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Medium engineering for enhanced production of undecylprodigiosin antibiotic in *Streptomyces coelicolor* using oil palm biomass hydrolysate as a carbon source

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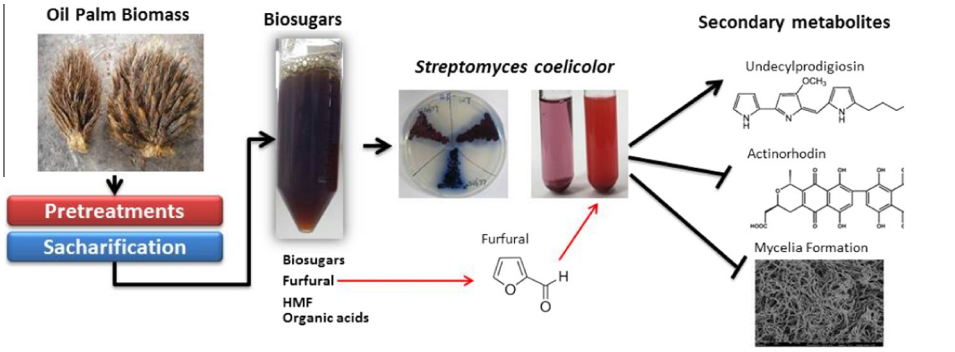
HIGHLIGHTS

- Oil palm biomass hydrolysate has furfural and hydroxymethylfurfural as inhibitors.
- Furfural effects xylose utilization in *S. coelicolor* adversely.
- Mycelia formation is decreased with reduction in actinorhodin production.
- Furfural elicits undecylprodigiosin production in *S. coelicolor* up to 52%.
- Engineered EFB hydrolysate media resulted a 88% higher undecylprodigiosin production.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, a biosugar obtained from empty fruit bunch (EFB) of oil palm by hot water treatment and subsequent enzymatic saccharification was used for undecylprodigiosin production, using *Streptomyces coelicolor*. Furfural is a major inhibitor present in EFB hydrolysate (EFBH), having a minimum inhibitory concentration (MIC) of 1.9 mM, and it reduces utilization of glucose (27%), xylose (59%), inhibits mycelium formation, and affects antibiotic production. Interestingly, furfural was found to be a good activator of undecylprodigiosin production in *S. coelicolor*, which enhanced undecylprodigiosin production by up to 52%. Optimization by mixture analysis resulted in a synthetic medium containing glucose:furfural:ACN:DMSO (1%, 2 mM, 0.2% and 0.3%, respectively). Finally, *S. coelicolor* was cultured in a fermenter in minimal medium with EFBH as a carbon source and addition of the components described above. This yielded 4.2 μg/mg dcw undecylprodigiosin, which was 3.2-fold higher compared to that in un-optimized medium.

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1. Introduction

Utilization of biomass for economical production of commercialized products is a subject of debate among the global scientific community (Pandey et al., 2000). Lignocellulosic raw materials are projected to play a major role in the development of various industrial bioprocesses (Patel et al., 2015). These raw materials are usually composed of cellulose, hemicellulose and lignin, which are associated with each other (Ahn et al., 2012; Jonsson et al., 2013). To use lignocellulose as a raw material, it must be converted to fermentable sugars by physio-chemical pretreatment and enzymatic hydrolysis. Lignin is a complex structure of polyalcohols, and is highly resistant to degradation by microbial enzymes (Arantes and Saddler, 2010). Pretreatment is necessary to improve enzyme accessibility and permeability to break down the complex cell wall, in which utilizable polysaccharides are embedded (Li et al., 2013). Various pretreatment methods using acids (such as sulfuric acid and phosphoric acid) or bases (such as ammonium hydroxide, sodium hydroxide, and calcium hydroxide) have been reported to promote conversion of the complex plant cell wall to fermentable sugars by hydrolytic enzymes (Kang et al., 2013; Sindhu et al., 2015). Lignocellulose pretreatment results in release of inhibitors; i.e., aldehydes (furan aldehydes), ketones, phenolics and organic acids, which influence microbial metabolism to utilize these free sugars as a carbon source for fermentation (Allen et al., 2010). Glucose and xylose degradation resulted in 5-hydroxymethylfurfural (HMF) and 2-furaldehyde (furfural), respectively (Kootstra et al., 2009). Furfural induces production of reactive oxygen species (ROS) in microorganisms, which cause cellular damage and affect growth (Allen et al., 2010). At present, little information about the microbial inhibitors released during pretreatment of lignocellulosic biomass, their mechanism of action, effects on carbon utilization and secondary metabolite production is available. Hence, the generation of inhibitors during the production of lignocellulosic hydrolysate and their effects on microbial fermentation warrant investigation.

Streptomyces coelicolor, an actinobacterium, produces various industrially important secondary metabolites (Borodina et al., 2008). *S. coelicolor* can synthesize two chemically distinct antibiotics as secondary metabolites—actinorhodin (a diffusible blue pigment) and the cell wall-associated, red-pigmented undecylprodigiosin (Kim et al., 2015a). Antibiotic production is controlled by many factors, such as transcriptional regulators, quorum-sensing molecules (γ-butyrolactone) concentrations, metabolic and nutritional status (van Wezel et al., 2000; Yang et al., 2005), and the proposed coupling of antibiotic synthesis and resistance genes (Hindra et al., 2010). These regulatory mechanisms can be altered by varying the culture conditions and addition of various factors (Schaberle et al., 2014). Undecylprodigiosin is a focus of interest due to its immunosuppressive and anticancer properties (Williamson et al., 2006).

In this study, the effects of biomass-derived inhibitors (furfural and hydroxymethylfurfural) on *S. coelicolor* growth, carbon source utilization and antibiotic production were investigated. Moreover, a minimal medium comprising EFB hydrolysate (EFBH) as a carbon source was designed to enhance production of undecylprodigiosin.

2. Methods

2.1. Microorganisms, media and culture conditions

All reagents were purchased from Difco Laboratories (Becton–Dickinson Franklin Lakes, NJ, USA) and other chemicals; i.e., furfural and hydroxymethylfurfural (HMF) from Sigma–Aldrich (St. Louis, MO, USA). *S. coelicolor* A3 (2) M145 used for antibiotic production was purchased from the Korean Culture Type Collection (KCTC, South Korea). For spore production *S. coelicolor* was cultivated on R5 agar plates for 7 days at 30 °C, harvested by scraping and suspended in 20% (v/v) glycerol and stored at −80 °C. Seed culture of *S. coelicolor* was prepared by inoculating spores in 50 ml of Luria–Bertani (LB) liquid medium, with five 3 mm glass beads, and incubated at 30 °C with shaking at 200 rpm. The germinated spores were harvested by centrifugation (3200*g*, 4 °C, 10 min) and resuspended in 5 ml of ion-free water. A 0.1 ml volume (2×10^6 CFU) of germinated seed culture was used as the inoculum. For production of undecylprodigiosin, *S. coelicolor* was cultured in minimal medium (Difco Laboratories) containing EFB hydrolysate (EFBH) as a carbon source. EFB hydrolysate was prepared by hot water treatment of empty fruit bunch at 190 °C for 15 min (Bench Top Reactor, Model 4526, Parr Instruments), mechanical refining of the pretreated slurry using a valley beater (L & W, Norway), enzymatic hydrolysis of the solid fraction of the pretreated EFB with Cellic CTec2 (Novozymes, Denmark), and finally solid–liquid separation of the hydrolysate by centrifugal filtration (Eom et al., 2015). An HPLC system equipped with a Bio-Rad Aminex HPX-87H column (Bio-Rad Co., Hercules, CA, USA) was used to analyze sugar, furfural and HMF contents. A mobile phase of 5 mM H_2SO_4 at a flow rate of 0.6 ml/min was used, and the column temperature was maintained at 50 °C. The EFBH concentration was adjusted to 1% glucose, and additional furfural (2 mM), DMSO (0.3%), and acetonitrile (0.2%) were added. *S. coelicolor* was cultured in 10 ml volumes in the capped tubes with a capacity of 25 ml at 30 °C, with agitation at 160 rpm for 72 h, and then subjected to analysis of antibiotic production.

2.2. Antibiotic extraction and quantification

At intervals of 24 h, 2 ml culture samples were removed, and biomass and antibiotic production were estimated. The samples were divided into two aliquots. For actinorhodin estimation, an equal volume of 1 N NaOH was added to the culture aliquot. The sample was then centrifuged at 4000*g* for 5 min and the actinorhodin concentration was determined by measuring the absorbance at 633 nm (Horinouchi and Beppu, 1984). Undecylprodigiosin is a membrane-associated red pigment, and was extracted from the cell pellet. An aliquot of *S. coelicolor* culture was harvested by centrifugation (4000*g* for 5 min), suspended in methanol and incubated at 37 °C with shaking at 200 rpm for 1 h. Cells were removed by centrifugation at 4000*g* for 5 min and 0.1 N HCl was added to adjust pH. To quantify undecylprodigiosin, absorbance at 533 nm was measured and the concentration calculated (Horinouchi and Beppu, 1984).

2.3. Targeting sub-lethal concentrations of furfural and HMF

EFB hydrolysate has furfural as the main inhibitory component, with trace amounts of HMF. The effect of these components on growth and antibiotic production by *S. coelicolor* was investigated. It is not possible to assess the effect of these inhibitors in complex EFBH, so a minimal medium containing glucose as carbon source was used. *S. coelicolor* was cultured in minimal medium in the presence of 1% glucose, 0.1% yeast extract and 0–4 mM inhibitors at 30 °C for 72 h. The minimum inhibitory concentration (MICs) of the inhibitors against *S. coelicolor* was calculated. Antibiotic extraction was also performed to estimate actinorhodin and undecylprodigiosin concentrations. To make stock solutions, compounds were dissolved in water and sterile filtered prior to their addition to the fermentation media.