# PRODUCTION OF SELENIUM ENRICHED *Saccharomyces boulardii* IN PILOT SCALE BIOREACTOR

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## ABSTRACT

Selenium (Se) yeast has been widely used as a Se supplementation for humans. Supplementation with Se-enriched yeast in animal and human diet has been proven to have beneficial health effects. One major disadvantage in Se yeast production is the complexity in the yeast production. The objective of this study is to optimize cell mass production of *Saccharomyces boulardii* for Selenium enrichment process. Production of *S. boulardii* cell mass was optimized by using both classical and statistical approach. Production of high cell mass of *S. boulardii* was upscaled using a 16-L stirred tanked bioreactor in batch and fed-batch cultivation strategies where the fed batch bioreactor cultivation with complete medium showed the highest cell mass production at 34.16 g $L^{-1}$. During Se enrichment process, effects of different Se concentration and addition time were examined to maximize the Se absorption process by *S. boulardii*. The production of Se yeast was further upscaled in a 16-L stirred tank bioreactor in batch and fed batch cultivation strategies. In Se enrichment process 90 mg $mL^{-1}$ Se which added at 16 hour of cultivation time for 24 hour was found to be best condition for Se enrichment in *S. boulardii*. The process was used in fed-batch cultivation in 16-L stirred tank bioreactor with full medium. Maximum cell biomass was at 24.97 g $L^{-1}$ with 0.177 $h^{-1}$ specific growth rate. The highest Se content was achieved at 41.65 µg $g^{-1}$ with 1.78 µg $g^{-1}$ $h^{-1}$ absorption rate. Therefore it can concluded that addition of Se in late exponential phase of *S. boulardii* growth is the most suitable condition to minimize the inhibition effect on *S. boulardii* cell mass production and at the same time maximize the absorption of Se process.

## ABSTRAK

Selenium yis telah digunakan secara meluas sebagai sumber tambahan Se kepada manusia. Yis yang diperkaya dengan Se terbukti dapat meningkatkan kesihatan apabila diambil dengan berkala. Masalah utama yang dihadapi dalam penghasilan yis diperkaya dengan Se adalah kerumitan dalam penghasilan yis yang mampu bertahan dengan kesan-kesan sampingan akibat penggunaan Se. Matlamat kajian ini adalah untuk mengoptimumkan penghasilan sel *S. boulardii* untuk penghasilan yis yang diperkaya dengan Se. Penghasilan sel *S. boulardii* dioptimumkan menggunakan pendekatan klasikal dan statistikal. Media yang telah dioptimumkan digunakan untuk penghasilan sel *S. boulardii* di dalam skala yang lebih besar iaitu 16 liter bioreaktor dengan kaedah kelompok dan suapan kelompok. Penghasilan sel *S. boulardii* yang tertinggi pada 34.16 g $L^{-1}$ berjaya di capai melalui kaedah suapan kelompok dengan menggunakan suapan media lengkap. Yis yang diperkaya dengan Se di uji dengan pelbagai kepekatan dan masa tambahan Se untuk mengoptimumkan penyerapan Se oleh sel. *S. boulardii* diperkaya dengan Se di hasilkan dalam skala besar 16 liter bioreaktor dengan kaedah kelompok dan suapan kelompok. Jumlah kandungan Se menunjukan 90 mg $mL^{-1}$ dengan waktu penambahan Se selepas 16 jam dan rawatan selama 24 jam adalah kaedah yang paling sesuai untuk pengasilan *S. boulardii* diperkaya dengan Se. Jumlah kandungan Se tertinggi di hasilkan melalui kaedah suapan kelompok di dalam 16-L bioreactor dengan jumlah kandungan Se adalah sebanyak 41.65 µg $g^{-1}$dengan kadar penyerapan Se pada 1.78 µg $g^{-1}$ $J^{-1}$. Melalui hasil penyelidikan ini, tambahan Se pada hujung fasa eksponen *S. boulardii* dapat mengurangkan kesan yang merencatkan pertumbuhan *S. boulardii* dan pada masa yang sama memaksimumkan daya penyerapan Se.

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| | | |
|---|---|---|
| AAD | - | Antibiotic Associated Diarrhea |
| ATCC-MYA | - | American Type Culture Collection, Manassaas |
| CD | - | Crohn's Disease |
| CDW | - | Cell dry weight |
| $CO_2$ | - | Carbon dioxide |
| DO | - | Dissolved Oxygen |
| FAO | - | Food and Agriculture Organization |
| GI | - | Gastrointestinal |
| OD | - | Optical density |
| OD540 | - | Optical density at 540 nm |
| OD600 | - | Optical density at 600 nm |
| pH | - | potential of hydrogen |
| RSM | - | Response Surface Methodology |
| SCF | - | The Scientific Committee for Food |
| sp. | - | Species |
| *S.* | - | *Saccharomyces* |
| UV | - | Ulcerative colitis |
| ICP-MS | - | Inductively coupled plasma mass spectrometry |

# LIST OF CHEMICALS

| | | |
|---|---|---|
| C | - | Carbon |
| $CaCl_2$. $2H_2O$ | - | Calcium chloride dehydrate |
| $CoCl_2.6H_2O$ | - | Cobalt Chloride, hexahydrate |
| $CuSO_4$. $5H_2O$ | - | Copper (II) sulfate pentahydrate |
| DNS | - | 3, 5-dinitro-salicylic acid |
| $FeCl_3.6H_2O$ | - | Iron (III) Chloride, hexahydrate |
| $FeSO_4$. $7H_2O$ | - | Iron (II) sulfate heptahydrate |
| $H_2O$ | - | Water |
| $H_2PO_4$ | - | Hydrogen phosphate |
| $H_3PO_3$ | - | Phosphorous Acid |
| HCl | - | Hydrochloric acid |
| $HNO_3$ | - | Nitric Acid |
| $K_2HPO_4$ | - | Dipotassium phosphate |
| $KH_2PO_4$ | - | Monopotassium phosphate |
| $MgCl_2$. $6H_2O$ | - | Magnesium sulfate hexahydrate |
| $MgSO_4$ | - | Magnesium sulfat |
| $MgS0_4.7H_2O$ | - | Magnesium sulfate heptahydrate |
| $MnSO_42H_2O$ | - | Manganese Sulfate |
| $Na_2SO4$ | - | Sodium sulfate |
| NaCl | - | Sodium Chloride |
| $NaNO_3$ | - | Sodium Nitrate |
| NaOH | - | Sodium hydroxide |
| $NH_4CI$ | - | Ammonium chloride |

| | | |
|---|---|---|
| $(NH_4)_2SO_4$ | - | Ammonium sulfate |
| Se | - | Selenium |
| YPD | - | Yeast Peptose Dextrose |
| $ZnSO_4$. $7H_2O$ | - | Zinc sulfate heptahydrate |

## LIST OF SYMBOLS

| | | |
|---|---|---|
| °C | - | Degree Celsius |
| μ | - | Specific growth rate [$h^{-1}$] |
| $t_d$ | - | Doubling time [$h^{-1}$] |
| % | - | Percentage |
| F | - | Feed Rate [g $L^{-1}$ $h^{-1}$] |
| v/v | - | Volume per volume |
| vvm | - | Volume per volume per minute |
| X | - | Biomass concentration [g $L^{-1}$] |
| H | - | Hour |
| Nm | - | Nanometer |

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# CHAPTER 1

# INTRODUCTION

## 1.1 Research Background

Probiotic organisms or biotherapeutic agent can be defined as live microorganisms which feed on or use in adequate amounts will beneficially affects the host by improving its intestinal microbial balance (Wohlgemuth *et al*., 2010). The gastrointestinal (GI) microflora is a complex ecosystem that has to be equilibrium with the host. Whereby the clinical disorder within the GI might occur once the equilibrium state has been disturb. *Lactobacilli* and *Bifidobacteria* is one of the most famous probiotics bacteria since they are the normal inhabitants of the human gut. The discovery of the yeast strain that can withstand and grow optimally in 37 °C has managed to discover a new strain that have large potential to the gastrointestinal (GI) microflora. Although yeast accounts for only a minority of the organisms which making up the microbiota, it has larger cell size compared to bacteria whereby up to 10 times larger. Therefore it might represent a significant stearic hindrance for bacteria.

*Saccharomyces boulardii* is the only probiotic that has been proven effective in the double-blind studies and commonly found as a health supplement (Sazawal *et al*., 2006). It was discovered by a French Microbiologist, Henri Boulard in 1923 when he was searching for a new yeast strain for making wine that withstand of the high temperature (Malgoire and Vandenplas, 2000).

Nowadays *Saccharomyces boulardii* has been used in many countries either as preventive or therapeutic agent for diarrhea and also other GI disorders which cause by administration of antimicrobial agents. The properties that residue by *S. boulardii* has made it a great potential for probiotic agent. It can survive the transit through the GI tract, 37 °C of optimum temperature and also it can inhibit the growth in some microbial pathogen. Yeast is a good candidate for probiotic studies as it resistance to the local stresses when enter the GI tract such as enzymes, bile salts, organic acids and variation of pH and temperature.

During the $20^{th}$ century many research has been conducted in order understanding the mechanisms of action and its benefits to the host organism. The research has been progress as they managed to understand the mechanism of action of the *S. boulardii* to the host organism. It has been discovered that *S. boulardii* has efficacy as an adjuvant agent for the treatment of diarrhea and also has the efficiency to prevent the antibiotic associated diarrhea (AAD).

Trace elements is important for human body in maintaining normal and yet complex physiological functions related to growth and development. Unlike major elements, such as carbon, hydrogen, nitrogen, oxygen, chlorine, phosphorous, potassium, sodium and so on which are present as the major constituent of body tissues, trace elements are present in body tissues at sub $\mu g\ g^{-1}$ levels but often acts as essential factors or co factors in biological process. Among there trace elements, Selenium (Se)

and Arsenic (As) are the only metalloid which are considered to be essential in life (Gissel-Nielsenetal, 1984). From the periodic Table selenium compound can be found to be metallic and non metallic in characteristic and can form cationic and anionic compounds.

Selenium has been known for it is toxicity at high concentration and affects to the central nervous system (Diaz-Alcaron *et al*., 1994). However recently selenium has been recognize to play a role as essential dietary supplement for the human. Deficiency in Selenium uptake has been associated with loss of hair pigment and macrocytosis in intravenously fed children (Navarro and Cabrera, 2008).

Inorganic selenium is generally toxic compared to organically bound forms. Therefore the organically production of selenium is very important as it has role in human diet. The production of selenium yeast is a key factor to obtain organically and safe selenium uptake by human. Moreover discovery of the *S. boulardii* has provided advantageous for researchers to further study on the production of selenium enriched yeast that able to perform as probiotic yeast as well.

## 1.2 Problem Statement

One major problem in the previous experimental of Se enriched yeast production was the complexity in the yeast production process. Previous research was focus more on process which intended for the production of only final product which is Se without consideration of cell mass production. Since, early stage of yeast growth is very critical in the fermentation process even though large fermenter with complex system control (pH, aeration, DO, etc) are used in order to grow yeast in good condition. This is even more difficult to manage when Se has to be incorporated to the yeast during cultivation process. More over yeast cultures inoculated with Se in previous studied, seemed to indicate a stunted primary growth stage which can be related with an increasing in toxicity of the Se therefore resulting a limited final biomass as well as low Se incorporation rate.

On the other hand, In spite of many literatures published concerning the importance of *S. boulardii* and its medical applications, very little information are available for cultivation and cell mass production. Thus through optimization of cell mass production of *S. boulardii* will provide platform for understanding more on *S. boulardii* cultivation process. Apart from that production of Selenium enriched *S. boulardii* will provide new dimension of Se enriched yeast production as up to date, no literature to be found on the production of Selenium enriched *S. boulardii*.

## 1.3 Objective of the Study

The objective of this study is to optimize high cell mass cultivation of *S. boulardii* and to achieve a good process for selenium enriched *S. boulardii* production.

## 1.4 Scopes of Research

The scope of this research are :

1 Study high cell mass production of *S. boulardii*:

a) Media optimization study for high cell mass production of *S. boulardii* using classical and statistical approach in shake flask cultivation

b) Comparison between optimize and non- optimized media on cell mass production of *S. boulardii*

c) Batch cultivation of *S. boulardii* in a 16-L stirred tanked bioreactor for high cell mass production under controlled and uncontrolled pH

d) Fed-batch cultivation of *S. boulardii* in a 16-L stirred tanked bioreactor for high cell mass production

2 Study the cultivation of *S. boulardii* in Selenium supplemented media.

a) Treatment of different Selenium concentration on *S. boulardii* growth and Selenium enrichment in shake flask cultivation level

b) Study the effects of Selenium enrichment protocols in *S. boulardii* at various points at cell cycle.

c) Study of Selenium absorption kinetic in *S. boulardii* growth curve

d) Enrichment of Selenium in post harvest *S. boulardii* cultivation

e) Batch cultivation of Se enriched *S. boulardii* in a 16-L stirred tank bioreactor for production of Selenium enriched yeast

f) Fed-batch cultivation of Se enriched *S. boulardii* in a 16-L stirred tank bioreactor for high Selenium enriched cell production

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