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, polyhydroxyalkanoates (PHAs) are regarded as the most potential substrate, which can be completely degraded to CO\(_2\) and H\(_2\)O (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.)
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$_4$)$_2$SO$_4$ 0.5, KH$_2$PO$_4$ 1.305, Na$_2$HPO$_4$·7H$_2$O 4.02, MgSO$_4$·7H$_2$O 0.45, CaCl$_2$ 2H$_2$O 0.02, FeSO$_4$·H$_2$O 0.02, and 1 mL L$^{-1}$ trace element solution. The trace element solution contained (g L$^{-1}$) MnSO$_4$·H$_2$O 4.9, ZnSO$_4$·7H$_2$O 2.6, CuSO$_4$·5H$_2$O 0.8, Na$_2$MoO$_4$·2H$_2$O 0.8, CoCl$_2$·6H$_2$O 0.8, and H$_3$BO$_3$ 0.6.

Mineral salt medium (MSM) (Qiu et al. 2014) contained (g L$^{-1}$) KH$_2$PO$_4$ 0.7, K$_2$HPO$_4$ 0.85, (NH$_4$)$_2$SO$_4$ 1.2, MgSO$_4$·7H$_2$O 0.1, CaCl$_2$ 0.01, FeSO$_4$·7H$_2$O 0.001, and 1 mL L$^{-1}$ trace element solution. The trace element solution contained (g L$^{-1}$) H$_2$BO$_3$ 6, CoCl$_2$·6H$_2$O 4, ZnSO$_4$·7H$_2$O 2, MnCl$_2$·4H$_2$O 0.6, Na$_2$MoO$_4$·2H$_2$O 0.6, NiCl$_2$·6H$_2$O 0.4, and CuCl$_2$·2H$_2$O 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 \[p \text{ value} \leq 0.05\]. 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 7.4232 g L\(^{-1}\) glucose or 7.918 g L\(^{-1}\) methanol respectively, were centrifuged at 2000 \(\times\) 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.

**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\(_4\)OH 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\(_2\)O 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\(_4\)OH 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 \(\times\) 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 (S cientz-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 \(\times\) g, 4 °C for 10 min, and then the supernatant was sampled for CoQ\(_{10}\) and PHB analysis.

**Table 1** Primers used in this study

| Genes | Primers | Sequence |
|-------|---------|----------|
| 16S rDNA | 16S-F | GGTGTTGGAACCTGCGAGTATGAG |
| | 16S-R | CCCAGGCCGAATGCCTCAAAG |
| ubiA | ubiA-F | GCTCTGCTTCTCTCCCTGG |
| | ubiA-R | GGCATCGGCATGACCCGTTC |
| ubiG | ubiG-F | CTGGACGCGGTCTCGATCTGC |
| | ubiG-R | CAAGCAAGCCGAGACCTATCC |
| ubiD | ubiD-F | CGTGACCTGTGCCAAAGCG |
| | ubiD-R | AACGAGACGTCTTGCTCGGTAG |
| ubiH | ubiH-F | TGGTCTCTCGTCGCTCCTTC |
| | ubiH-R | TGGAAAGCTCGAAACGTGATG |
| ubiX | ubiX-F | AAGAGAGCCCGCGGAGGAGG |
| | ubiX-R | CCGCTGCTCGTGATCTGTTC |
| hmgl | hmgl-F | CGTCAAGACGCTCGGCAAGAG |
| | hmgl-R | GAGGCTCTCCATCACGGTAAAC |
| phaC-3 | phaC-3-F | ACGCGCGAAGATGCTGCTG |
| | phaC-3-R | TGCAGGCTCCTCGGATG |

**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 | Trition 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 (OD600). OD600 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 H2SO4 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). Firstly, 1 mL culture samples were centrifuged for 10 min at 4 °C; the cells were washed twice in distilled water, centrifuged 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.

CoQ10 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 CoQ10 standard. The molecular structure of CoQ10 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 CoQ10 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
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 1H- and 13C-NMR spectra of PHB standards and PHAs from Methylobacterium sp. 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 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 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 CoQ₁₀ standard and the sample extracted from Methylobacterium sp. XJLW cells are 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 Methylobacterium sp. XJLW strain exhibited a molecular peak (m/s, 885.6) same to that of CoQ₁₀ standard. The result suggested that the Methylobacterium sp. XJLW has the ability of CoQ₁₀ biosynthesis. However, further purification of the sample CoQ₁₀ and enhanced production of CoQ₁₀ 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 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 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 thatphaC-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 CoQ₁₀ 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
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 *Methylobacterium* sp. XJLW.

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

Both medium M3 and MSM are recommended as suitable medium for *Methyloptroph* 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 CoQ10 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 CoQ10 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;
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 CoQ10 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 CoQ10 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 CoQ10 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.

| 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-Cyclophosphate 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-ethyl-diphosphate synthase | 425.885         | 296.847          | -0.52074       | Down       |
| A3862_RS10000 | ispH    | 4-Hydroxy-3-methylbut-2-ethyl diphosphate reductase                      | 1071.31         | 683.363          | -0.64865        | Down       |
| A3862_RS18005 | gpps    | 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_RS03925 | 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 monoxygenase                                                | 65.993          | 170.113          | 1.366108        | Up         |
| A3862_RS18735 | ubiE    | Bifunctional dimethylmenaquinone methyltransferase/2-methoxy-6-polyrenyl-1,4-benzoquinol methylase UbiE | 99.1489         | 146.834          | 0.566517        | Up         |
| A3862_RS22085 | ubIF   | UbiH/UbiF family hydroxylase                                              | 84.6668         | 194.111          | 1.197014        | 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 CoQ10 and PHB biosynthesis, meanwhile the productivities of the Triton X-100 group

| 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-acetyltransferase | 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 | hmgI    | Hydroxymethylglutaryl-CoA lyase | 64.564         | 145.106         | 1.168305       | Up         |
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 CoQ₁₀ 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. 5 Cell growth (a) and PHB/CoQ₁₀ production (b) of XJLW in M3 and MSM, respectively. Significant differences from MSM group are indicated by * p < 0.05; ** p < 0.01

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 CoQ₁₀ biosynthesis. Significant differences from selected group (7.918 g L⁻¹ methanol group for a, 0.5 g L⁻¹ (NH₄)₂SO₄ 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 CoQ₁₀ 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 CoQ₁₀ 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 CoQ₁₀ increased in the same trend, implying both PHB and CoQ₁₀ 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 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 0.15 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...
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 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; 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. 2012; Mozumder et al. 2014). For M. 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 CoQ10 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; 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; OD₆₀₀: 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 ¹³C spectra (A) and ¹⁴C-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 PHB.
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

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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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. https://doi.org/10.1007/BF00169931

Bule MV, Singhal RS (2011) Fermentation kinetics of production of ubiquinone-10 by Paracoccus distniitrici NRRL B-3758: effect of type and concentration of carbon and nitrogen sources. Food Sci Biotechnol 20(3):607–613. https://doi.org/10.1007/s10068-011-0085-6

Cardoso LOB, Karelski B, Gracioso LH, CAO d N, Perpetuo EA (2020) Increased P3HB accumulation capacity of Methylorubrum sp. in response to discontinuous methanol addition. Appl Biochem Biotechnol 192(3):846–860. https://doi.org/10.1007/s12010-020-03569-9

Chen L, Qiao GQ, Shuai BW, Song KN, Yao WX, Jiang XR, Chen GQ (2019) Engineering self-flocculating Halomonas campanensis for wastewaterless open and continuous fermentation. Biotechnol Bioeng 116(4): 805-815.

Ernst L, Dallner G (1995) Biochemical, physiological and medical aspects of ubiquinone function. Biochimica Et Biophysica Acta 1271(1):195–204. https://doi.org/10.1016/0025-2691(95)00028-3

Gamez-Perez J, Penkhwue W, Jendrossek D, Khonongnuch C, Pathom-aree W, Asawata A, Behrens RL, Lumphong S (2020) Response surface method for polyhydroxybutyrate (PHB) bioplastic accumulation in Bacillus dr minutus BP17 using pineapple peel. Plos One 15(6):023048

Holper 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(1):70. https://doi.org/10.1186/1475-2859-9-70

Kalayezhini 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. https://doi.org/10.1080/08927052.2014.87582

Khosravi-Darani K, Mohktari ZB, Amai T, Tanaka K (2013) Microbial production of poly(hydroxybutyrate) from C(1) carbon sources. Appl Microbiol Biotechnol 97(4):1607–1424. https://doi.org/10.1007/s00253-012-4649-0

Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Saberg SL (2013) TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol 14(4):R36. https://doi.org/10.1186/gb-2013-14-4-36

Lai LS, Tsai TH, Wang TC (2002) Application of oxygen vectors to Aspergillus niger cultivation. J Bioсис Biogен 94(4):453–459. https://doi.org/10.1007/S1389-172320920249-9

Lu W, Shi Y, He S, Fei Y, Yu K, Yu H (2013) Enhanced production of CoQ10 by constitutive overexpression of 3-demethyl ubinonine-9-3-methyltransferase under tac promoter in Rhodobacter sphaeroides. Biochem Eng J 72:42–47. https://doi.org/10.1016/j.ijbiotech.2012.12.019

Mohandas SP, Balan L, Lekshmi S, Cubello 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):688–707. https://doi.org/10.1111/jam.13159

Mohktari-Hosseini ZB, Vaskheghani-Farahani E, Heidarzadeh-Zafiekhaharan A, Shojaosadati SA, Karimzadeh R, Darani KK (2009) Statistical media optimization for growth and PHB production from methanol by a methylotrophic bacterium. Bioresource Technol 100(8):2436–2443. https://doi.org/10.1016/j.biortech.2008.11.024

Mostafa YS, Alnumaman AS, Otai KF, Mostafa MS, Alaffy AM (2020) Bioplastic (poly-3-hydroxybutyrate) production by the marine bacterium Pseudonodonghicola xianemansens through date syrup valorization and structural assessment of the biopolymer. Sci Rep 10(1):8815. https://doi.org/10.1038/s41598-020-65858-5

Mozumder MS, De Wever H, Volcke EP, 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. https://doi.org/10.1016/j.procbio.2013.12.004

Mozumder MS, Garcia-Gonzalez L, De Wever H, Volcke EP (2015) Effect of sodium accumulation on heterotrophic growth and polyhydroxybutyrate (PHB) production by Cupriavidus necator. Bioresource Technol 191:213–218. https://doi.org/10.1016/j.biortech.2015.04.110

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. Bioresource Technol 99(13):5749–5755. https://doi.org/10.1016/j.biortech.2007.10.017

Pal A, Priabhu A, Kumar AA, Rajagopal B, Dadhe K, Ponnamma V, Shivakumar S (2009) Optimization of process parameters for maximum poly-3-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 A9 in recombinant Escherichia coli containing the decapenyl diphosphate synthase gene from Glucobacter suboxydans. Appl Microbiol Biotechnol 67(2):192–196. https://doi.org/10.1007/s00253-004-1743-y

Parveez GK, Bahariya B, Ayub NH, Masani MY, Rased OA, Tamizi 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 methanol and nitrogen limitation increases PHB production in a methanotrophic community. Bioresour Technol 107(0): 385-392. DOI: https://doi.org/10.1016/j.biortech.2011.12.044

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. https://doi.org/10.1007/s00449-013-1110-4

Qiu L, Ding H, Wang W, Kong Z, Li X, Shi Y, Zhong W (2012) Coenzyme Q(10) production by immobilized Sphingomonas sp. ZUTE02 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
