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Improvement of welan gum biosynthesis and transcriptional analysis of the genes responding to enhanced oxygen transfer by oxygen vectors in *Sphingomonas* sp.

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ABSTRACT

Oxygen transfer (k_La) is one of the most essential factors affecting microbial cell growth and metabolite formation. In this study, low-cost methods capable of effectively enhancing oxygen transfer were investigated by adding oxygen vectors into the fermentation broth. Upon addition of *n*-dodecane and *n*-hexadecane, the k_La of the fermentation medium reached remarkably higher values than that of the control. In response to the enhanced oxygen transfer, welan gum concentration and yield reached maxima of 33.9 ± 0.56 g/L and 0.761 ± 0.010 g/g, respectively. The DCW reached a maximum of 7.82 ± 0.18 g/L with *n*-dodecane addition. Welan gum productivity and yield were significantly affected by k_La under *n*-hexadecane addition because of the promotion of the highest transcriptional levels of genes in the metabolic flux for welan gum biosynthesis. In addition, transcription levels of TCA and electron transfer chain (ETC) genes exhibited maximum up-regulation under *n*-dodecane, which resulted in enhanced cell growth. These results suggest a mechanism for enhanced k_La using oxygen vectors to improve welan gum biosynthesis through regulation of transcriptional levels of ETC, TCA, and metabolic pathways for welan gum biosynthesis genes during fermentation.

1. Introduction

Welan gum is a microbial polysaccharide produced by fermentation with *Sphingomonas* sp. It is widely used in food, medicine, concrete additives, oil recovery, and in various other fields [1–3]. Oxygen is one of the crucial factors influencing *Sphingomonas* sp. growth and welan gum production during aerobic fermentation [4]. However, welan gum fermentation is accompanied by a dramatic increase in broth viscosity because of extracellular accumulation of the biopolymer, which leads to a significant decrease in the oxygen mass transfer rate. The dissolved oxygen (DO) concentration becomes the limiting nutrient and the oxygen mass transfer rate (k_La) functions as the controlling step rate for the overall process [5]. In previous studies, enhancements in oxygen transfer rates have traditionally involved enhancements in either the stirring or aeration rate; such increases cause mechanical stress to the sensitive organism and uncontrolled foam formation in the reactor, which lead to an uneconomical fermentation process [6,7]. Therefore, the development of low-cost methods capable of enhancing oxygen transfer is crucial to improve the cost effectiveness of industrial biotechnology.

Addition of a water-immiscible organic phase, more commonly known as oxygen vector, induces significant increases in oxygen transfer rate from the air to microorganisms with no supplementary intensification of mixing [8]. These vectors have higher oxygen solubilisation capacities than water [9]. Numerous studies describe the effect on oxygen mass transfer of various organic compounds such as alkanes, perfluorocarbons, silicone oils, and vegetable oil [10]. Oxygen solubility in these compounds is approximately 15–20 times higher than that in water. In addition, oxygen-vectors have no toxicity against the cultivated microorganisms at the levels tested [11].

In our previous study, we evaluated the effect of relatively high dissolved oxygen on welan gum production in *Sphingomonas* sp. by changing the metabolic flux [4]. Oxygen availability strongly affected product formation by influencing metabolic pathways and

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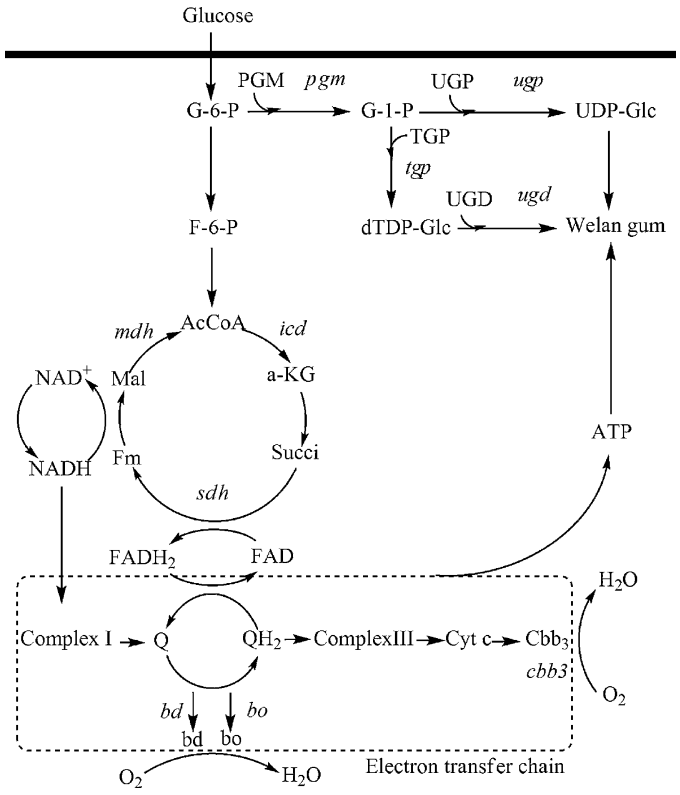

Fig. 1. Proposed glucose metabolic pathway for *Sphingomonas* sp. including TCA cycle, ETC, and welan gum biosynthesis. *G-6-P*, glucose-6-phosphate; *G-1-P*, glucose-1-phosphate; *UDP-Glc*, UDP-glucose; *dTDP-Glc*, dTDP-glucose; *F-6-P*, fructose-6-phosphate; *AcCoA*, acetyl coenzyme A; *α-KG*, α-ketoglutarate; *Succi*, succinate; *Fm*, fumarate; *Mal*, malate; *pgm*, phosphoglucomutase; *ugp*, uracil-diphosphate (UDP)-glucose pyrophosphorylase; *tgp*, dTDP-glucose pyrophosphorylase; *ugd*, UDP-glucose dehydrogenase; *mdh*, malate dehydrogenase; *icd*, isocitrate dehydrogenase; *sdh*, succinate dehydrogenase; *bd*, cytochrome bd terminal oxidase subunit I; *bo*, cytochrome bo terminal oxidase subunit I, *cbb3*, cytochrome cbb3-type terminal oxidase subunit III.

changing metabolic fluxes. However, to the best of our knowledge, few scale-down studies have reported on the effect of relatively high oxygen transfer rates on energy requirements for the improvement of welan gum biosynthesis. Similar to the production of various other bacterial exopolysaccharides, energy efficiency is the major constraint for the improvement of exopolysaccharide production, because ATP is needed during precursor regeneration in the syntheses of many microbial polysaccharides [12]. ATP regeneration is essentially undertaken through the tricarboxylic acid (TCA) cycle and electron transfer chain (ETC) in the presence of oxygen [13]. Considering that oxygen vectors induce significant enhancements in k_La during fermentation, cells are often exposed to high DO environments, which can affect their metabolic conditions in the TCA cycles, ETCs, and metabolic pathways for welan gum biosynthesis. Gene transcription is a particularly relevant molecular event because it is one of the first cellular responses to high DO stress [14]. Therefore, exploring the effect of enhanced k_La on transcriptional changes in selected genes (Fig. 1), especially those related to carbohydrate metabolism and ATP regeneration in *Sphingomonas* sp. will facilitate the understanding of the metabolic control mechanism of enhanced DO concentrations at the molecular level. This information is essential for the improvement of high welan gum productivity bioprocess.

The objectives of this study were to investigate the effects of enhanced oxygen transfer on gene transcriptional changes in *Sphingomonas* sp. by oxygen vectors addition and obtain data using quantitative reverse transcription-polymerase chain reaction (RT-PCR). To the best of our knowledge, this study is the first to report the impact of relatively high k_La on gene transcriptional changes during welan gum biosynthesis. Our experimental results are especially useful for understanding the welan gum production and metabolic responses of *Sphingomonas* sp. under relatively high DO conditions at the molecular level. Such information is important for designing improved fermentation processes for welan gum or other biopolymers through enhanced DO concentrations.

2. Materials and methods

2.1. Microorganism and medium

This study used *Sphingomonas* sp. CGMCC1737 deposited at the General Microbiological Culture Collection Centre in China. Seed medium (pH 7.2–7.4) was prepared with the following components (per litre): glucose, 20 g; yeast extract, 1 g; peptone, 3 g; $K_2HPO_4{\cdot}3H_2O$, 2 g; and $MgSO_4$, 0.1 g. Fermentation medium (pH 7.2–7.4) contained the following components (per litre): glucose, 50 g; yeast extract, 8 g; $K_2HPO_4{\cdot}3H_2O$, 3 g; and $MgSO_4$, 0.4 g.

2.2. Cultivation conditions

Sphingomonas sp. CGMCC1737 was first inoculated into 135 mL of seed medium in a 1 L flask and incubated at 30 °C for 16 h with shaking at 200 rpm. The seed culture was then transferred to a 7.5 L bioreactor (BioFlo 110, New Brunswick Scientific, USA) with 4.5 L of fermentation medium. The pH was adjusted to 7.4 with 3 M NaOH and the media were autoclaved prior to use. The fermentation culture was incubated at 30 °C for 72 h. The aeration rate was maintained at 1.0 vvm with an agitation speed of 600 rpm.

In addition to the control, sterilised *n*-dodecane and *n*-hexadecane with concentrations of 0.2%, 0.8%, 1%, 2%, 4%, 6%, and 8% were added to the medium at the beginning of fermentation.

2.3. Analytical methods

Dry cell weight (DCW) was determined from 10 mL cell sample suspensions harvested by centrifugation, washed with distilled water, and dried at 105 °C. Glucose content was measured using a biosensor equipped with a glucose oxidase electrode (SBA-40C, Shandong Academy of Sciences, China). Welan gum concentration was measured as previously reported [15]. Fermentation broth viscosity was measured using a rotational viscometer (NDJ-1, Shanghai Hengping Scientific Instrument Company, China) with rotor number 4 at 60 rpm. DO concentrations and pH were measured using inductors of the bioreactor. Volumetric mass transfer coefficient (k_La) was measured using the dynamic gas-out/gas-in method [16]. Each experiment was repeated thrice, and the experimental errors were <4%.

2.4. Transcriptional analysis of selected genes via RT-PCR

Cells were collected and harvested at the exponential phase (20 h) of fermentation by centrifugation. Total RNA was isolated using RNAiso Plus (TaKaRa Biotechnology Company, China) and then used in the synthesis of cDNA using PrimeScript™ RT Master Mix (TaKaRa Biotechnology Company, China). Real-time PCR (StepOnePlus™ Real-Time PCR System, Applied Biosystems, USA) was performed with 2 μL cDNA in a 20 μL PCR system [containing 10 μL 2× SYBR® Premix Ex Taq Tli RNaseH Plus, 0.4 μL of each primer (25 pmol/μL), 0.4 μL 50× ROX reference dye, 2 μL cDNA, and 6.8 μL sterile distilled water]. Information collected from these genes was analysed; gene primers are listed in Table 1. 16S rRNA was used as an endogenous control gene. After an initial denaturation period at 95 °C for 30 s, the reaction mixture was cycled 40