Production of xylonic acid by recombinant Escherichia coli

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**KEYWORDS**
- Xyloonic acid
- Recombinant E. coli

**ABSTRACT**
In this paper, the effect of cultural condition on xylonic acid secretion by recombinant Escherichia coli (E. coli) was investigated. Then, the optimized cultural condition was determined to elevate xylonic acid secretion. To achieve this, analysis on xylonic acid secretion by using hydroxamate assay and High Performance Liquid Chromatography (HPLC) was performed. From the experimental works, it was understood that post induction temperature, inducer concentration, substrate concentration, medium pH and type of medium have affected the secretion of xylonic acid.

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1. Introduction

Over the years, processing of biomass for the production of value-added chemicals has become important to both biological and chemical engineering research (Fitzpatrick et al., 2010). Xyloonic acid, a five-carbon organic acid has gained outstanding interest due to its potential as an important platform chemical. It has extensive applications in food, pharmaceutical and chemical industries. Xyloonic acid can be produced by oxidation of xylose with microorganism (Cao et al., 2013).

Xyloonic acid has been produced by the recombinant Escherichia coli, Saccharomyces cerevisae and Kluyveromyces lactis (Toivari et al., 2012). Xyloonic acid productivity and yield by recombinant E. coli are both higher as compared to Saccharomyces cerevisae or Kluyveromyces lactis (Nygård et al., 2011; Toivari et al., 2010).

E. coli has native metabolic pathways for both xylose and xylonic acid, unfortunately it is incapable of converting xylose directly to xylonic acid. Liu et al., (2012) in their studies, genetically modified E. coli to produce xyloonic acid by introducing a xylose dehydrogenase gene from Caulobacter crescentus. Both xylose and xylonic acid catabolic pathways were blocked to prevent the conversion from xylose or xylonic acid to biomass.

Diverse approaches have been studied to enhance extracellular production of recombinant proteins in E. coli. These approaches include improving culture conditions, such as varying the medium composition, nutritional feeding design, induction mode, utilization of media additives (Cheng et al., 2011; Fang et al., 2011).

The most common strategy engaged in molecular biology is by changing each variable at a time while holding the others constant, assuming that each variable is independent to evaluate the influence of such variables on heterologous protein expression (Larentis et al., 2014).

In this study, parameters affecting xylonic acid production by recombinant E. coli was studied.

2. Experimental procedure

2.1 Bacterial strain and chemicals

The recombinant E. coli strains carrying the genes to express the secretion of xylonic acid were chosen as the biological hosts in this research. The strain is grown overnight and 80 % glycerol is prepared. Immobilization technique was used to express the secretion of xylonic acid. Most of the chemicals and reagents that are used in this research are analytical and molecular biology grade, purchased from various companies such as Merck, Sigma, Fluka and Calbiochem.

2.2 Expression conditions and analytical methods

Parameters affecting xylonic acid secretion were determined at five different conditions for each parameter. Xylose and xylonic acid were measured by utilizing High Performance Liquid Chromatography fitted with Bio-Rad Aminex HPX-87H column (300 x 7.8 mm).
Xylonic acid concentrations was determined by hydroxamate method. The conversion of xylonic acid to xylonolactone was done by heating the diluted sample. Then, 1 mL hydroxylamine reagent (2 M hydroxylamine HCl in 2 M NaOH) was added to 500 µl of the diluted sample. Addition of HCl (650 µl, 3.2 M) was then done followed with addition of 500 µl FeCl₃ (100 g L⁻¹ FeCl₃ in 0.1 M HCl). Absorbance was measured at 550 nm.

3. Results and discussion

3.1 Parameters affecting xylonic acid synthesis

Post induction temperature is an important factor in determining the production of xylonic acid by immobilized recombinant E. coli. Five different temperatures were investigated. The results indicated that xylonic acid secretion in immobilized cells was improved significantly by increasing the post induction temperature. According to Hohenblum et al. (2004), the metabolic stress generated by heterologous gene expression can be slightly relieved by using lower temperatures to perform expression.

Then, immobilized cells were induced using five different concentrations of inducer. Highest amount of xylonic acid was obtained at an intermediate amount of inducer concentration. If the amount is small, it is not enough for expression. While high amount of inducer concentration reduce xylonic acid secretion by immobilized recombinant E. coli that was caused by the toxic effect of the inducer and the metabolic burden imposed on the cells due to heterologous gene expression (Larentis et al., 2014).

Substrate concentration plays an important role in determining the secretion of xylonic acid by using immobilized cell. In this research, xylose was used as the substrate. The different concentration of substrate seems to have affected the synthesis of xylonic acid by immobilized cell. According to Maier (2009), cell growth is dependent on the substrate concentration, when substrate concentration is low, the rate of growth will be low as well.

The effects of pH on xylonic acid secretion from immobilized recombinant E. coli are studied. It was found that an environment that is too acidic or too alkaline causes low yield of xylonic acid. This is because it could impose too much stress on the metabolic activities of cells (Che et al., 2015).

Next, five different expression media, were screened to determine their effect on xylonic acid production. The production of xylonic acid was found when the expression medium consist high amount of yeast and tryptone. As mentioned by Studier (2005), increasing the amount of peptone/tryptone or yeast extract will lead to higher cell densities thus this in turn elevates the secretion of xylonic acid.

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Reference


