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Growth and lovastatin production by *Aspergillus terreus* under different carbohyrates as carbon sources

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ABSTRACT

Carbon source is a key component of metabolites synthesis in microorganisms. This work examined the effects of selected carbon sources in the form of carbohydrates, on the growth of *Aspergillus terreus* ATCC 20542 and the production of lovastatin. Slowly metabolised carbohydrates, such as D-galactose (consumption rate, r = 3.11), produced a high microbial biomass, X_{FINAL} (9.44 g/L) compared to other carbohydrates, but with a low biomass yield coefficient ($Y_{LOV/X}$ = 1.68). In contrast, D-ribose ($Y_{LOV/X}$ =) which showed moderate biomass growth (X_{FINAL} = 8.78 g/L) and consumption rate (r = 5.44 g/day), produced the highest lovastatin amount (51.81 mg/L, day 6). These indicate little correlation between biomass growth and lovastatin production. Notably, culture consisting of pellets with short hairy surface feature is associated with enhanced lovastatin production. Our findings suggest that the production of lovastatin by *Aspergillus terreus* is highly influenced by the choice of carbohydrates that will shape the pellet morphology rather than the rate of carbohydrates metabolism.

1. Introduction

Lovastatin ($C_{24}H_{36}O_5$) is a commonly prescribed cholesterol-lowering drug belonging to the statins group that acts by competitively inhibiting the formation of 3-hydroxy-3-methyl glutaryl CoA (HGM-CoA) reductase, a rate limiting enzyme of cholesterol biosynthesis in the liver. This compound is produced naturally by a few fungus species including the commonly known *Aspergillus terreus* ATCC 20542 (Manzoni et al., 1998). Although unclear, lovastatin is thought to have a protective role in fungus on the basis that it is effective at reducing sterols important for the growth of other microbes (Debakey and Endo, 2008). Recent studies also suggest that lovastatin may have pharmacological potential beyond cholesterol-lowering ability, such as for the treatment of cancer (Hindler et al., 2006), Alzheimer's disease (Sparks, 2011) and osteoporosis (Gonyeau, 2005).

The factors that influence the production of lovastatin include the types of carbon and nitrogen source (Hajjaj et al., 2001), vitamin B content (Bizukojc et al., 2007) and physical factors such as aeration rate (Bizukojc and Ledakowicz, 2008), dissolved oxygen concentration (Casas López et al., 2004), pH (Lai et al., 2005), and types of fermentation (Barrios-González and Miranda, 2010). One of the most important aspects, carbon source, has been widely studied in terms of its impact on the growth and metabolism of *A. terreus* (Casas López et al., 2003; Hajjaj et al., 2001; Jia et al., 2009a, 2009b; Kumar et al., 2000). A higher ratio of carbon to nitrogen (also known as 'nitrogen starvation') is thought to be an important factor in lovastatin production (Casas López et al., 2003). Similarly, slowly-metabolised carbon sources, such as α-lactose and glycerol, are better carbon choices which favour higher lovastatin production (Casas López et al., 2003). The use of rapidly-metabolised carbon sources, such as D-glucose, may lead to reduced lovastatin production via catabolite repression mechanism (Lai et al., 2007). It also favours the production of primary metabolite such as itaconic acid (Lai et al., 2007). Despite this, some studies have not reported repressed lovastatin yield when these carbons (D-glucose and fructose) were used (Casas López et al., 2003; Hajjaj et al., 2001).

The present study investigated the effects of different sources of carbon in the form of carbohydrate on the growth and metabolite production of *A. terreus* under similar culture conditions. Here, we evaluated the effects of different carbohydrates comprised of monosaccharides (D-glucose, D-xylose, D-fructose, D-galactose, D-mannose, D-ribose, D-arabinose) and disaccharides (α-lactose, maltose, D-sucrose) on lovastatin production and the morphology of *A. terreus*.

Abbreviations: r, consumption rate; $Y_{LOV/X}$, biomass yield coefficient; $Y_{LOV/S}$, substrate yield coefficient; X_{FINAL}, biomass growth; u_{max}, specific growth rate

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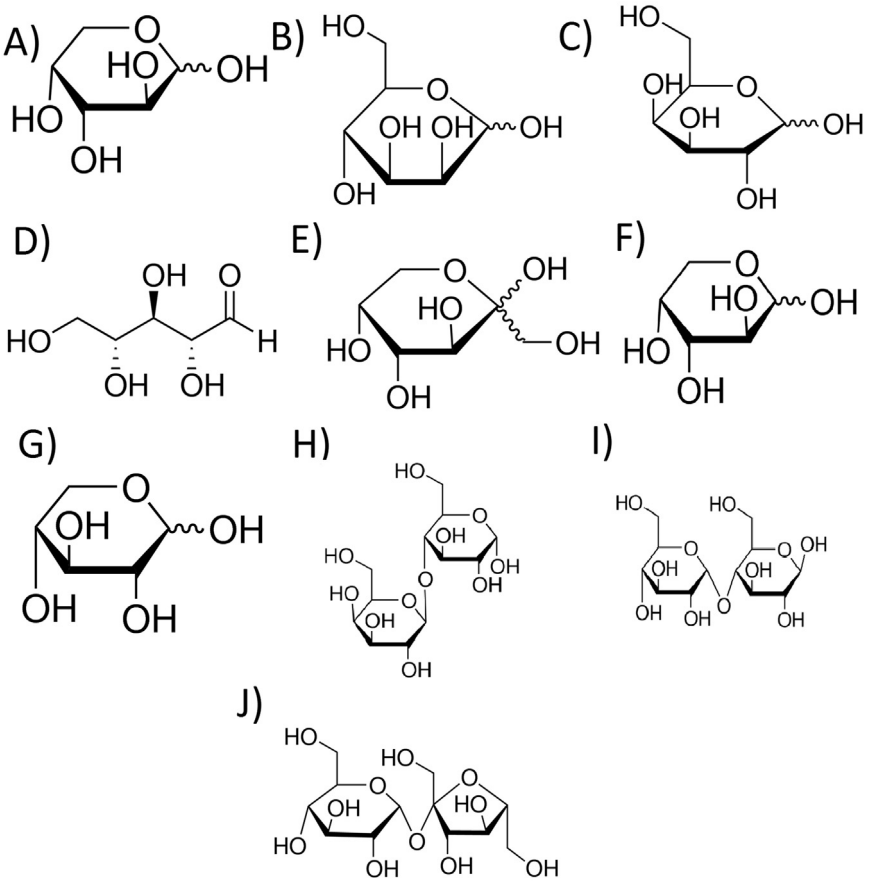

Fig. 1. The chemical structure of monosaccharides and disaccharides (pentoses and hexoses) used in this study. The chemical structures have been shown to influence the carbon consumption and metabolism in several microorganisms (Fernandes and Murray, 2010; Peterson et al., 1922). From top left: A) D-glucose, B) D-mannose, C) D-galactose, D) D-ribose, E) D-fructose, F) D-arabinose, G) D-xylose, H) α-Lactose, I) D-maltose, J) D-sucrose.

2. Materials and methods

2.1. Culture condition

The culture conditions used in this study have been described previously with slight modifications (Abd Rahim et al., 2015). The basal media contained minimal salt media (containing KH_2PO_4, 0.4g/L, $MgSO_4{\bullet}_7H_2O$, 0.2g/L, and $NaCl_2$ 0.4g/L) to ensure that the effect on the yield and morphology is due to the carbon sources. 1 mL aliquot of spore suspension (10^7 spores/mL) was inoculated into 125 mL conical flask containing 50 mL of media at 30 °C, 180 rpm in a shaking incubator. Seed culture was performed 24–30 h prior to the actual experiment, using 8g/L of yeast extract and 10g/L of α-lactose in the basal salt media to allow the fungus to achieve exponential growth before cultivation.

Ten different carbohydrates comprised of monosaccharides (D-glucose, D-xylose, D-galactose, D-mannose, D-arabinose, D-fructose, D-ribose) and disaccharides (D-maltose, α-lactose, D-sucrose) were used in this study at 20 g/L (Fig. 1). Glycerol was used in control experiment. Initial pH was kept at 6.5. No further pH control was applied during the duration of the experiment.

2.2. Dry cell weight determination

The biomass yield was determined gravimetrically. The recovery of fungus biomass was carried out through filtration on No. 2 Whatman filter paper. The biomass was washed twice with distilled water, followed by drying at 80 °C for 24 h or until constant weight is achieved.

2.3. Analytical method

Lovastatin (Sigma Aldrich, Sydney, Australia) standard was prepared according to manufacturer's instruction. The main analysis was performed using High Performance Liquid Chromatography (HPLC), Agilent 1200, using a UV detector at a wavelength of 238 nm, with a reference wavelength of 360 nm. The column used was XDB Eclipse Zorbax C-18. Parameters used for HPLC were as follows: Column temperature at 30 °C, sample chamber temperature at 4 °C, flow rate at 1 mL/min, injection volume at 10 μL and 95% acetonitrile and 0.1% phosphoric acid solution as mobile phases. The resultant HPLC peaks are shown in Fig. 2.

Glycerol concentration was determined using free glycerol calorimetric detection kit (Sigma-Aldrich), which is based on fluorescence reading at 575 nm. The sugar content in the samples was determined using phenol-sulfuric acid method (Masuko et al., 2005) with slight modification.

2.4. Image capture procedure

The images of the fungal pellet were taken using Digital Blue QX7 Computer Microscope (Digital Blue, Atlanta, USA), a light microscope equipped with camera. The fungal pellets were harvested from the fermentation system at day 4 (where the fully formed morphology was observed). The pellets were placed on a microscope slide and the excess liquid around the pellet was dried using Kimtech Wipes. The images were taken immediately following the drying procedure.