UNIVERSITI PUTRA MALAYSIA

THE ISOLATION OF KOJIC ACID PRODUCING FUNGI AND THE OPTIMIZATION OF KOJIC ACID PRODUCTION

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BY

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SPECIALLY DEDICATED TO;

HAJJAH KALTHOM ABDUL MAJID (EMAK)

HAJI SALLEH BIN SATAK (ABAH)

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HAJJAH KAMARIAH, ISMAIL, ISHAK AND ILIAS

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"THANKS FOR YOUR LOVING SUPPORT DURING THE DIFFICULT TIME'

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h	:	Hour
QO_2	:	Specific Oxygen Uptake Rate (mmol O_2/ g Cell)
P	:	Product Concentration (g/L)
X	:	Cell Concentration (g/L)
t	:	Time (hour)
DOT	:	Dissolved Oxygen Tension (%)

Abstract of thesis submitted to the Senate of Universiti Pertanian Malaysia as fulfilment of the requirements for the degree of Master of Science.

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MADIHAH MD SALLEH

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Chairman : Professor Mohammed Ismail Abdul Karim, PhD.

Faculty : Food Science and Biotechnology

A local strain of fungus, which was found to be a high kojic acid producer has been isolated from Morning glory flower (*Bixa orellana*) using glucose-peptone medium. The pure strain was obtained through several steps of monospore isolation procedures using spread plate technique and identified by International Mycological Institute as *Aspergillus flavus* Link.

Optimization of medium composition and cultural conditions for kojic acid production by this fungus were carried out in shake flask. The development of dissolved oxygen tension (DOT) control strategies aimed at improving kojic acid production by this strain was carried out using 1.5 L stirred tank reactor with automatic pH and DOT control systems. Initial culture pH 3 was found to

be optimum for kojic acid production. This strain was able to grow and produce kojic acid in various carbon sources such as glucose, starch, sucrose and xylose. However, the highest production of kojic acid was obtained at 100 g/L (w/v) glucose. In addition, 5 g/L of yeast-extract was found to be the best nitrogen source for fungal growth and enhancement of kojic acid production. Addition of 4% (v/v) of methanol to glucose yeast-extract medium increased kojic acid production by two times. The optimized medium for kojic acid production for this strain was proposed and the medium consisted of 100 g/L glucose, 1.0 g/L KH_2PO_4, 0.5 g/L $MgSO_4$. $7H_2O$, 5.0 g/L yeast-extract and 4% methanol. Using this optimal medium the maximum kojic acid production in batch fermentation using shake flask was 39 g/L. This fermentation gave yield and productivity of 0.04 g/g and 0.07 g/L/h, respectively and is comparable to that reported in the literature for industrial strain.

The DOT control strategy for the improvement of the kojic acid production by this strain was also developed. By controlling DOT at 80% saturation during growth phase and then switched to 30% saturation during production phase, increased the production of kojic acid by about two times as compared to uncontrol fermentation in the stirred tank fermenter which only produced maximum concentration of kojic acid of 15 g/L.

The effect of the addition of yeast-extract during the fermentation was also investigated using a constant fed-batch culture. The addition of 15 g/L yeast-extract at constant flow rate of 3.2 ml/h during the production phase caused excessive mycelial growth and decreased kojic acid production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi keperluan Ijazah Master Sains

PEMENCILAN FUNGI YANG MENGHASILKAN ASID KOJIK DAN PENGOPTIMUMAN PENGHASILAN ASID KOJIK

OLEH

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Kulat tempatan yang berupaya menghasilkan asid kojik yang tinggi telah dipencilkan daripada bunga Kembang Pagi (*Bixa orellana*) dengan menggunakan media glukosa-pepton. Strain tulin yang didapati melalui beberapa peringkat pemencilan spora tunggal dengan menggunakan teknik plat sebaran telah dikenalpasti oleh Institut Antarabangsa Mikologi sebagai *Aspergillus flavus* Link.

Pengoptimuman komposisi media dan keadaan kultur untuk penghasilan asid kojik oleh kulat ini telah dijalankan dalam kelalang penggoncang. Strategi pengawalan kepekatan oksigen terlarut (DOT) dengan tujuan untuk

meningkatkan asid kojik oleh strain ini telah dijalankan dengan menggunakan fermenter berpengaduk dengan sistem kawalan pH dan oksigen terlarut secara automatik. pH awalan optimum bagi penghasilan asid kojik adalah 3. Strain ini berupaya tumbuh dan menghasilkan asid kojik dalam pelbagai sumber karbon seperti glukosa, kanji, sukrosa dan xilosa. Walaubagaimanapun, penghasilan asid kojik tertinggi didapati pada 100 g/L glukosa. Selain daripada itu, 5 g/L yis-ekstrak adalah sumber nitrogen terbaik untuk pertumbuhan kulat dan peningkatan penghasilan asid kojik. Penambahan 4% metanol ke dalam media glukos yis-ekstrak meningkatkan penghasilan asid kojik sebanyak dua kali ganda. Media optimum untuk penghasilan asid kojik oleh strain ini adalah terdiri daripada 100 g/L glukosa, 1.0 g/L KH_2PO_4, 0.5 g/L $MgSO_4$. $7H_2O$, 5.0 g/L yis-ekstrak dan 4% metanol. Penggunaan media optimum dalam kultur sesekelompok dengan menggunakan kelalang penggoncang menghasilkan maksimum asid kojik 39.9 g/L. Fermentasi ini juga memberikan 0.04 g/g kadar penghasilan serta 0.07 g/L/h produktiviti dan hasil kajian ini adalah sama seperti strain industri yang telah dilaporkan oleh kajian bahan bertulis.

Strategi pengawalan oksigen terlarut untuk meningkatkan penghasilan asid kojik telah dijalankan. Pengawalan oksigen terlarut pada ketepuan 80% semasa fasa pertumbuhan dan diubah ke 30% semasa fasa penghasilan meningkatkan penghasilan asid kojik dua kali ganda berbanding dengan

fermentasi tanpa kawalan di dalam fermenter berpengaduk yang menghasilkan kepekatan asid kojik maksimum 15 g/L.

Kesan penambahan yis-ekstrak semasa fermentasi juga dikaji dengan menggunakan kultur suapan sesekelompok tetap Penambahan 15 g/L yis - ekstrak pada kadar aliran tetap 3.2 ml/j semasa fasa penghasilan menyebabkan peningkatan pertumbuhan kulat dan penurunan penghasilan asid kojik.

CHAPTER I

INTRODUCTION

Kojic acid is secreted by several microorganisms such as *Aspergillus oryzae* and *Aspergillus flavus*. This crystalline substance was first isolated by Saito in 1907 from the mycelia of *Aspergillus oryzae* grown on steamed rice. The chemical structure was determined as 5-hydroxy-2-hydroxymethyl-δ pyrone by Yabuta (1924). Although kojic acid can be synthesized from chemical conversion of various substrates, commercially it is produced by aerobic fermentation of *Aspergillus* species.

Kojic acid has several economic uses in various fields. In medical field, kojic acid is used as an antibacterial and antifungal agents. In the food industries, kojic acid is used as an antispeck and antimelanosis (blackening) agents for agricultural products. Since Malaysia has many agricultural products, the use of kojic acid will be economically important in the post harvest process. In addition, kojic acid is also used as a chelating agent and activator in insecticide production. Recently, a new application of kojic acid is found in the cosmetic industry. It is used as a whitening agent and ultraviolet filter in skin care products.

Although kojic acid has been produced and applied industrially, attempts to improve kojic acid production are still been intensively studied. Two main areas which are normally considered for the improvement of kojic acid production are the improvement of strain and development of the fermentation process. Screening of high kojic acid producer from various sources and also improvement of strain through mutation process had been conducted in the last few years. Although works on optimization of medium composition and environmental condition for kojic acid production by microorganisms have been studied extensively, the effect of each nutrient component on kojic acid production had not been well described.

Recently, the use of immobilized viable cells as an approach to enhance kojic acid production has also been reported (Kwak et al., 1991). However, work on the improvement of the production of kojic acid using different free cell fermenter operations such as fed-batch culture in bioreactor is not reported. Information on the influence of aeration condition on kojic acid production is also scarce.

The objectives of the research is to (1) screen and isolate potential kojic acid producer from local sources including flowers, fruits, fermented food and hot spring (2) optimize the culture medium condition using various nitrogen and carbon sources and stimulant, (3) study on the effect of dissolved oxygen

tension (DOT) during kojic acid fermentation in batch culture using without DOT control, single phase DOT control and two phases DOT control and (4) investigate the effect of the addition of nitrogen source (yeast-extract) during kojic acid production phase in fed-batch culture.

CHAPTER II

LITERATURE REVIEW

Properties of Kojic Acid

Kojic acid is a monocyclic pyrone consisting of carbon ring with two double bonds (Figure 1) The hydroxyl group at position carbon 5 have weakly acidic characteristic that are capable of producing salts with few metals The side chain of C5 also behave as a primary alcohol whose reactivity was enhanced by the adjacent oxygen atom in the nucleus (Beelik, 1956) Kojic acid forms a complex with ferric ions to produce reddish purple colour which has maximum absorption at 500 nm (Bently, 1957; Crueger et al , 1984) This colorization is widely used as a principle method for the quantitative determination of kojic acid

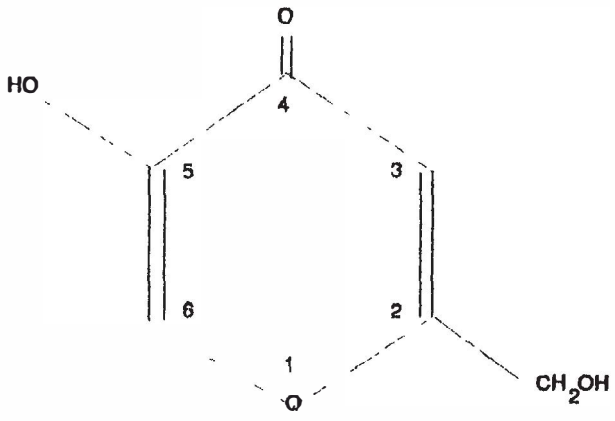

Figure 1 : Structure of Kojic Acid

(Source · Beelik, 1956)