UNIVERSITI PUTRA MALAYSIA

BIOCONVERSION OF GELATINISED SAGO STARCH TO FERMENTABLE SUGAR USING RECOMBINANT *SACCHAROMYCES CEREVISIAE*

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By

AZLIAN MOHAMAD NAZRI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of Requirements for the Degree of Master of Science

March 2004

Specially dedicated to,

mak, ayah
and my family

I love you all

Yang 2004

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

BIOCONVERSION OF GELATINISED SAGO STARCH TO FERMENTABLE SUGAR USING RECOMBINANT *SACCHAROMYCES CEREVISIAE*

By

AZLIAN BINTI MOHAMAD NAZRI

March 2004

Chairman: Suraini Abd-Aziz, Ph.D.

Faculty: Food Science and Biotechnology

Bioconversion of sago starch to fermentable sugar was investigated using three genetically modified *Saccharomyces cerevisiae* strains, YKU107 (expressing α-amylase), YKU131 (expressing glucoamylase) and YKU 132 (expressing α-amylase and glucoamylase). Alpha-amylase (YKU107) and glucoamylase (YKU131) was partial purified using acetone and ammonium sulphate precipitation, respectively before characterisation studies were carried out. The enzymes were purified by about 2.78 and 1.08 fold with recovery of 41.93% and 33.64%, respectively. Through DEAE-cellulose column chromatography, only 26.31% α-amylase and 36.68% glucoamylase were recovered with purification fold of 6.90 and 1.81. Futher characterisation showed that both enzymes were stable at pH 5.5, temperature 30^{o}C and ionic strength of 0.05 M, evidenced with residual activity higher than 90%. Optimum pH, temperature and initial starch concentration for glucose production were determined as 5.5, 30^{o}C and 20gL^{-1}, respectively. From influence of various starches studied, potato starch was hydrolysed efficiently, followed by corn, sago, cassava and rice starch. However, the maximum yield of glucose based on utilised

starch followed the sequence: sago > corn > potato > cassava > rice starch. Batch fermentation using 2 L fermenter showed that strains YKU107, YKU131 and YKU132 were able to hydrolyse about 97.82%, 86.86% and 88.06%, respectively during 60 hours cultivation with maximum glucose concentration of 9.32 gL^{-1}, 3.63 gL^{-1} and 0.85 gL^{-1}, respectively. Based on maximum glucose production, YKU107 was selected for futher studies. The influence of rpm examined by this strain indicated that the glucose production consistently increased with rpm. Repeated-batch fermentation at maximum glucose concentration produced 6.91 gL^{-1} of glucose and 12.35 gL^{-1} of biomass. The continuous culture was performed in order to increase the glucose production. The maximum glucose concentration of 7.80 gL^{-1} was obtained at 0.075 h^{-1} dilution rate and suggested that the optimum operating conditions for glucose production is just at the critical dilution rate. The plasmid was categorised as stable even after 348 hours of continuous cultivation (43 residence times).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

BIOPENUKARAN KANJI SAGU KEPADA GULA FERMENTASI OLEH REKOMBINAN *SACCHAROMYCES CEREVISIAE*

Oleh

AZLIAN BINTI MOHAMAD NAZRI

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Pengerusi: **Suraini Abd-Aziz, Ph.D.**

Fakulti: **Sains Makanan dan Bioteknologi**

Biopenukaran kanji sagu kepada gula fermentasi telah dijalankan dengan menggunakan strain rekombinan yis *Saccharomyces cerevisiae* iaitu YKU107 (menghasilkan α-amilase), YKU131(menghasilkan glucoamilase) dan YKU 132 (menghasilkan α-amilase dan glucoamilase). Alpha-amilas (YKU107) dan glucoamilas (YKU131) telah ditulenkan pada peringkat pertama menggunakan kaedah pemendakan Aceton dan Sodium sulfat masing-masing sebelum kajian tentang pencirian dilakukan. Setelah dimendakkan, enzim-enzim ini telah ditulenkan kira-kira 2.78 dan 1.08 kali indeks dengan peratus pendapatan semula 41.93 dan 33.64, masing-masing. Melalui kromatografi turus DEAE-selulosa, hanya 26.31% α-amilase dan 36.68% glucoamilase didapati semula dengan indeks penulenan masing-masing 6.90 dan 1.81. Pencirian menunjukkan bahawa kedua-dua adalah stabil pada pH, suhu dan kekuatan ion masing-masing 5.5, 30^{o}C dan 0.05 M dibuktikan dengan aktiviti melebihi 90%. PH, suhu dan kepekatan awal kanji optima untuk penghasilan glukosa telah didapati seperti 5.5, 30^{o}C dan 20gL^{-1} masing-masing. Dari kajian kesan pelbagai jenis kanji, kanji kentang telah dihidrolisis dengan berkesan diikuti oleh jagung, sagu, ubi kayu dan beras. Hasil maksima glukosa berdasarkan kanji yang

telah diguna adalah mengikut turutan sagu > jagung > kentang > ubi kayu > beras. Fermentasi sesekelompok menggunakan fermenter 2 L menunjukkan strain YKU107, YKU131 dan YKU132 masing-masing boleh menghidrolisis sebanyak 92.82%, 86.86% dan 88.06% dalam 60 jam dengan kepekatan glukosa maksima 9.32 gL^{-1}, 3.63 gL^{-1} dan 0.85gL^{-1}, masing-masing. Berdasarkan kepada keupayaan penghasilan maksimum glukosa, strain YKU107 telah dipilih untuk kajian selanjutnya. Kajian kesan goncangan ke atas strain ini menunjukkan penghasilan glukosa adalah berkadar terus dengan kadar goncangan. Fermentasi sesekelompok-berulang pada kepekatan glucosa maksima menghasilkan 6.91 gL^{-1} glucose dan 12.35 gL^{-1} sel. Fermentasi selanjar telah dijalankan bertujuan untuk meningkatkan penghasilan glukosa. Kepekatan glukosa iaitu 7.80 gL^{-1} telah diperolehi pada kadar dilusi 0.075 h^{-1} seterusnya mencadangkan bahawa keadaan operasi optima untuk penghasilan glucosa secara selanjar adalah pada kadar dilusi kritikal. Plasmid dikategorikan adalah stabil walaupun setelah menjalani fermentasi selanjar selama 348 jam (43 kali masa residen).

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I certify that an Examination Committee me on 5 March 2004 to conduct the final Examination of Azlian binti Mohamad Nazri on her Master of Science thesis entitled "Bioconversion of Gelatinised Sago Starch to Fermentable Sugar using Recombinant *Saccharomyces cerevisiae*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follow:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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DECLARATION

I here declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

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AZLIAN MOHAMAD NAZRI

Date:

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LIST OF ABBREVIATIONS

E.C.	Enzyme Commission
GA	Glucoamylase
rpm	Rotation per minutes
DEAE	diethylaminoethyl
μ_{max}	Maximum specific growth rate
$Y_{x/s}$	Yeild of cell on the basis of hydrolysed starch
$Y_{p/s}$	Yeild of glucose on the basis of hydrolysed starch
$Y_{p/x}$	Yield of glucose on the basis of biomass
dS/dt_{max}	Maximum starch hydrolysis rate during fermentation
P_{max}	Maximum glucose concentration during fermentation
X_m	Maximum cell concentration
t_m	Fermentation time, the time needed to reach the maximum glucose concentration
DOT	Dissolved oxygen tension
OD	Optical density
pH	Hydrogen potential
$(NH_4)_2\ SO_4$	Ammonium sulphate

CHAPTER 1

INTRODUCTION

The use carbohydrates as the carbon sources in microbial fermentation processes are common practice in the industry. In Malaysia, sago starch was reported to have the greatest potential for commercial production of glucose due to its relatively low prices and availability. Sago is an important source of industrial starch for local food industries. Glucose obtained from sago starch is used as a substrates for the fermentation industries as well as for the production of high fructose syrup. In industry, sago starch is also used as an ingredient in the production of monosodium glutamate and caramel. Sago starch is also used in the animal feed industry, the manufacturing of high fructose syrup as an alternative of sucrose and in gasohol fuel production (Zulpilip *et al*., 1990).

The hydrolysis of starch to glucose has been carried out in many studies and usually is made up of two distinct steps performed by two different enzymatic reactions using different conditions in a batch system (Berghoeer and Sarhaddar, 1988). The present study is to explore the possibility of converting sago starch to fermentable sugar biologically using recombinant yeast and also to determine the physicochemical properties of the starch.

A large variety of starches are used for this production around the world. In Asia it is not uncommon for the industries to use sago or tapioca starches for syrup production, depending on the availability and price, (Schenck and Habeda, 1992).

At present, the use of sago starch in Malaysia has been increasing and has a great potential to be utilized for the production of glucose due to its relatively low prices and abundance. Thus, the possibility of producing glucose from sago starch should be explored.

The yeast, *Saccharomyces cerevisiae*, is recognized as an ideal eukaryotic microorganism for biological studies and has been widely used as a host cell for foreign gene products due to the abundance of information that are available following the early development of recombinant DNA techniques for the microorganism. Furthermore, yeast has an ability to produce mature foreign protein from plants or animals.

The recombinant *Saccharomyces cerevisiae* obtained from the host strain YKU 76 named YKU 107 (expressing α-amylase), YKU 131 (expressing glucoamylase) and YKU 132 (expressing α-amylase and glucoamylase) were used for glucose production from sago starch, due to its abundance in Malaysia and relatively low prices.

Design of fermentation medium for glucose production must take into consideration factors beyond simple nutrition. It is not just the presence of a given nutrient in the medium that is important but also how it acts in terms of cell growth, the microorganism physiology and its ability to produce the enzyme. The medium composition is a communication code used to achieve the objectives of the fermentation processes, its effects must be well understood.

The work reported in this thesis has been aimed at the performance of these recombinant *Saccharomyces cerevisiae* strains using sago starch as substrate and to gain the optimum condition for bioconversion of starch to fermentable sugar by recombinant yeasts. Experiments have been carried out in shake flasks and 2L stirred tank fermenter and results have been obtained relating to the different parameters of cultivation conditions.

The objectives of the study are:

1) To investigate the performance of the recombinant yeast to hydrolyze sago starch into fermentable glucose;

2) To study the influence of initial starch concentration, pH, temperature, ionic strength and various types of starches on the glucose accumulated, activity and stability of α- as well as glucoamylase secreted by the recombinant yeast;

3) To select the best strain from kinetic analysis in relation to cell growth, substrate consumption, enzyme accumulation and glucose production;

4) To study the feasibility of using different agitation speeds, mode of fermentation operation, repeated batch and continuous culture for the improvement of sago starch hydrolysis by recombinant yeast.

CHAPTER 2

LITERATURE REVIEW

2.1 The Yeast and Its Properties

The yeast *Saccharomyces cerevisiae*, is recognized as an ideal eukaryotic microorganism for biological studies. Some of the properties that make yeast particularly suitable for biological studies include rapid growth, a budding pattern resulting in dispersed cells, the ease of replica plating and mutant isolation, a well-defined genetic system, and the most importantly, a highly versatile DNA transformation system. Moreover, it has been widely used as a host cell for foreign gene products due to the abundance of genetic information, microbiological and biochemical (Beggs, 1978; Hinnen *et al*., 1978). Besides, yeast has similar transcription, translation and secretion systems that ability to produce mature foreign protein from plants or animals. Therefore, yeast become an attractive host for production of useful animal or plant proteins, cheaply, maturely and in large amounts (Brunt, 1986; Romanos *et al*., 1992). Accordingly, protein production by recombinant yeasts is important in bioindustry, because yeasts perform many of the post-translational modifications characteristic of eukaryotes.

The used of *Saccharomyces cerevisiae* begun since prehistoric times in the making of breads and wines, but their cultivation and use in large quantities was put on a scientific basis by the work of the French microbiologist Louis Pastuer in the 19th century. Today they are used industrially in a wide range of fermentation processes, medicinally, as a source of B-complex vitamins and thiamine and as a