Analysis of metabolites in the fermentation of *Pichia pastoris* for the production of phospholipase C (PLC)

Kyung-Ah Han[1,3], Yu Sun Baek[2,3] and Jong Il Rhee[1,3]
Department of Material and Biochemical Engineering[1], Faculty of Applied Chemical Engineering[2], Bioprocess Technology lab[3], Chonnam National University, Gwangju 500-757, Korea
Tel : +82-062-530-0847, Fax : +82-062-530-0846

Abstract

Analysis of extra- and intra-cellular metabolites is very important to study cell metabolism. The production and consumption of metabolites also depends on the activity of enzymes within cells. We made fermentation of *Pichia pastoris* using various carbon sources (ex. glycerol, xylitol, dextrose and etc.) in shake flasks. After centrifugation of cells the harvested cells are disrupted by ultrasonication (30 sec, 5 times). The supernatants were used to analyze intra/extra-cellular metabolites by HPLC system. For HPLC analysis, Bio-rad Aminex HPX-87H column, Waters Sugar-Pak column, Shodex KS-800 column and Atlatis™ dC18 column were used with UV and RID detector. For the activity determination of intracellular enzymes 96-well microplate reader is used based on the kinetic or end-point method. Other metabolites were measured by spectrophotometer. And the carbon sources were analyzed with thin layer chromatography (TLC) by ninhydrin reaction. The phospholipase C (PLC) activity was measured photometically with microplate reader.

References

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