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Productivity of γ-Linoleic acid by oleaginous fungus *Cunninghamella echinulata* using a pulsed high magnetic field

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ABSTRACT

γ-Linolenic acid (GLA) plays a crucial role in brain function and normal growth and development. High percentage of the population cannot effectively produce GLA, resulting in the use of GLA dietary supplements. Several oleaginous fungi can produce low level of GLA. The present study aimed to improve the production of GLA using physical mutagenesis. Spore suspensions of *Cunninghamella echinulata* were treated with pulsed high magnetic field. A total of 64 strains were isolated and screened using Nile red staining and fluorescence detection. Three mutants were selected after fermentation screening. GLA yields of Mut-29, Mut-15, and Mut-64 increased by 46.2%, 23.1%, and 19.2%, respectively, compared with that of the parent strain. Biomass, lipid content and yield, GLA content, and GLA yield of Mut-29 increased significantly ($P < 0.05$) by 3.60%, 22.2%, 26.4%, 15.4%, and 46.2%, respectively, compared with those of the parent strain. The effects of different factors on lipid and GLA production were investigated to estimate the stability of the mutants. GLA yields from Mut-29 were 1.31 g L^{-1} and 1.28 g L^{-1} in medium with 1% soybean powder or 0.5% potassium nitrate, respectively. Result indicates that mutagenesis induced by pulsed high magnetic field can effectively improve GLA yield in *C. echinulata*.

1. Introduction

γ-Linoleic acid (GLA, 18:3 omega-6) is an essential polyunsaturated fatty acid (PUFA), commonly used in foods, cosmetics, and pharmaceuticals. GLA selectively kills cancer cells (Kenny et al., 2000) and also plays a role in several other diseases, such as osteoporosis, allergic rhinitis, and heart diseases (Goud, Neogi, & Saumya, 2007). GLA can control several other metabolic pathways and minimize cholesterol levels (Tauk-Tornisielo, Vieira, Cecília, Carneiro & Govone, 2007). GLA is a precursor of many biologically active compounds, such as prostaglandin E1 (Ronda & Lele, 2008; Somashekar, Venkateshwaran, Sambaiah, & Lokesh, 2003).

Although most commercial GLA products are derived from plant oil, mainly from evening primrose oil, plant-based GLA products cannot meet the increasing market demand. Microorganisms, such as molds and microalgae, can also produce GLA. The first commercial microbial GLA product was produced from *Mucor circinelloides* in the United Kingdom (Sinden, 1987). Thus, microorganisms are considered to be promising alternatives for GLA production. Zygomycetes, including *Mortierella*, *Cunninghamella*, *Rhizopus*, *Mucor*, and *Thamnidium*, are efficient GLA-producing microorganisms (Fakas et al., 2008; Kennedy, Reader, & Davies, 1993). *Mortierella* and *Mucor* have been used for industrial scale production of GLA in Japan (Conti, Stredansky, Stredanska, & Zanetti, 2001). Oleaginous fungi can also synthesize other PUFAs with biological activities similar to those of GLA, such as arachidonic acid (AA, C20:4) and eicosapentaenoic acid (EPA, C20:5) (Totani, Watanabe, & Oba, 1987). However, GLA, even from microorganisms, is expensive because of low yield. Thus, screening high GLA strains using either natural selection or mutagenesis may be beneficial.

Physical mutagenesis, including UV, X-ray mutagenesis and so on, is one of the most important and efficient technique for breeding of industrial microorganisms. A magnetic field is an inescapable environmental factor for living organisms on the Earth. Krylov and Tarakonova (1960) firstly reported its effects on plants. There are some reports about magnetic field effects on microorganisms. The growth of *Escherichia coli* was inhibited in the presence of magnetic field of up to 0.6 T (Zhang, Wei, Zhang, Mao, & Liu, 2002). Yeast proliferation rate decreased after exposure to high magnetic field of 14 T (Iwasaka, Ikehata, Miyakoshi & Ueno, 2004). A strong magnetic field is a more dominant factor influencing changes in the production of secondary metabolites of *Streptomyces avermitilis*, and magnetic field at 12 T resulted in sporulation suppression and mycelial biomass decrease with

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