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Co-production of polyhydroxybutyrate (PHB) and coenzyme Q_{10} (CoQ_{10}) via no-sugar fermentation—a case by *Methylobacterium* sp. XJLW

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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 methanol as sole carbon and energy source.

Methods: The metabolic pathways of PHB and CoQ_{10} biosynthesis in *Methylobacterium* sp. XJLW 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 *Methylobacterium* sp. XJLW were evaluated in Erlenmeyer flask 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 analysis showed that the PHB and CoQ_{10} biosynthesis pathways co-exist in *Methylobacterium* sp. XJLW. Transcriptomics analysis showed that the transcription level of key genes in both pathways responding to methanol was significantly higher than that responding to glucose. Correspondingly, strain *Methylobacterium* sp. 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 *Methylobacterium* sp. XJLW was in M3 medium containing 7.918 g L^{-1} 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 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^{-1}) with the highest yields of PHB and CoQ_{10}, reaching 6.94 g L^{-1} and 22.28 mg L^{-1}, respectively.

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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 *Methylobacterium* sp. XJLW. It also suggests a potential strategy to develop efficiently co-producing other high-value metabolites using methanol-based bioprocess.

Keywords: *Methylobacterium* sp. XJLW, Metabolic pathway mining, Methanol-based process, PHB, CoQ_{10}, Fed-batch fermentation

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 attention 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 polymer production. For showing similar thermoplastic, elastomeric, and other physical–chemical properties to conventional plastics, polydroxyalkanoates (PHAs) are regarded as the most potential substituent, which can be completely degraded to CO_2 and H_2O (Sukruansuwan and Napathorn 2018; Mostafa et al. 2020). However, the high cost of PHA 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 PHA-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 methylotroph fermentation processes for value-added chemical production (Zaldivar Carrillo et al. 2018; Zhang et al. 2019). Hence, methanol-based fermentation for PHA production is still a highly promising process without sugar consumption.

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 Q_{10} (CoQ_{10}) 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 (Ernster and Dallner 1995; Qiu et al. 2012; Lu et al. 2013). Thus, PHB and CoQ_{10} 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. 2019a). Its completed genome has been sequenced (Shao et al. 2019b). Comparative genomic analysis exhibited *Methylobacterium* sp. XJLW contains both pathways of CoQ_{10} and PHB biosynthesis (Fig. 1), suggesting the possibility to develop a new fermentation process to realize co-production of PHB and CoQ_{10} 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 study, the aim was to (1) verify the potential of PHB and CoQ_{10} co-production by *Methylobacterium* sp. XJLW with different carbon sources, glucose, and methanol; (2) elucidate the expression difference of the key genes in both pathways of PHB and CoQ_{10} biosynthesis in *Methylobacterium* sp. XJLW response to methanol and glucose; (3) evaluate the effects of medium composition and cultivation conditions on PHB and CoQ_{10} co-production in Erlenmeyer flasks and in a 5-L stirred bioreactor employing methanol fed-batch strategy. This study provided a new reference of strategy for improving value-added product productivity with methanol-based fermentation process employing methylotrophs.

Materials and methods

Chemicals

PHB (purity above 95%, CAS no: 26063-00-3) and CoQ_{10} (purity above 99.9%, CAS no: 303-98-0) were purchased from Sigma-Aldrich, China. Alcohol (HPLC grade, purity above 99.5%) was purchased from Tjshield fine chemicals Co., Ltd. (Tianjin, China). Other

Fig. 1 (See legend on next page.)

(See figure on previous page.)

Fig. 1 Genetic organization of genes and core pathway responsible for CoQ_{10} (**a**, **c**) and PHB (**b**, **d**) synthesis in XJLW strain via comparative genomic analysis. The EC No. in yellow-backed textboxes in **a** and **b** meant that they cannot be found in genomic data of XJLW strain. The green color and (+) symbol-labelled genes were upregulated expressed in the methanol group, while the red color and (-) symbol-labelled genes were downregulated expressed in methanol compared with glucose. The black-backed gene in **c** suggests that the expression level of this gene was not affected by methanol or glucose

chemicals were analytical reagents and purchased from a local company.

Microorganism and maintenance

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.

After the broth OD_{600} of strain XJLW cultured in liquid M3 mineral medium containing 1.0% methanol reaches about 0.6, about 750 μL broth was mixed with 250 μL 80% sterile glycerol in a 1.5-mL centrifuge tube, and then stored in − 80 °C refrigerator. When activation is required, the stored strains are taken out and thawed, inoculated into an M3 liquid medium containing methanol, and activated on a shaker at 30 °C and 180 rpm.

Culture condition

Medium M3 (Bourque et al. 1995) contained (g L^{-1}) $(NH_4)_2SO_4$ 0.5, KH_2PO_4 1.305, $Na_2HPO_4{\cdot}7H_2O$ 4.02, $MgSO_4{\cdot}7H_2O$ 0.45, $CaCl_2$ $2H_2O$ 0.02, $FeSO_4{\cdot}$ H_2O 0.02, and 1 mL L^{-1} trace element solution. The trace element solution contained (g L^{-1}) $MnSO_4{\cdot}H_2O$ 4.9, $ZnSO_4{\cdot}7H_2O$ 2.6, $CuSO_4{\cdot}5H_2O$ 0.8, $Na_2MoO_4{\cdot}2H_2O$ 0.8, $CoCl_2{\cdot}6H_2O$ 0.8, and H_3BO_3 0.6.

Mineral salt medium (MSM) (Qiu et al. 2014) contained (g L^{-1}) KH_2PO_4 0.7, K_2HPO_4 0.85, $(NH_4)_2SO_4$ 1.2, $MgSO_4{\cdot}7H_2O$ 0.1, $CaCl_2$ 0.01, $FeSO_4{\cdot}$ $7H_2O$ 0.001, and 1 mL L^{-1} trace element solution. The trace element solution contained (g L^{-1}) H_3BO_3 6, $CoCl_2{\cdot}6H_2O$ 4, $ZnSO_4{\cdot}7H_2O$ 2, $MnCl_2$ $4H_2O$ 0.6, $Na_2MoO_4{\cdot}2H_2O$ 0.6, $NiCl_2{\cdot}4H_2O$ 0.4, and $CuCl_2{\cdot}2H_2O$ 0.2.

Initial pH of the above media was adjusted to 7.0 with 1 mol L^{-1} NaOH. Methanol, 7.918 g L^{-1}, was added to the two media used as sole carbon source after being autoclaved at 115 °C for 30 min. Fifty microliters suspension of frozen stock *Methylobacterium* sp. XJLW was inoculated into a 250-mL Erlenmeyer flask containing 50 mL medium M3, and incubated for 96 h. Then 2-mL culture was inoculated into 250-mL Erlenmeyer flasks containing 50 mL fermentation medium and incubated for 5 days in a rotary incubator (SPH-2102, SHIPING, China) with the parameter settings at 30 °C and 400 rpm, respectively.

Cell morphology observation via transmission electron microscope

Cells in 1 mL culture broth was harvested by centrifugation at 5790 × g for 10 min at 4 °C in a high-speed freezing centrifuge (TGL-16M, Bioridge, China), and then were suspended 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) at the magnification of 15,000 ×.

Physiological characteristic analysis combined with RNA-seq and RT-qPCR

The cell growth and simultaneous production ability of PHB and CoQ_{10} was detected in M3 medium supplemented with 7.4232 g L^{-1} glucose or 7.918 g L^{-1} methanol, respectively. Meanwhile, the cells were harvested for RNA-seq and RT-qPCR.

RNA-Seq data analysis

After culture in M3 containing methanol or glucose as carbon source, respectively, at 30 °C to log phase (OD_{600} 0.8), *Methylobacterium* sp. XJLW cells were harvested via centrifugation at 2000 × g for 10 min at 4 °C in a high-speed freezing centrifuge (TGL-16M, Bioridge, China). Then, cell 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 (Thermo Fisher Scientific, USA) to preliminary quantify. Insert size was assessed using the Bioanalyzer 2100 system (Agilent, USA), after the insert size is consistent with expectations, qualified fragment was 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 base ≤ 10 and occupied less than 50% of the entire read) for subsequent analysis. Firstly, Tophat2 (Kim et al. 2013) was used to evaluate the sequencing data by comparison with the genomic sequences of reference strains. Based on the Tophat2 alignment results, Cufflinks-2.2.1 (Trapnell et al. 2010) was used to perform quantitative gene expression analysis. Gene expression is calculated as

follows: FPKM (expected number of Fragments Per Kilobase of transcript sequence per Million of sequenced base pairs). In general, the screening criteria for significantly differentially expressed genes are: $|\log_2\text{fold change}| \geq 1$ and p value ≤ 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 7.4232 g L^{-1} glucose or 7.918 g L^{-1} methanol respectively, were centrifuged at 2000 × *g* for 10 min at 4 °C in a high-speed freezing centrifuge (TGL-16M, Bioridge, China). 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 the quantitative PCR with Bio-Rad CFX real-time PCR system was performed. The expression level of the 16S rRNA gene was used as internal reference. Each reaction was repeated at least three times. The primers used for RT-qPCR are listed in Table 1.

Effect of culture conditions on *Methylobacterium* sp. XJLW fermentation in Erlenmeyer flask

Firstly, to choose a better initial medium, the cell growth and biosynthesis of target products of *Methylobacterium* sp. XJLW 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 *Methylobacterium* sp. XJLW growing and target metabolites biosynthesis. The value ranges of the above mentioned culture condition variables are listed in Table 2**.**

Table 1 Primers used in this study

Genes	Primers	Sequence
16S rDNA	16S-F	GTTGGTGGAACTGCGAGTGTAGAG
	16S-R	CCCCAGGCGGAATGCTCAAAG
ubiA	*ubiA*-F	GCTGGTGGCTCCTCCTCCTG
	ubiA-R	GGCATCGGCATGACCCGTTTC
ubiG	*ubiG*-F	CTGGACGGGCTTTCGATCTGC
	*ubiG*R	CAGCCAGCCGAGCACGTATTC
ubiD	*ubiD* -F	CGTGACCCTGTGCCCAAAGC
	ubiD -R	AACTGAGCGGTTTCTGCGGATG
ubiH	*ubiH*-F	TGGTGCTCTCGCTCGCCTATC
	ubiH-R	TGGAAGCTCGGAAACGTGATGATG
ubiX	*ubiX* -F	AAGAGAGCCGCGAGGGTGAG
	ubiX-R	CCCTGCTCCTGTACCTGATCTGG
hmgl	*hmgl*-F	CGTCAAGCAGCTCGCCAAGAG
	hmgl-R	GAGGCTCTCCATCACGTTGAACAC
phaC-3	*phaC-3*-F	ACGCCGAAGGATCTGGTCTGG
	phaC-3-R	TTCGCCGCCTCCTGGATGAC

Cultivation of *Methylobacterium* sp. XJLW on bench bioreactor using fed-batch strategy

After investigation of fermentation conditions in Erlenmeyer 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 ddH_2O and were autoclaved in the bioreactor. To start the fermentation, 30 mL methanol and 300 mL inoculum suspension (OD_{600} = 3.0) 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 element 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 CoQ_{10} 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 (Scientz-IID, China) at 500 W for 12 min with a pulse of 15 s on and 10 s off. After cell disruption, the suspension was centrifuged at 8000 × *g*, 4 °C for 10 min, and then the supernatant was sampled for CoQ_{10}

Table 2 Ranges of the culture condition variables

Variables	Ranges of values
Methanol concentration (%, v/v)	0.5, 1.0, 1.5, 2.0, 2.5
Ammonium sulfate (%, w/v)	0.5, 1.0, 1.5, 2.0, 2.5
Temperature (°C)	25, 30, 37
Initial pH	5, 6, 7, 8, 9
Oxygen carriers	Triton X-100, Tween 80, H_2O_2
Sodium chloride (g/L)	1.0, 5.0, 10.0

analysis, while the precipitation was sampled and kept in a 45 °C oven to a constant weight before PHB extraction.

For PHB extraction, 10 mL chloroform was added to a digestion tube with threaded cap containing less than 100 mg of 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 twice to remove the pigment. The purified PHB was obtained after drying at 45 °C.

Assay 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 was nitrogen, and column flow was 3.0 mL/min, split ratio of 10/L, and a sampling quantity of 1 μL.

Cell biomass was measured by analyzing the optical density at 600 nm using UV1800 spectrophotometer (Shimadzu, Japan). Firstly, 1 mL culture samples were centrifuged at 6000 × *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 weight (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 *Methylobacterium* sp. 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 spectrometry (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}), 1 μL 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 m × 0.25 mm, film thickness 0.25 μm; 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 was helium and column flow was 40 cm/s.

Proton (^1H) and carbon (^{13}C) NMR spectra were recorded by using an 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 deuterochloroform ($CDCl_3$) 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 4 h 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 4000–600 cm^{-1}.

CoQ_{10} concentration was analyzed by high-performance liquid chromatography (HPLC; Agilent 1200, America) equipped with Agilent SB-C18 (5 μm, 4.6 × 150 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 μL; 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 *Methylobacterium* sp. XJLW was identified by liquid chromatography-mass spectrometry (LC-MS) method using Esquire 6000 (Bruker Daltonics, Germany).

Statistical analyses

The mean and standard deviation were calculated from samples in triplicate using Microsoft Excel 2013.

Results

Methylobacterium sp. XJLW can produce PHB and CoQ_{10} simultaneously

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

After isolation and purification, the exact structure of PHAs from *Methylobacterium* sp. XJLW was identified via GC-MS, NMR, and IR analysis methods, respectively. Fig. S1A shows the GC spectra of PHA extracts of

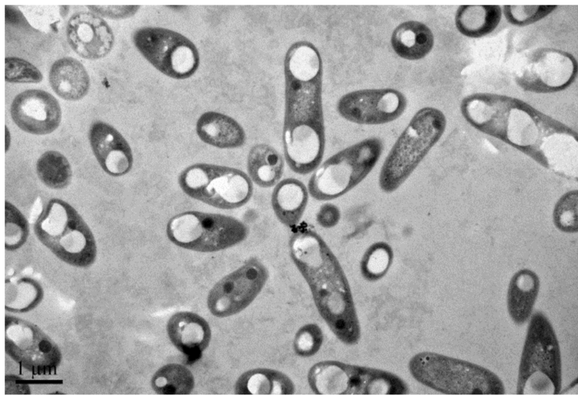

Fig. 2 Micrography of the *Methylobacterium* sp. XJLW under transmission electron microscope (15,000 ×, HITACHI H-7650 TEM)

Methylobacterium sp. XJLW strain, and the 7.59-min peak 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 are 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 ^{1}H- and ^{13}C-NMR spectra of PHB standards and PHAs produced by *Methylobacterium* sp. XJLW are shown in Fig. S2. The ^{1}H-NMR spectra show the presence of three signals in both spectra of the two polymer samples, which corresponded to the methyl group (CH_3 at 1.28 ppm), methylene group (CH_2 at 2.61 ppm), and methine group (CH at 5.26 ppm), respectively (Fig. S2A). The methyl group (CH_3), methylene group (CH_2), 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 ^{1}H- and ^{13}C-NMR of PHAs from *Methylobacterium* sp. XJLW are in good agreement with the data of PHB standards. IR spectra of PHB standards and PHAs from *Methylobacterium* sp. 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 *Methylobacterium* sp. XJLW and PHB standards referring to the terminal hydroxyl. Meanwhile, the great similarity of IR spectra characteristic indicates chemical group composition in PHAs from *Methylobacterium* sp. XJLW is the same to that of PHB standards. All the above evidences demonstrate PHB should be produced by *Methylobacterium* sp. XJLW.

LC-MS results of CoQ_{10} standard and the sample extracted from *Methylobacterium* sp. XJLW cells are shown in Fig.S4. It was found that the peak of CoQ_{10} in sample appeared at the retention time same to that of CoQ_{10} standard. Although the target peak area of sample looked lower than that of other unidentified peaks, the mass-to-charge ratio of CoQ_{10} sample extracted from *Methylobacterium* sp. XJLW strain exhibited a molecular peak (*m/s*, 885.6) same to that of CoQ_{10} standard. The result suggested that the *Methylobacterium* sp. XJLW has the ability of CoQ_{10} biosynthesis. However, further purification of the sample CoQ_{10} and enhanced production of CoQ_{10} in *Methylobacterium* sp. XJLW are required in future research.

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

As shown in Fig. 3, *Methylobacterium* sp. XJLW exhibited much higher biomass and yield of both PHB and CoQ_{10} 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 CoQ_{10} biosynthesis of *Methylobacterium* sp. 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 (Tables 3 and 4). 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 conditions. In the RT-qPCR analysis, *phaC-3* encoding poly(R)-hydroxyalkanoic acid synthase (class III) was chosen for analysis, results showed that *phaC-3* was significantly upregulated by methanol, which was identified with RNA-seq results. However, *phaC-1* catalyzing the same step in the pathway was downregulated 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 *Methylobacterium* sp. XJLW showing different responses to methanol (Table 4), which indicated that there was a more complex regulation system in *Methylobacterium* sp. XJLW responsible for PHB production. From genomic data mining, it was also found no gene encoding hydroxybutyrate-dimer hydrolase (EC: 3.1.1.22) and hydroxymethylglutaryl-CoA synthase (EC: 2.3.3.10) existing in *Methylobacterium* sp. XJLW strain, suggesting PHB were mainly synthesized through FadJ-catalyzed branch pathway. Besides, in CoQ_{10} synthetic pathway of *Methylobacterium* sp. XJLW, it was also found no gene encoding decaprenyl-diphosphate synthase (EC: 2.5.1.91) existed in the genomic data, but the LC-MS had strickly verified the product of CoQ10 from

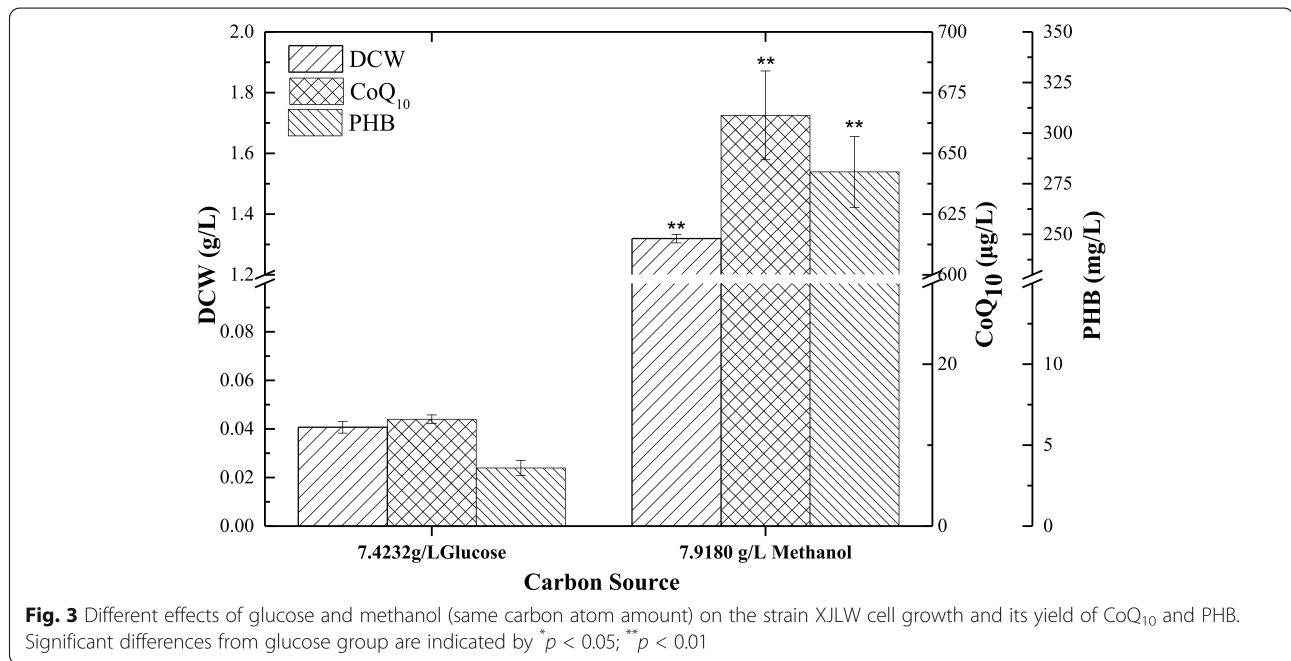

Fig. 3 Different effects of glucose and methanol (same carbon atom amount) on the strain XJLW cell growth and its yield of CoQ_{10} and PHB. Significant differences from glucose group are indicated by $^{*}p < 0.05$; $^{**}p < 0.01$

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 CoQ_{10}, biosynthesis in *Methylobacterium* sp. XJLW.

Effects of medium composition and cultivation conditions on cell growth, PHB, and CoQ_{10} productivity in Erlenmeyer flask level

Both medium M3 and MSM are recommended as suitable medium for *Methylotroph* strain cultivating (Bourque et al. 1995) with methanol as sole carbon and energy source. Thus, the growth behaviors of *Methylobacterium* sp. XJLW in M3 and MSM were evaluated in Erlenmeyer flasks. The results (Fig. 5a) showed that M3 medium exhibited more superiority for cell growth than MSM, and 5 days was the best harvest time with maximum dry cell density. Meanwhile, the ability of PHB and CoQ_{10} production by *Methylobacterium* sp. XJLW in M3 and MSM was also evaluated respectively. The results (Fig. 5b) also showed that *Methylobacterium* sp. XJLW exhibited better PHB and CoQ_{10} biosynthesis capacity in medium M3 than in MSM. M3 was then selected as initial medium for the optimization of *Methylobacterium* sp. 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 reports (Wei et al. 2012;

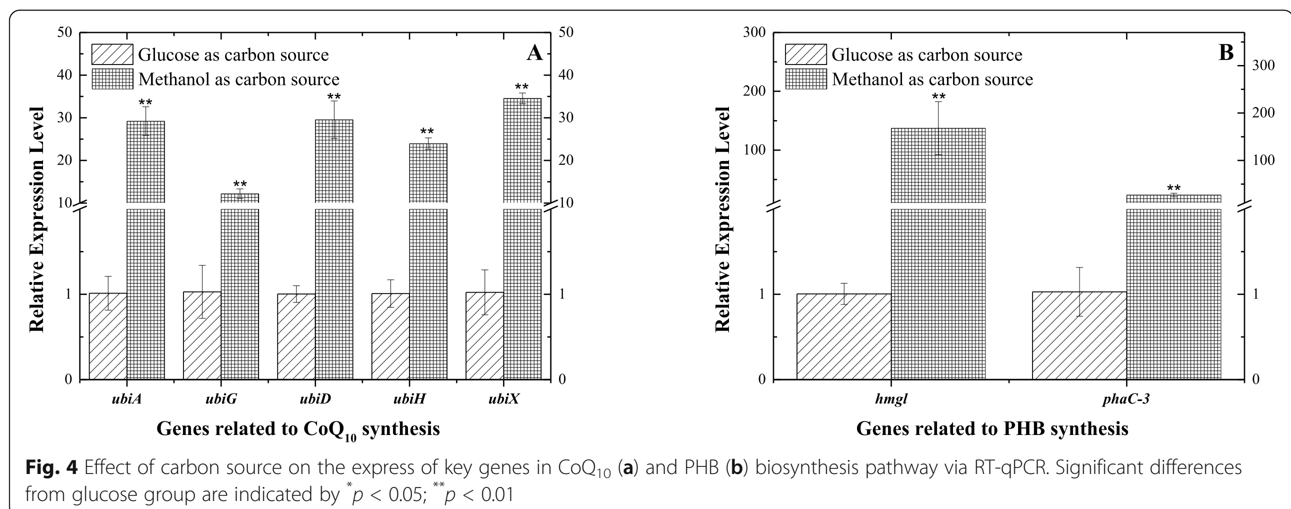

Fig. 4 Effect of carbon source on the express of key genes in CoQ_{10} (**a**) and PHB (**b**) biosynthesis pathway via RT-qPCR. Significant differences from glucose group are indicated by $^{*}p < 0.05$; $^{**}p < 0.01$

Table 3 FPKM values of CoQ_{10} 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	*dxr*	1-Deoxy-D-xylulose-5-phosphate reductoisomerase	99.9563	148.212	0.568293	Up
A3862_RS20315	*ispDF*	Bifunctional 2-C-methyl-D-erythritol 4-phosphate Cytidylyltransferase/2-C-methyl-D-erythritol 2,4-Cyclodiphosphate synthase	92.8581	149.282	0.684941	Up
A3862_RS03995	*ispE*	4-(Cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase	64.3157	141.036	1.132821	Up
A3862_RS12025	*ispG*	Flavodoxin-dependent (E)-4-hydroxy-3-methylbut-2-enyl-diphosphate synthase	425.885	296.847	− 0.52074	Down
A3862_RS10000	*ispH*	4-Hydroxy-3-methylbut-2-enyl diphosphate reductase	1071.31	683.363	− 0.64865	Down
A3862_RS18005	*ggps*	Geranylgeranyl diphosphate synthase, type II	30.1079	127.672	2.084228	Up
A3862_RS28415	*ispA*	Polyprenyl synthetase family protein	200.033	143.273	− 0.48147	Down
A3862_RS04015	*ispB*	Polyprenyl synthetase family protein	95.6316	164.97	0.786644	Up
A3862_RS03825	*ubiA*	4-Hydroxybenzoate octaprenyltransferase	73.9099	148.495	1.006575	Up
A3862_RS05140	*ubiX*	UbiX family flavin prenyltransferase	43.2101	92.1585	1.092749	Up
A3862_RS05150	*ubiD*	UbiD family decarboxylase	30.4548	120.907	1.989156	Up
A3862_RS18730	*ubiI*	2-Polyprenylphenol 6-hydroxylase	54.8386	146.31	1.415765	Up
A3862_RS01610	*ubiG*	Bifunctional 2-Polyprenyl-6-hydroxyphenol methylase/3-demethylubiquinol 3-O-methyltransferase UbiG	68.382	118.171	0.789188	Up
A3862_RS13590	*ubiH*	FAD-dependent monooxygenase	65.993	170.113	1.366108	Up
A3862_RS18735	*ubiE*	Bifunctional demethylmenaquinone methyltransferase/2-methoxy-6-polyprenyl-1,4-benzoquinol methylase UbiE	99.1489	146.834	0.566517	Up
A3862_RS22985	*ubiF*	UbiH/UbiF family hydroxylase	84.6668	194.111	1.197014	Up

Mozumder et al. 2014). Thus, the effect of carbon and nitrogen sources is also very necessary to be evaluated for the optimization of *Methylobacterium* sp. 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. 6a) and ammonium sulfate (Fig. 6b) on PHB and CoQ_{10} productivity of *Methylobacterium* sp. XJLW was evaluated respectively in the present study. It was found that 7.918 g L^{-1} methanol led to maximal CoQ_{10} concentration of 1.26 mg L^{-1} while the optimal biomass and PHB concentration was obtained under 11.877 g L^{-1} methanol. The phenomenon may result from the different biosynthesis pathways of CoQ_{10} and PHB. In order to avoid cell intoxication caused by high methanol concentration, 7.918 g L^{-1} methanol was selected as the optimal carbon source concentration in further research. However, no significant increase of PHB and CoQ10 yield was detected when ammonium sulfate concentration ranged from 0.5 g L^{-1} to 1.5 g L^{-1}, thus 0.5 g L^{-1} was selected for the following study. Besides medium components, cultural condition such as culture temperature and initial pH also play important roles in microbial fermentation. Thus, the effect of culture temperature and initial pH on *Methylobacterium* sp. XJLW fermentation was then evaluated in Erlenmeyer flask. The results (Fig. 6c and d) 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 *Methylobacterium* sp. 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.

Table 4 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	*acat-1*	Acetyl-CoA C-acetyltransferase	181.947	195.674	0.104934	Up
A3862_RS05695	*acat-2*	Acetyl-CoA C-acetyltransferase	1101.5	455.904	− 1.27267	Down
A3862_RS09310	*acat-3*	Beta-ketothiolase BktB	117.494	160.747	0.452205	Up
A3862_RS25790	*acat-4*	Acetyl-CoA C-acyltransferase	72.2701	163.337	1.176381	Up
A3862_RS27615	*acat-5*	Acetyl-CoA acetyltransferase	302.52	199.266	− 0.60233	Down
A3862_RS05690	*phbB*	Acetoacetyl-CoA reductase	775.466	472.896	− 0.71354	Down
A3862_RS05930	*phaC-1*	Class I poly(R)-hydroxyalkanoic acid synthase	266.759	198.599	− 0.42568	Down
A3862_RS11350	*phaC-2*	Polyhydroxyalkanoic acid synthase	111.896	382.12	1.771867	Up
A3862_RS19105	*phaC-3*	Class III poly(R)-hydroxyalkanoic acid synthase subunit PhaC	90.7047	116.952	0.366667	Up
A3862_RS19110	*phaE*	Poly-beta-hydroxybutyrate polymerase subunit	133.443	134.851	0.015143	Up
A3862_RS05165	*paaH-1*	3-Hydroxybutyryl-CoA dehydrogenase	60.5688	125.351	1.049327	Up
A3862_RS17305	*paaH-2*	3-Hydroxybutyryl-CoA dehydrogenase	487.68	218.354	− 1.15927	Down
A3862_RS21635	*paaH-3*	3-Hydroxyacyl-CoA dehydrogenase family protein	33.7683	105.064	1.637527	Up
A3862_RS02250	*fadJ*	Enoyl-CoA hydratase/isomerase family protein	155.264	191.337	0.301393	Up
A3862_RS15330	*fadN-1*	3-Hydroxyacyl-CoA dehydrogenase/enoyl-CoA	93.6416	170.494	0.864499	Up
A3862_RS25795	*fadN-2*	Enoyl-CoA hydratase/isomerase family protein	40.528	110.806	1.451045	Up
A3862_RS06255	*scoA*	Succinyl-CoA--3-ketoacid-CoA transferase/CoA transferase subunit A	737.041	126.763	− 2.53961	Down
A3862_RS06260	*scoB*	Succinyl-CoA--3-ketoacid-CoA transferase/CoA transferase subunit B	1515.36	206.479	− 2.87559	Down
A3862_RS14320	*bdh*	3-Hydroxybutyrate dehydrogenase	426.046	159.7	− 1.41564	Down
A3862_RS09710	*phaZ-1*	Polyhydroxyalkanoate depolymerase	397.233	383.709	− 0.04997	Down
A3862_RS17340	*phaZ-2*	Polyhydroxyalkanoate depolymerase	150.142	139.807	− 0.10289	Down
A3862_RS12335	*hmgl*	Hydroxymethylglutaryl-CoA lyase	64.564	145.106	1.168305	Up

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 enhance 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 carriers was added to *Methylobacterium* sp. XJLW fermentation system, respectively. The results (Fig. 6e) showed that Tween 80 exhibits positive effects especially in the level of CoQ_{10} and PHB biosynthesis, meanwhile the productivities of the Triton X-100 group

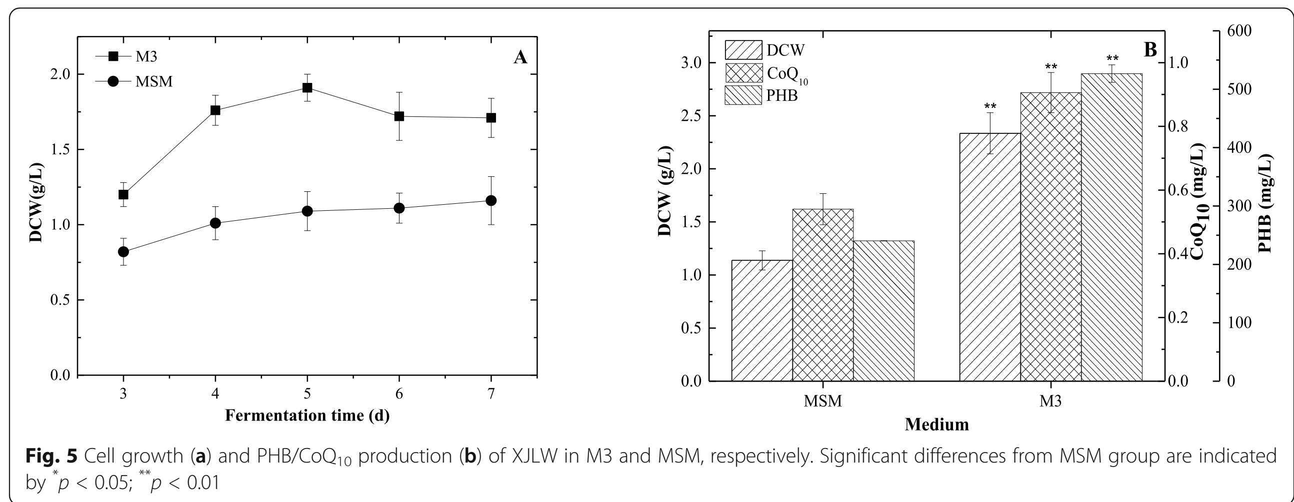

Fig. 5 Cell growth (**a**) and PHB/CoQ_{10} production (**b**) of XJLW in M3 and MSM, respectively. Significant differences from MSM group are indicated by $^{*}p < 0.05$; $^{**}p < 0.01$

and the 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 *Methylobacterium* sp. XJLW. 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 metabolite 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 *Methylobacterium* sp. XJLW metabolism were discussed through adding different concentrations of sodium chloride. The results (Fig. 6f) showed that the group adding 1.0 g L^{-1} of sodium chloride exhibited the highest cell yield and target product concentration, so this regulation strategy was chosen in the subsequent research.

Based on the above, the optimal medium and cultural conditions for CoQ_{10} and PHB co-production through *Methylobacterium* sp. XJLW strain fermentation were M3 medium containing 7.918 g L^{-1} methanol, 0.5 g L^{-1} of ammonium sulfate, 0.1% (v/v) of Tween 80, and 1.0 g

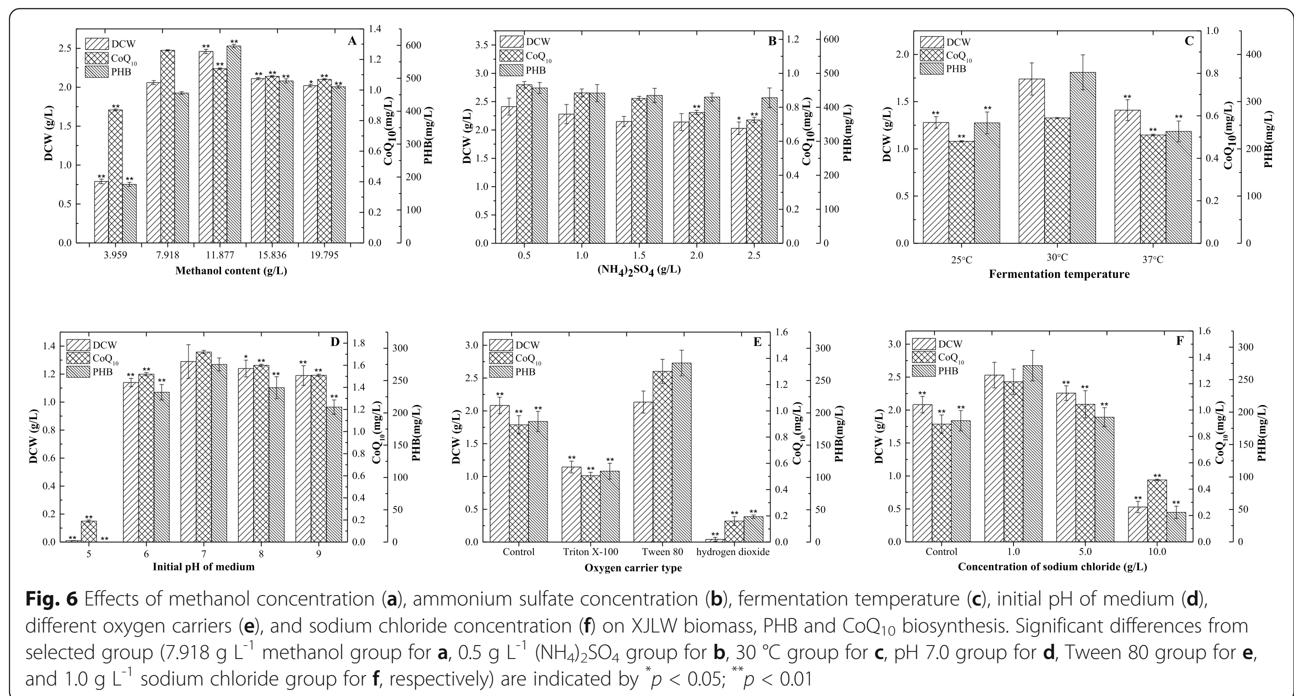

Fig. 6 Effects of methanol concentration (**a**), ammonium sulfate concentration (**b**), fermentation temperature (**c**), initial pH of medium (**d**), different oxygen carriers (**e**), and sodium chloride concentration (**f**) on XJLW biomass, PHB and CoQ_{10} biosynthesis. Significant differences from selected group (7.918 g L^{-1} methanol group for **a**, 0.5 g L^{-1} $(NH_4)_2SO_4$ group for **b**, 30 °C group for **c**, pH 7.0 group for **d**, Tween 80 group for **e**, and 1.0 g L^{-1} sodium chloride group for **f**, respectively) are indicated by $^{*}p < 0.05$; $^{**}p < 0.01$

L^{-1} of sodium chloride under the fermentation temperature and initial medium pH of 30 °C and 7.0, respectively.

Methylobacterium sp. XJLW fermentation in a 5-L fermenter

Based 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 online monitors, and the acquisition curves are shown in Fig. 7a. Meanwhile, the changes of methanol concentration, biomass, and PHB and CoQ_{10} productivity during the whole process are shown in Fig. 7b. 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.

It was also found that low content of PHB and CoQ_{10} were detected during the first 36 h, suggesting initially added methanol was almost completely exhausted for cell respiration and growth. Later, along with feeding substrates, concentration of biomass, PHB, and CoQ_{10} increased in the same trend, implying both PHB and CoQ_{10} were biosynthesized in association with cell growth. During the whole process, the total exhausted methanol volume is 830 mL, coupled with feeding 113.05 mL ammonium hydroxide. Finally, a maximum DCW value of 46.31 g L^{-1} was obtained, and the highest yields of PHB and CoQ_{10} reached 6.94 g L^{-1} and 22.28 mg L^{-1}, respectively. Thus, the final productivities of PHB and CoQ_{10} in this fed-batch fermentation system reached 0.15 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 *Methylobacterium* sp. 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 PHA production, there will be many polymer particles inside the cell suggesting PHA 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 PHA-producing strains.

For Methylotrophs 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 *Methylobacterium* sp. XJLW cell growth than MSM. As medium components, carbon source and nitrogen

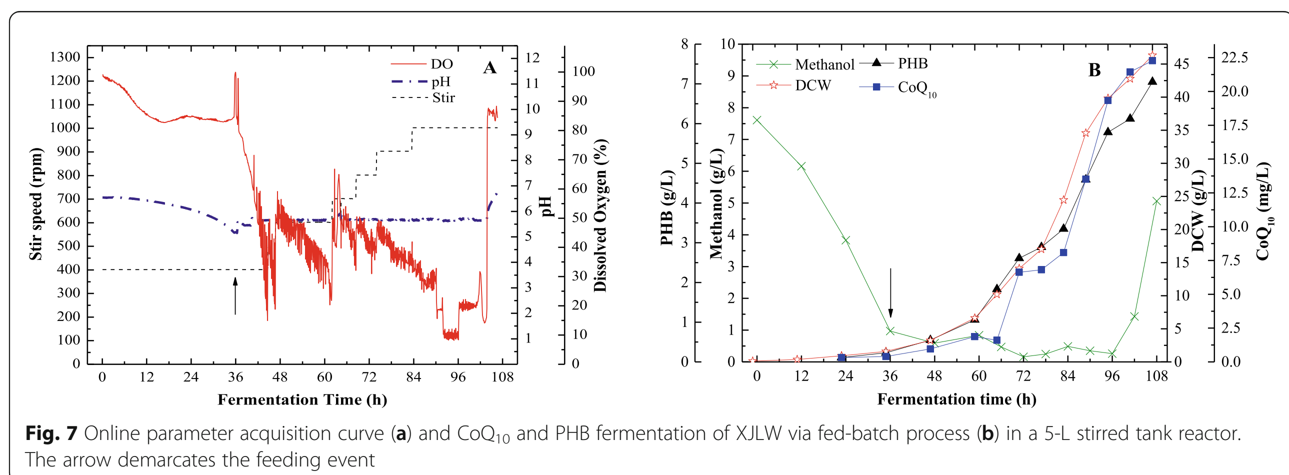

Fig. 7 Online parameter acquisition curve (**a**) and CoQ_{10} and PHB fermentation of XJLW via fed-batch process (**b**) in a 5-L stirred tank reactor. The arrow demarcates the feeding event

source usually play the significant role in the fermentation productivity according to previous reports (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 CoQ_{10} 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; Kalaiyezhini and Ramachandran. 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 et al. 2019), *Bacillus drentensis* (Gamez-Perez et al. 2020). After process and culture condition 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, CoQ_{10} 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 CoQ_{10}. However, no publication was found about CoQ_{10} synthesis in methylotrophic bacteria. In this study, it was found that both metabolic pathways of PHB and CoQ_{10} biosynthesis exist in *Methylobacterium* sp. XJLW 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, *Methylobacterium* sp. XJLW can produce PHB and CoQ_{10} 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 CoQ_{10} production simultaneously by methylotrophic bacteria.

After optimization of medium composition and the culture conditions on PHB and CoQ_{10} biosynthesis, a cell density of DCW 46.31 g L^{-1} with a PHB concentration of 6.94 g L^{-1}, and a CoQ_{10} 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 Erlenmeyer flasks, respectively. Although the productivity of CoQ_{10} 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 and Ramachandran. 2015), the volumetric yield of 22.3 mg L^{-1} of *Methylobacterium* sp. XJLW was higher than that of several previous reported strains including the mutant strain of *Rhodobacter sphaeroides* (14.12 mg L^{-1}) (Bule and Singhal. 2011), *Paracoccus dinitrificans* NRRL B-3785 (10.81 mg L^{-1}) (Tian et al. 2010), and *Sphingomonas* sp. ZUTEO3 (1.14 mg L^{-1}) (Zhong et al. 2009). Meanwhile, *Methylobacterium* sp. XJLW could accumulate PHB at the productivity level of 0.15 g g^{-1} of DCW. The PHB yield of *Methylobacterium* sp. XJLW 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 *Methylobacterium* sp. XJLW 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, compared with the cost of chemical polymers and the productivity of PHB or CoQ10 high yield strains, it is still necessary to further optimize fermentation process, and genetically modify strain pathway, for enhanced production of PHB and CoQ_{10} simultaneously by *Methylobacterium* sp. XJLW. This study also presented a potential strategy to develop efficiently co-producing other high-value metabolites using methanol-based bioprocess.

Abbreviations

CGMCC: China General Microbiological Culture Collection Center; CoQ_{10}: Coenzyme Q_{10}; 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; OD_{600}: Optical density at 600 nm; PCR: Polymerase chain reaction; NMR: Nuclear magnetic resonance; PHAs: Polyhydroxy-alkanoates; PHB: Poly-β-hydroxybutyrate

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13213-021-01632-w.

Additional file 1: Fig. S1 GC-MS spectra of PHAs extracted from *Methylobacterium* sp. XJLW, A is the GC spectra while B shows the MS spectra of the 7.59 min peak in A. Fig. S2 Comparison of ^{1}H- spectra (A) and ^{13}C-NMR spectra (B) between PHB standards and PHAs extracted from *Methylobacterium* sp. XJLW. Fig. S3 Comparison of IR spectra between PHB

standards and PHAs extracted from *Methylobacterium* sp. XJLW. Fig. S4 LC-MS spectra of CoQ_{10} standards (A) and CoQ_{10} sample extracted from *Methylobacterium* sp. XJLW (B).

Additional file 2:. Supplementary Material-Table of Samples.

Authors' 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. The authors read and approved the final manuscript.

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Availability of data and materials
The genome of *Methylobacterium* sp. XJLW is available in GenBank (accession no. CP016429), while its transcriptomics data are available in this article (Supplemental Material-Table of Samples FPKM).

Declarations

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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