700 FT-IR spectrophotometer (Thermo, America) in the range $4000\text{--}600 \text{ cm}^{-1}$.

CoQ_{10} concentration was analyzed by high-performance liquid chromatography (HPLC; Agilent 1200, America) equipped with Agilent SB-C18 ($5 \mu\text{m}$, $4.6 \times 150 \text{ mm}$) (Park et al. 2005). The conditions for HPLC analysis were: temperature, 40°C ; mobile phase, 100% alcohol (HPLC grade); flow rate, 1.0 mL/min ; injection volume, $20 \mu\text{L}$; and detector, UV detector at 275 nm. A standard curve was created by serial dilutions of CoQ_{10} standard. The molecular structure of CoQ_{10} from strain XJLW was identified by liquid chromatography-mass spectrometric (LC-MS) method using Esquire 6000 (Bruker Daltonics, Germany).

Statistical analyses

The mean and standard deviation were calculated from at least three independent experiments in triplicate.

Results

Strain XJLW can produce PHB and CoQ₁₀ simultaneously

Transmission electron microscope observation results (Fig. 2) showed that there were many white particles with high refraction inside strain XJLW cells, occupying nearly half or more space. It suggested high content of PHAs inside the XJLW cells.

After isolation and purification, the exact structure of PHAs from XJLW was identified via GC-MS, NMR and IR analysis methods, respectively. Fig. S1A shows the GC spectra of PHAs extracts of XJLW strain and the 7.59 min peak is corresponded to the hydrolyzed product of PHB according to standards. In order to obtain an exact structure of this polyester, a further MS analysis of the 7.59 min peak fragment was carried out and the spectra were shown in Fig. S1(B). The 101.0 *m/z* molecular fragment was identical to the 3-hydroxybutyrate, while the molecular fragments of 85.0 *m/z* represented butyrate. The ¹H- and ¹³C-NMR spectra of PHB standards and PHAs produced by strain XJLW are shown in Fig. S2. The ¹H-NMR spectra show the presence of three signals in both spectra of the two polymer samples, which are corresponded to the methyl group (CH₃ at 1.28 ppm), methylene group (CH₂ at 2.61 ppm) and methine group (CH at 5.26 ppm), respectively (Fig. S2A). The methyl group (CH₃), methylene group (CH₂), methyne group (CH), and carbonyl group (C=O) are found at 19.8, 40.8, 67.6 and 169.2 ppm respectively (Fig. S2B). The chemical shifts of both ¹H- and ¹³C-NMR of PHAs from XJLW are in good agreement with the data of PHB standards. IR spectra of PHB standards and PHAs from XJLW are shown in Fig. S3. It shows mainly two intense absorption bands at about 1280-1291 cm⁻¹, 1725 cm⁻¹ and 2925-2978 cm⁻¹ corresponding to C-O, C=O and C-H stretching groups respectively. The 3436.8 cm⁻¹ absorption band indicates a small number of O-H existing in PHAs from XJLW and PHB standards referring to the terminal hydroxyl. Meanwhile, the great similarity of IR spectra characteristic indicates chemical group composition in PHAs from XJLW is the same to that of PHB standards. All the above evidences demonstrate PHB should be produced by XJLW strain.

LC-MS results of CoQ₁₀ standard and the sample extracted from strain XJLW cells were shown in Fig.S4. It was found that the peak of CoQ₁₀ in sample appeared at the retention time same to that of CoQ₁₀ standard. Although the target peak area of sample looked lower than that of other unidentified peaks, the mass-to-charge ratio of CoQ₁₀ sample extracted from XJLW strain exhibited a molecular peak (*m/s*, 885.6) same to that of CoQ₁₀ standard. The result suggested that the strain XJLW has the ability of CoQ₁₀ biosynthesis. However, further purification of the sample CoQ₁₀ and enhanced production of CoQ₁₀ in the strain XJLW are required in future research.

Higher biomass, PHB and CoQ10 yield in M3 with methanol than with glucose

As shown in Fig.3, XJLW exhibited much higher biomass and yield of both PHB and CoQ₁₀ when incubated in M3 medium supplemented with methanol than glucose as sole carbon source, respectively. It is interesting that the expression level of some genes coding the key enzymes in the pathway of PHB and CoQ₁₀ biosynthesis of strain XJLW in methanol medium was also significantly higher than that in glucose medium (Fig. 4). The expression level of much more genes was also compared based on the RNA-seq results (Table 2, 3). Besides, the data of quantitative RT-qPCR of selected genes involved in PHB synthesis pathway indicated that PHB may be synthesized by different pathways or be regulated by different isoenzymes under different substrates or different cultivating condition. In the RT-qPCR analysis, we chose *phaC-3* encoding poly(R)-hydroxyalkanoic acid synthase (class III) for analysis, results showed that *phaC-3* was significantly up regulated by methanol, which was identified with RNA-seq results. However, *phaC-1* catalyzing the same step in the pathway was down regulated by methanol, indicating different isoenzymes were regulated by different factors. Meanwhile, totally 5 *acat* genes, 3 *paaH* genes, 2 *fadN* genes and 2 *phaZ* genes were found in PHB synthesis pathway in XJLW showing different responses to methanol (Table 3), which indicated that there was a more complex regulation system in XJLW responsible for PHB production. From genomic data mining, we also found no gene encoding hydroxybutyrate-dimer hydrolase (EC: 3.1.1.22) and hydroxymethylglutaryl-CoA synthase (EC:2.3.3.10) existing in XJLW strain, suggesting PHB were mainly synthesized through FadJ catalyzed branch pathway. Besides, in CoQ₁₀ synthetic pathway of XJLW, we also found no gene encoding decaprenyl-diphosphate synthase (EC: 2.5.1.91) existed in the genomic data, but the LC-MS had strictly verified the product of CoQ₁₀ from this strain. So, it is very possible that there is another new branch pathway or unannotated gene responsible for decaprenyl-diphosphate, an important precursor of CoQ₁₀, biosynthesis in XJLW.

Effects of medium composition and cultivation conditions on cell growth, PHB and CoQ₁₀ productivity in flask level

Both medium M3 and MSM are recommended as suitable medium for *Methylotrophs* strain cultivating (Bourque et al. 1995) with methanol as sole carbon and energy source. Thus, the growth behaviors of XJLW in M3 and MSM were evaluated in flasks. The results (Fig. 6A) showed that M3 medium exhibited more superiority for cell growth than MSM and 5 d was the best harvest time with maximum dry cell density. Meanwhile, the ability of PHB and CoQ₁₀ production by XJLW in M3 and MSM was also evaluated respectively. The results (Fig. 6B) also showed that XJLW exhibited better PHB and CoQ₁₀ biosynthesis capacity in medium M3 than in MSM. M3 was then selected as initial medium for the optimization of XJLW fermentation in the following experiments.

As medium components, carbon source and nitrogen source play the significant role in the fermentation productivity according to previous report (Wei et al. 2012; Mozumder et al. 2014). Thus, the effect of carbon and nitrogen sources is also very necessary to be evaluated for the optimization of XJLW fermentation process. In the previous publications, methanol and ammonium sulfate had been approved

to be the suitable carbon and nitrogen source for *Methylobacterium* (Bourque et al. 1995; Yezza et al. 2006). Therefore, the effect of different concentrations of methanol (Fig. 7A) and ammonium sulfate (Fig. 7B) on PHB and CoQ₁₀ productivity of strain XJLW was evaluated respectively in the present study. It was found that 1% (v/v) methanol led to maximal CoQ₁₀ concentration of 1.26 mg L⁻¹ while the optimal biomass and PHB concentration was obtained under 1.5% (v/v) methanol. The phenomenon may result from the different biosynthesis pathway of CoQ₁₀ and PHB. In order to avoid cell intoxication caused by high methanol concentration, 1% (v/v) methanol was selected as the optimal carbon source concentration in further research. It was also found that 0.5 g L⁻¹ of ammonium sulfate resulted in the highest yield of biomass, PHB and CoQ₁₀. Therefore, 0.5 g L⁻¹ of ammonium sulfate was selected as the optimal nitrogen source concentrate in the following experiments.

Besides medium components, cultural conditions such as culture temperature and initial pH also play important role in microbial fermentation. Thus, the effect of culture temperature and initial pH on XJLW fermentation was then evaluated in flask. The results (Fig.7C and Fig.7D) showed that the best cultural temperature is 30 °C, and the optimal initial pH is 7.0. As fermentation broth may turn to lower pH caused by carbon metabolism of XJLW, feeding ammonium hydroxide to neutralize the excess formic acid derived from methanol metabolism is very important. Thus, the optimal initial pH and cultural temperature were selected as 7.0 and 30 °C respectively.

Due to the poor solubility of oxygen in aqueous medium, the dissolved oxygen (DO) supply is another key factor affecting the productivity in aerobic fermentation process, and one of the most effective strategies for improving oxygen mass transfer efficiency is adding oxygen carrier to the aerobic fermentation system (Lai et al. 2002; Xia 2013; Vieira et al. 2015). In this study, three different oxygen carriers were chosen to enhancing the oxygen supply, including two different surfactants (Triton X-100 and Tween 80) and hydrogen dioxide. Compared with the control group, 0.1% (v/v) of different oxygen carrier was added to the XJLW strain fermentation system respectively. The results (Fig.7E) showed that Tween 80 exhibits positive effects especially in the level of CoQ₁₀ and PHB biosynthesis, meanwhile the productivities of Triton X-100 group and hydrogen dioxide group were both lower than the control group. Perhaps excessive emulsification of Triton X-100 and denaturation of membrane protein caused by hydrogen dioxide can both inhibit normal metabolism of XJLW strain. Tween-80, a non-ionic surfactant, could improve the cell membrane permeability and the specific surface area of oxygen at appropriate concentration, so it may also exhibit positive promotion for intracellular metabolites biosynthesis. According to these data, 0.1% (v/v) of Tween 80 was chosen as the best oxygen carrier in the following research.

As an important environmental factor, osmotic pressure may affect the mass transfer and the accumulation level of metabolites in many microorganisms (Xu et al. 2013; Mozumder et al. 2015), so the effects of osmotic pressure on XJLW metabolism were discussed through adding different concentration of sodium chloride. The results (Fig.7F) showed that the group adding 1.0 g L⁻¹ of sodium chloride

exhibited the highest cell yield and target products concentration, so this regulation strategy was chosen in the subsequent research.

Based on the above, the optimal medium and cultural conditions for CoQ₁₀ and PHB coproduction through XJLW strain fermentation were M3 medium containing 1% (v/v) of methanol, 0.5 g L⁻¹ of ammonium sulfate, 0.1% (v/v) of Tween 80, and 1.0 g L⁻¹ of sodium chloride under the fermentation temperature and initial medium pH of 30°C and 7.0, respectively.

XJLW fermentation in a 5 L fermenter

The cultural medium components and fermentation conditions in shake flask fermentation experiments are easy to control and regulate without complicated operation. However, there are also many great difficulties in dealing with the problems occurring in shake flask level. These problems include but not limited to higher resistance in mass transfer and heat transfer, lower oxygen transfer efficiency, unpractical on line regulation and feeding strategies. Thus, it is very necessary to explore the metabolic behavior of strain XJLW in a 5 L stirred tank reactor. Base on the above results, a methanol feeding strategy coupled with pH and dissolved oxygen (DO) controlling was employed in a 5 L stirred tank reactor for a high-density fermentation. During the whole cultivation period, DO, stir speed, and pH were captured by on line monitors, and the acquisition curves were shown in Fig. 8A. Meanwhile, the changes of methanol concentration, biomass, PHB and CoQ₁₀ productivity during the whole process were shown in Fig. 8B. During the first 36 h, the consumption of methanol added before fermentation was speeded up gradually until DO rebounding to 100%, meaning that there was no methanol enough for cell growth in the medium. From then on, methanol was fed at a pulsed pace to ensure sufficient carbon source in the fermentation system without toxicity caused by excessive methanol. With cell density increasing, the limited dissolved oxygen became another key factor affecting cell growth. Thus, stir speed also gradually increased to ensure the DO level between 10% and 50%. During the whole fed-batch process, pH of broth was controlled at 5.7 approximately rather than 7.0, for excessive ammonium hydroxide used for adjusting pH may inhibit PHB accumulation according to previous report (Pieja et al. 2012). After 106 h when methanol accumulation occurred, methanol feeding ceased, and DO quickly rose up to 100%, indicating the respiration intensity of XJLW cells weakened sharply with little methanol consumption in the final period. Thus, it was time for the harvest.

It was also found that low content of PHB and CoQ₁₀ were detected during the first 36 h, suggesting initial adding methanol was almost completely exhausted for cell respiration and growth. Later, along with feeding substrates, concentration of biomass, PHB and CoQ₁₀ increased in the same trend, implying both of PHB and CoQ₁₀ were biosynthesized associated with cell growth. During the whole process, the total exhausted methanol volume is 830 mL, coupled with feeding 113.05 mL ammonium hydroxide. Finally we got a maximum DCW value (46.31 g L⁻¹), and the highest yields of PHB and CoQ₁₀ reached 6.94 g L⁻¹ and 22.28 mg L⁻¹, respectively. Thus, the final productivities of PHB and CoQ₁₀ in this fed-batch

fermentation system reached 0.14 g g^{-1} of DCW and 0.48 mg g^{-1} of DCW, respectively. These results suggest that the feeding methanol coupled with DO controlled through adding ammonium hydroxide strategy should be an effective method to increase the cell density and productivities in XJLW submerged fermentation system.

Discussion

As carbon source storage in microbial cells, PHAs are usually synthesized and accumulated under imbalanced growth conditions by limiting a nutritional element, such as nitrogen, phosphate, or oxygen (Mozumder et al. 2014). PHAs could accumulate inside a membrane enclosed inclusion in many bacteria at a high content up to 80% of the dry cell weight (Khosravi-Darani et al. 2013). Thus, if a strain has the potential for PHAs production, there will be many polymer particles inside the cell suggesting PHAs existence. In this study, the cell morphology of *Methylobacterium* sp. XJLW under a transmission electron microscope (TEM) also showed a high content of polymer particles (Fig.1), which is similar to most PHAs producing strains.

For Methylophils cultivating with methanol as sole carbon and energy source, both medium M3 and MSM are recommended as suitable medium (Bourque et al. 1995). However, M3 medium exhibited superiority for strain XJLW cell growth than MSM. As medium components, carbon source and nitrogen source usually play the significant role in the fermentation productivity according to previous report (Wei et al. 2012; Mozumder et al. 2014). For *Methylobacterium* strains, methanol and ammonium sulfate had been approved to be the suitable carbon and nitrogen source (Bourque et al. 1995; Yezza et al. 2006). In the present study, a methanol utilized strain *Methylobacterium* sp. XJLW, which was isolated as formaldehyde degrading strain in our previous study (Qiu et al. 2014), also grows better in the M3 than in BSM containing methanol as sole carbon source (Fig. 5).

In order to develop its potential applications in biotechnological industry, PHB and CoQ₁₀ were selected as representatives of biopolymers and quinone metabolites, respectively, to evaluate the potential for their co-production via methanol-based culture process of *Methylobacterium* sp. XJLW. An increasing number of PHB producing strains have been reported, including *Methylobacterium extorquens* (Ueda et al. 1992; Bourque et al. 1995), *Paracoccus denitrificans* (Ueda et al. 1992; Kalaiyehzini et al 2015), *Alcaligenes latus* (Yamane et al. 1996), *Methylobacterium* sp. ZP24 (Nath et al. 2008), *Bacillus thuringiensis* (Pal et al. 2009), *Cupriavidus necator* (Mozumder et al. 2015), *Halomonas campaniensis* (Chen Let al. 2019), *Bacillus drentensis* (Gamez-Perez et al. 2020). After process and culture conditions optimization, the yield of PHB has reached a high level more than 100 g L^{-1} PHB from methanol via high-cell-density fed-batch culture of methylotrophic bacteria (Ueda et al. 1992; Yamane et al. 1996). Based on the above, methylotrophic bacteria seem the potential industrial strains for PHB production via methanol-based biotechnology.

Meanwhile, CoQ₁₀ is another important compound which can be widely used as potent antioxidative dietary supplement in treating cardiovascular disease, cancer, periodontal disease, and hypertension

acting (Hofer et al. 2010; Lu et al. 2013). There are also a number of strains capable of producing CoQ₁₀. However, no publication was found about CoQ₁₀ synthesis in methylotrophic bacteria. In this study, it was found that both metabolic pathways of PHB and CoQ₁₀ biosynthesis exist in XJLW strain based on the genomic and comparative transcriptomics information (Fig.1). RT-qPCR results also showed the transcription level of key genes in both pathways response to methanol was significantly higher than that response to glucose (Fig.4). Correspondingly, strain XJLW can produce PHB and CoQ₁₀ simultaneously with higher yield using methanol than using glucose as sole carbon and energy source (Fig.3). To our knowledge, it is the first report on PHB and CoQ₁₀ production simultaneously by methylotrophic bacteria.

After optimization of medium composition and the culture conditions on PHB and CoQ₁₀ biosynthesis, a cell density of DCW 46.31 g L⁻¹ with a PHB concentration of 6.94 g L⁻¹, and a CoQ₁₀ concentration of 22.28 mg L⁻¹ were achieved in a 5 L bioreactor, which were 30 fold, 6 fold, and 17 fold higher than that in flasks, respectively. Although the productivity of CoQ₁₀ was 0.48 mg g⁻¹ of DCW, which was lower than that of previous reported strains such as *Rhodobacter sphaeroides* (2.01 mg g⁻¹ of DCW) (Kalaiyezhini 2015), the volumetric yield of 22.3 mg L⁻¹ of XJLM was higher than that of several previous reported strains including the mutant strain of *Rhodobacter sphaeroides* (14.12 mg L⁻¹) (Bule 2011), *Paracoccus dinitrificans* NRRL B-3785 (10.81 mg L⁻¹) (Tian et al. 2010), and *Sphingomonas* sp. ZUTE03 (1.14 mg L⁻¹) (Zhong et al. 2009). Meanwhile, XJLM could accumulate PHB at the productivity level of 0.14 g g⁻¹ of DCW. The PHB yield of XJLM was lower than several reported strains such as *Methylobacterium extorquens* DSMZ 1340 (0.62 g g⁻¹ of DCW) (Mokhtari-Hosseini et al. 2009) and *Methylobacterium extorquens* ATCC 55366 (0.46 g g⁻¹ of DCW) (Bourque et al. 1995), but the volumetric yield of PHB of XJLM in this study (6.94 g L⁻¹) was higher than that of *Methylobacterium* sp. ZP24 (3.91 g L⁻¹) (Nath et al. 2008).

Conclusions

In summary, it is feasible to develop a co-production process of two valuable metabolites by *Methylobacterium* sp. XJLW from methanol. However, it is still necessary to further optimize fermentation process, and genetically modify strain pathway, for enhanced production of PHB and CoQ₁₀ simultaneously by XJLW. This study also presented a potential strategy to develop efficiently co-producing other high value metabolites using methanol-based bio-process.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The XJLW genome is available in GenBank (accession no. CP016429), while its transcriptomics data is available in this article (Supplemental Material-Table of Samples FPKM).

Competing interests

The authors declare that they have no competing interests.

Funding

This work was financially supported by National Natural Science Foundation of China (81973211), Innovation Platform and Talent Plan of Hunan Province (2015JC3073), Scientific Research Project of Hunan Education Department (18B249).

Author's contributions

WZ Conceived of the study, PC, YS, YW, RZ, and HZ designed and performed the experiments, YS and WZ supervised and implemented the statistical analysis, PC and WZ wrote the manuscript. All authors read and approved the final manuscript.

Consent for publication

Not applicable.

Abbreviations

CGMCC: China General Microbiological Culture Collection Center;

CoQ10: coenzyme Q10;

DCW: Dry cell weight;

DO: dissolved oxygen;

FT-IR: Fourier transformation infrared spectrum;

GC-MS: Gas chromatography/mass spectrometry;

HPLC: High performance liquid chromatography;

LC-MS: Liquid chromatography/mass spectrometry;

PCR: Polymerase chain reaction;

NMR: nuclear magnetic resonance;

PHAs: polyhydroxy- alkanates;

PHB: poly- β -hydroxybutyrate.

References

Bourque D, Pomerleau Y, Groleau D (1995) High cell density production of poly-beta-hydroxybutyrate (PHB) from methanol by *Methylobacterium extorquens*: Production of high-molecular-mass PHB. Appl Microbiol Biotechnol **44**(3-4): 367-376.

Bule MV, Singhal RS (2011) Fermentation kinetics of production of ubiquinone-10 by *Paracoccus dinitrificans* NRRL B-3785: Effect of type and concentration of carbon and nitrogen sources. Food Sci Biotechnol **20**(3): 607-613.

Cardoso LOB, Karolski B, Gracioso LH, do Nascimento CAO, Perpetuo EA (2020) Increased P3HB Accumulation Capacity of *Methylobacterium* sp. in Response to Discontinuous Methanol Addition. Appl Biochem Biotechnol **192**(3): 846-860.

- Gamez-Perez J, Penkhrue W, Jendrossek D, Khanongnuch C, Pathom-aree W, Aizawa T, Behrens RL, Lumyong S. (2020) Response surface method for polyhydroxybutyrate (PHB) bioplastic accumulation in *Bacillus drentensis* BP17 using pineapple peel. Plos One **15**(3): e0230443.
- Hofer P, Choi YJ, Osborne MJ, Miguez CB, Vermette P, Groleau D (2010) Production of functionalized polyhydroxyalkanoates by genetically modified *Methylobacterium extorquens* strains. Microb Cell Fact **9**: 70.
- Kalaiyezhini D, Ramachandran KB (2015) Biosynthesis of poly-3-hydroxybutyrate (PHB) from glycerol by *Paracoccus denitrificans* in a batch bioreactor: effect of process variables. Prep Biochem Biotechnol **45**(1): 69-83.
- Khosravi-Darani K, Mokhtari ZB, Amai T, Tanaka K (2013) Microbial production of poly(hydroxybutyrate) from C(1) carbon sources. Appl Microbiol Biotechnol **97**(4): 1407-1424.
- Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL (2013) TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol **14**(4): R36.
- Lai LS, Tsai TH, Wang TC (2002) Application of oxygen vectors to *Aspergillus terreus* cultivation. J Biosci Bioeng **94**(5): 453-459.
- Chen L, Qiao GQ, Shuai BW, Song KN, Yao WX, Jiang XR, Chen GQ (2019) Engineering self-flocculating *Halomonas campaniensis* for wastewaterless open and continuous fermentation. Biotechnol Bioeng **116**(4): 805-815.
- Lu W, Shi Y, He S, Fei Y, Yu K, Yu H (2013) Enhanced production of CoQ₁₀ by constitutive overexpression of 3-demethyl ubiquinone-9 3-methyltransferase under tac promoter in *Rhodobacter sphaeroides*. Biochem Eng J **72**: 42-47.
- Mohandas SP, Balan L, Lekshmi N, Cubelio SS, Philip R, Bright Singh IS (2017) Production and characterization of polyhydroxybutyrate from *Vibrio harveyi* MCCB 284 utilizing glycerol as carbon source. J Appl Microbiol **122**(3): 698-707.
- Mokhtari-Hosseini ZB, Vasheghani-Farahani E, Heidarzadeh-Vazifekhoran A, Shojaosadati SA, Karimzadeh R, Darani KK (2009) Statistical media optimization for growth and PHB production from methanol by a methylotrophic bacterium. Bioresour Technol **100**(8): 2436-2443.
- Mostafa YS, Alrumman, Alamri SA, Otaif KA, Mostafa MS, Alfaify AM (2020) Bioplastic (poly-3-hydroxybutyrate) production by the marine bacterium *Pseudodonghicola xiamenensis* through date syrup valorization and structural assessment of the biopolymer. Sci Rep **10**(1):8815
- Mozumder MSI, De Wever H, Volcke EIP, Garcia-Gonzalez L (2014) A robust fed-batch feeding strategy independent of the carbon source for optimal polyhydroxybutyrate production. Process Biochem **49**(3):

365-373.

Mozumder MSI, Garcia-Gonzalez L, De Wever H, Volcke EIP (2015) Effect of sodium accumulation on heterotrophic growth and polyhydroxybutyrate (PHB) production by *Cupriavidus necator*. *Bioresour Technol* **191**: 213-218.

Nath A, Dixit M, Bandiya A, Chavda S, Desai AJ (2008) Enhanced PHB production and scale up studies using cheese whey in fed batch culture of *Methylobacterium* sp. ZP24. *Bioresour Technol* **99**(13): 5749-5755.

Pal A, Prabhu A, Kumar AA, Rajagopal B, Dadhe K, Ponnammam V, Shivakumar S (2009) Optimization of process parameters for maximum poly(-beta)-hydroxybutyrate (PHB) production by *Bacillus thuringiensis* IAM 12077. *Pol J Microbiol* **58**(2): 149-154.

Park YC, Kim SJ, Choi JH, Lee WH, Park KM, Kawamukai M, Ryu YW, Seo JH (2005) Batch and fed-batch production of coenzyme Q₁₀ in recombinant *Escherichia coli* containing the decaprenyl diphosphate synthase gene from *Gluconobacter suboxydans*. *Appl Microbiol Biotechnol* **67**(2): 192-196.

Parveez GK, Bahariah B, Ayub NH, Masani MY, Rasid OA, Tarmizi AH, Ishak Z (2015) Production of polyhydroxybutyrate in oil palm (*Elaeis guineensis* Jacq.) mediated by microprojectile bombardment of PHB biosynthesis genes into embryogenic calli. *Front Plant Sci* **6**: 598.

Pieja AJ, Sundstrom ER, Criddle CS (2012) Cyclic, alternating methane and nitrogen limitation increases PHB production in a methanotrophic community. *Bioresour Technol* **107**(0): 385-392.

Qiu L, Chen W, Zhong L, Wu W, Wu S, Chen J, Zhang F, Zhong W (2014) Formaldehyde biodegradation by immobilized *Methylobacterium* sp. XJLW cells in a three-phase fluidized bed reactor. *Bioprocess Biosyst Eng* **37**(7): 1377-1384.

Qiu L, Ding H, Wang W, Kong Z, Li X, Shi Y, Zhong W (2012) Coenzyme Q(10) production by immobilized *Sphingomonas* sp. ZUTE03 via a conversion-extraction coupled process in a three-phase fluidized bed reactor. *Enzyme Microbiol Technol* **50**(2): 137-142.

Shao Y, Li J, Wang Y, Yi F, Zhang Y, Cui P, Zhong W (2019a) Comparative genomics and transcriptomics insights into the C1 metabolic model of a formaldehyde-degrading strain *Methylobacterium* sp. XJLW." *Mol Omics* **15**(2): 138-149.

Shao Y, Wang Y, Yi F, Zhang Y, Liu W, Yang C, Meng H, Cui P, Zhong W (2019b) Gaseous Formaldehyde Degrading by *Methylobacterium* sp. XJLW. *Appl Biochem Biotechnol* **15**: 138-149

Sharma N (2019) Polyhydroxybutyrate (PHB) Production by Bacteria and its Application as Biodegradable Plastic in Various Industries. *Academic Journal of Polymer Science* **2**(3): 555587

- Sukruansuwan V, Napathorn SC (2018) Use of agro-industrial residue from the canned pineapple industry for polyhydroxybutyrate production by *Cupriavidus necator* strain A-04. *Biotechnol Biofuels* **11**: 202.
- Tian Y, Yue T, Yuan Y, Soma PK, Lo YM (2010) Improvement of cultivation medium for enhanced production of coenzyme Q₁₀ by photosynthetic *Rhodospirillum rubrum*. *Biochem Eng J* **51**(3): 160-166.
- Ueda S, Mataumoto S, Takagi A, Yamane T (1992) Synthesis of Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) from Methanol and N-amyl alcohol by the Methylophilic Bacteria *Paracoccus denitrificans* and *Methylobacterium extorquens*. *Appl Environ Microbiol* **58**(11): 3574-3579.
- Vieira ES, de Oliveira Fontes TK, Pereira MM, Alexandre HV, da Silva DP, Soares CM, Lima AS (2015) New strategy to apply perfluorodecalin as an oxygen carrier in lipase production: minimisation and reuse. *Bioprocess Biosyst Eng* **38**(4): 721-728.
- Wei ZH, Bai L, Deng Z, Zhong JJ (2012) Impact of nitrogen concentration on validamycin A production and related gene transcription in fermentation of *Streptomyces hygroscopicus* 5008. *Bioprocess Biosyst Eng* **35**(7): 1201-1208.
- Xia Z (2013) Effect of Tween 80 on the production of curdlan by *Alcaligenes faecalis* ATCC 31749. *Carbohydr Poly* **98**(1): 178-180.
- Xu YN, Xia XX, Zhong JJ (2013) Induced effect of Na⁺ on ganoderic acid biosynthesis in static liquid culture of *Ganoderma lucidum* via calcineurin signal transduction. *Biotechnol Bioeng* **110**(7): 1913-1923.
- Yamane T, Fukunaga M, Lee YW (1996) Increased PHB productivity by high-cell- density fed-batch culture of *Alcaligenes latus*, a growth-associated PHB producer, *Biotechnol Bioeng* **50**(2):197-202.
- Yezza A, Fournier D, Halasz A, Hawari J (2006) Production of polyhydroxyalkanoates from methanol by a new methylophilic bacterium *Methylobacterium* sp. GW2. *Appl Microbiol Biotechnol* **73**(1): 211-218.
- Zaldivar Carrillo JA, Stein LY, Sauvageau D (2018) Defining Nutrient Combinations for Optimal Growth and Polyhydroxybutyrate Production by *Methylosinus trichosporium* OB3b Using Response Surface Methodology. *Front Microbiol* **9**:1513.
- Zhang M, Yuan XJ, Zhang C, Zhu LP, Mo XH, Chen WJ, Yang S (2019) Bioconversion of Methanol into Value-added Chemicals in Native and Synthetic Methylophilic. *Curr Issues Mol Biol* **33**: 225-236.
- Zhong W, Fang J, Liu H, Wang X (2009) Enhanced production of CoQ₁₀ by newly isolated *Sphingomonas* sp. ZUTEO3 with a coupled fermentation-extraction process. *J Ind Microbiol Biotechnol* **36**(5): 687-693.

Tables

Table 1 Primers used in this study

Genes	Primers	Sequence
16S rDNA	16S-F	GTTGGTGGAAGTGCAGGTGTAGAG
	16S-R	CCCCAGGCGGAATGCTCAAAG
<i>ubiA</i>	<i>ubiA</i> -F	GCTGGTGGCTCCTCCTCCTG
	<i>ubiA</i> -R	GGCATCGGCATGACCCGTTTC
<i>ubiG</i>	<i>ubiG</i> -F	CTGGACGGGCTTTCGATCTGC
	<i>ubiG</i> -R	CAGCCAGCCGAGCACGTATTC
<i>ubiD</i>	<i>ubiD</i> -F	CGTGACCCTGTGCCCAAAGC
	<i>ubiD</i> -R	AACTGAGCGGTTTCTGCGGATG
<i>ubiH</i>	<i>ubiH</i> -F	TGGTGCTCTCGCTCGCCTATC
	<i>ubiH</i> -R	TGGAAGCTCGGAAACGTGATGATG
<i>ubiX</i>	<i>ubiX</i> -F	AAGAGAGCCGCGAGGGTGAG
	<i>ubiX</i> -R	CCCTGCTCCTGTACCTGATCTGG
<i>hmgI</i>	<i>hmgI</i> -F	CGTCAAGCAGCTCGCCAAGAG
	<i>hmgI</i> -R	GAGGCTCTCCATCACGTTGAACAC
<i>phaC-3</i>	<i>phaC-3</i> -F	ACGCCGAAGGATCTGGTCTGG
	<i>phaC-3</i> -R	TTCGCCGCCTCCTGGATGAC

Table 2 FPKM values of CoQ₁₀ synthesis related genes based on RNA-seq analysis

Locus	Genes	Enzymes	FPKM in Glucose	FPKM in Methanol	Log2 FPKM (M/G)	up or down
A3862_RS14500	<i>dxr</i>	1-deoxy-D-xylulose-5-phosphate reductoisomerase	99.9563	148.212	0.568293	up
A3862_RS20315	<i>ispDF</i>	bifunctional 2-C-methyl-D-erythritol 4-phosphate cytidyltransferase/2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	92.8581	149.282	0.684941	up
A3862_RS03995	<i>ispE</i>	4-(cytidine 5'-diphospho)-2-C-methyl-D- erythritol kinase	64.3157	141.036	1.132821	up
A3862_RS12025	<i>ispG</i>	flavodoxin-dependent (E)-4-hydroxy-3-methylbut-2- enyl-diphosphate synthase	425.885	296.847	-0.52074	down
A3862_RS10000	<i>ispH</i>	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	1071.31	683.363	-0.64865	down
A3862_RS18005	<i>ggps</i>	geranylgeranyl diphosphate synthase, type II	30.1079	127.672	2.084228	up
A3862_RS28415	<i>ispA</i>	polyprenyl synthetase family protein	200.033	143.273	-0.48147	down
A3862_RS04015	<i>ispB</i>	polyprenyl synthetase family protein	95.6316	164.97	0.786644	up
A3862_RS03825	<i>ubiA</i>	4-hydroxybenzoate octaprenyltransferase	73.9099	148.495	1.006575	up
A3862_RS05140	<i>ubiX</i>	UbiX family flavin prenyltransferase	43.2101	92.1585	1.092749	up
A3862_RS05150	<i>ubiD</i>	UbiD family decarboxylase	30.4548	120.907	1.989156	up
A3862_RS18730	<i>ubiI</i>	2-polyprenylphenol 6-hydroxylase	54.8386	146.31	1.415765	up
A3862_RS01610	<i>ubiG</i>	bifunctional 2-polyprenyl-6-hydroxyphenol methylase/3-demethylubiquinol 3-O- methyltransferase UbiG	68.382	118.171	0.789188	up
A3862_RS13590	<i>ubiH</i>	FAD-dependent monooxygenase	65.993	170.113	1.366108	up
A3862_RS18735	<i>ubiE</i>	bifunctional demethylmenaquinone methyltransferase/2-methoxy-6-polyprenyl-1,4- benzoquinol methylase UbiE	99.1489	146.834	0.566517	up
A3862_RS22985	<i>ubiF</i>	UbiH/UbiF family hydroxylase	84.6668	194.111	1.197014	up

Table 3 FPKM values of PHB synthesis related genes based on RNA-seq analysis

Locus	Genes	Enzymes	FPKM in Glucose	FPKM in Methanol	Log2 FPKM (M/G)	up or down
A3862_RS02265	<i>acat-1</i>	acetyl-CoA C-acetyltransferase	181.947	195.674	0.104934	up
A3862_RS05695	<i>acat-2</i>	acetyl-CoA C-acetyltransferase	1101.5	455.904	-1.27267	down
A3862_RS09310	<i>acat-3</i>	beta-ketothiolase BktB	117.494	160.747	0.452205	up
A3862_RS25790	<i>acat-4</i>	acetyl-CoA C-acyltransferase	72.2701	163.337	1.176381	up
A3862_RS27615	<i>acat-5</i>	acetyl-CoA acetyltransferase	302.52	199.266	-0.60233	down
A3862_RS05690	<i>phbB</i>	acetoacetyl-CoA reductase	775.466	472.896	-0.71354	down
A3862_RS05930	<i>phaC-1</i>	class I poly(R)-hydroxyalkanoic acid synthase	266.759	198.599	-0.42568	down
A3862_RS11350	<i>phaC-2</i>	polyhydroxyalkanoic acid synthase	111.896	382.12	1.771867	up
A3862_RS19105	<i>phaC-3</i>	class III poly(R)-hydroxyalkanoic acid synthase subunit PhaC	90.7047	116.952	0.366667	up
A3862_RS19110	<i>phaE</i>	poly-beta-hydroxybutyrate polymerase subunit	133.443	134.851	0.015143	up
A3862_RS05165	<i>paaH-1</i>	3-hydroxybutyryl-CoA dehydrogenase	60.5688	125.351	1.049327	up
A3862_RS17305	<i>paaH-2</i>	3-hydroxybutyryl-CoA dehydrogenase	487.68	218.354	-1.15927	down
A3862_RS21635	<i>paaH-3</i>	3-hydroxyacyl-CoA dehydrogenase family protein	33.7683	105.064	1.637527	up
A3862_RS02250	<i>fadJ</i>	enoyl-CoA hydratase/isomerase family protein	155.264	191.337	0.301393	up
A3862_RS15330	<i>fadN-1</i>	3-hydroxyacyl-CoA dehydrogenase/enoyl-CoA	93.6416	170.494	0.864499	up
A3862_RS25795	<i>fadN-2</i>	enoyl-CoA hydratase/isomerase family protein	40.528	110.806	1.451045	up
A3862_RS06255	<i>scoA</i>	succinyl-CoA--3-ketoacid-CoA transferase/CoA transferase subunit A	737.041	126.763	-2.53961	down
A3862_RS06260	<i>scoB</i>	succinyl-CoA--3-ketoacid-CoA transferase/CoA transferase subunit B	1515.36	206.479	-2.87559	down
A3862_RS14320	<i>bdh</i>	3-hydroxybutyrate dehydrogenase	426.046	159.7	-1.41564	down
A3862_RS09710	<i>phaZ-1</i>	polyhydroxyalkanoate depolymerase	397.233	383.709	-0.04997	down
A3862_RS17340	<i>phaZ-2</i>	polyhydroxyalkanoate depolymerase	150.142	139.807	-0.10289	down
A3862_RS12335	<i>hmgI</i>	hydroxymethylglutaryl-CoA lyase	64.564	145.106	1.168305	up

Figures

Figure 1

Genetic organization of genes and core pathway responsible for CoQ10 (A, C) and PHB (B, D) synthesis in XJLW strain via comparative genomic analysis. The EC No. in yellow-backed textboxes in Fig. 1A and Fig. 1B means that they cannot be found in genomic data of XJLW strain. The green color and (+) symbol labeled genes were up-regulated expressed in methanol group while the red color and (-) symbol labeled

genes were down-regulated expressed in methanol compared to glucose. The black-backed gene in Fig. 1C suggest that the expression level of this gene was not affected by methanol or glucose.

Figure 2

Micrography of the *Methylobacterium* sp. XJLW under transmission electron microscope (15000 ×, HITACHI H-7650 TEM).

Figure 3

Different effect of glucose and methanol (same carbon atom amount) on the strain XJLW cell growth and its yield of CoQ10 and PHB.

Figure 4

Effect of carbon source on the express of key genes in CoQ₁₀ (A) and PHB (B) biosynthesis pathway via RT-qPCR.

Figure 5

Cell growth (A) and PHB/CoQ₁₀ production (B) of XJLW in M3 and MSM respectively.

Figure 6

Effects of methanol concentration (A), ammonium sulfate concentration (B), fermentation temperature (C), initial pH of medium (D), different oxygen carriers (E) and sodium chloride concentration (F) on XJLW biomass, PHB and CoQ10 biosynthesis.

Figure 7

On-line parameters acquisition curve (A) and CoQ10 and PHB fermentation of XJLW via fed-batch process (B) in a 5-l stirred tank reactor. The arrow demarcates the feeding event.

Supplementary Files

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

- [20210217SupplementalMaterialFigures.docx](#)
- [20210217SupplementalMaterialTableofSamplesFPKM.xls](#)