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Optimization of various parameters for selenium enriched yeast production

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Abstract

Selenium is an essential trace element for humans, animals and poultry. It belongs to body's antioxidant defense system. There are two major sources of selenium i.e. organic and inorganic. Selenium enriched yeast is the finest source of organic selenium. Yeast production can easily be achieved in laboratory as it utilizes soluble sugars and organic acids to yield biomass with high protein content. In this study the experiments were designed for optimizing culture conditions for the production of selenium enriched yeast. The statistical analysis of the data was performed on statistical software JMP predicted variables for optimization. The data of variables covered a wide range of values for fermentation temperature, shaking speed, pH, incubation time, Selenium concentration and selenium adding time to built-in response for variables to specific yeast biomass. Morphological characteristics such as colonial morphology (colony shape, colour, and surface appearance) and yeast biochemical identification on metabolized glucose sucrose, lactose, cellibiose express genes that activate the synthesis of yeast. The optimized values for Temp 25°C, pH 4, Se conc. 30 µg/ml, addition of Se after 9 hr of incubation, shaking speed 130rpm and incubation time 48 hrs yielded total biomass (65.59g/L) and selenium accumulation (46.23mg/kg) respectively. It was concluded that using a culture medium supplemented with 30 µg/mL sodium selenite identified the optimum fermentation conditions initial sodium selenite (Na2SeO3) concentration, initial pH and temperature) from Saccharomyces cerevisiae for maximal total Se yield of selenium-enriched yeast.

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Introduction

Selenium is a vital trace element for humans, animals and poultry. Its deficiency is linked with various disease conditions including immune impairment. Selenium has distinctive place among feed-derived natural antioxidants (Aggarwal and Upadhyay, 2013). Being a part of specific seleno-proteins, selenium compounds improve immune system by producing certain cytokines secreted by immune cells of the body to combat antioxidant status, hence preventing tissues damages (Avery and Hoffmann, 2018). As part of glutathione peroxidase (GSH-Px) Se belongs to the first and second major levels of antioxidant defense in the cell. Selenium supplementation also increases feed intake, body weight gain, feed efficiency, egg quality and production under heat-stressed conditions (Surai, 2002). Nutritional approaches intended to reduce the adverse effects of heat stress by supplementing micronutrients to fulfill the special requirements during heat stress have been proven beneficial. Nutrition under heat stress is one of the major areas of research receiving great attention for several years.In poultry industry higher production performance and feed conversion efficacy makes today's chickens more vulnerable to heat stress than ever before. Enhancing the bird's thermo-tolerance is one of the most promising management approach in promoting the heat resistance in chickens (Diarra and Tabuaciri, 2014). There are two major sources of Se for poultry i.e. organic selenium, mainly in the form of seleno-methionine (SeMet) found in feed ingredients in varying concentrations and inorganic selenium, mainly selenite or selenate, which is widely used for dietary supplementation.

Selenium yeast is the finest source of organic selenium. The major difference in metabolism and efficiency of both forms of selenium, with SeMet being more effective. In fact SeMet possesses antioxidant properties, however, in some conditions selenite can be a pro-oxidant. In general, adequate Se supplementation is considered to be a crucial factor in maintaining high productive and reproductive characteristics of commercial poultry (Briens *et al*., 2013).

Saccharomyces cerevisiae growing in Se-enriched media, yeast can accumulate large amount of Se and incorporate them into organic Se-containing compounds, mainly Se-Met, Na_2SeO_3 that are bio-transformed to organic form and being absorbed by the yeast. Through this practice the low bio available and more toxic the inorganic Se can be transformed to highly bioactive and more safer organic source with improved nutritional properties. It also contains all the nutrients of yeast i.e. high quality protein, vitamins, minerals, dietary fiber and essential amino acids. Yeasts have a great amount of protein as compared to other plant sources. It can integrate Se by replacement of sulphur atom in protein. Yeast production can easily be managed in laboratory as it utilizes soluble sugars and organic acids to yield biomass with high protein content (Esmaeili *et al*., 2012).

Several culture conditions of Se-enriched yeast production has been described but none of these research were based on the simultaneous screening of these variables.

The current experimental designs grounded on arithmetical scheming provided foundation for optimizing culture conditions of cell growth and the production of selenium enriched yeast. The leading parameters of optimization for fermentation included temperature, pH, incubation time, shaking speed, selenium concentration and selenium adding time. Therefore the present trial was designed to optimize the leading parameters of fermentation for the production of Se enriched yeast.

Materials and methods

This study was conducted at Center of Biotechnology and Microbiology (COBAM) and Centralized Resource laboratory (CRL) University of Peshawar during summer 2018-19. This study aimed to optimize experimental conditions for the production of selenium enriched yeast.

The *Saccharomyces cerevisiae* already isolated at this center was used in current study. Sodium selenite used for the enrichment of *Saccharomyces cerevisiae* was obtained from Sigma Chemical Company Karachi.

All other chemicals used for enrichment of culture included ammonium nitrate, zinc chloride sugar sources, and magnesium chloride pantothenates were procured from Merck, Rawalpindi, Pakistan.

Morphological, Biochemical and Molecular identification of Saccharomyces cerevisiae

The inoculated yeast extract peptone dextrose (YEPD) media plates on spread plate technique were incubated at 30°C for 48 hours. The morphological identification of colonies on yeast extract peptone dextrose (YEPD) agar were performed.

Colonies were observed for the different characteristics including shape size color (Nasir *et al*., 2017). Microscopic examination was done with gram staining and wet mount slide method (McDonald, 1963). Sugar fermentation tests were conducted for further confirmation (Oliveira *et al*., 2008).

The results were predicted on the basis of taxonomic classification as illustrated in previous study (Kurtzman *et al*., 2011). Molecular identification *S. cerevisiae* specific 301-bp sequence in polymerase chain reaction (PCR) was used. The following primers were used (Khan, 2017).

- SC1d 5\-ACATATGAAGTATGTTTCTATATAACG GGTG-3\
- SC1r 5\-TGGTGCTGGTGCGGATCTA-3\

PCR products was observed via electrophoresis to obtain clear bands of both PCR products and 1kb DNA ladder and the gel patterns were observed under UV light.

Optimization of various parameters for growth of Saccharomyces cerevisiae isolates

Optimization of different growth parameters, experiments were designed using statistical software, JMP trail 14.3.0 (64 bit) (Khan, 2017). Table (1) presents the high and low level of each variable. All variables were executed in triplicate and mean values were recorded. Shaking incubator, 3016R Lab Tech was used withdifferent parameters such as pH, temperature, shaking speed, Selenium adding time, Selenium concentration and incubation time were optimized. Confirmation for production of optimum microbial biomass, trail was performed under the optimum conditions as predicted by the model.

Table 1. Variables to be monitored in JMP model for Selenium enriched yeast mass production.

Factors	Variables	High Value	Low Value
Temperature	A	35°C	25°C
pH	B	6	4
Shaking Speed	C	130 rpm	110rpm
Selenium concentration added	D	30µg/ml	15 µg/ml
Selenium adding time	E	09hrs	03hrs
Incubation time	F	48hrs	24hrs

Table 2. Twelve experiments to study of six variable factors in total biomass yield and organic Se formation.

S.No	Temperature (°C)	Concentration of selenium added (µg/ml)	Selenium adding time (Hrs)	Incubation time (Hrs)	pH	Shaking speed (rpm)
1	25	15	3	24	6	130
2	25	30	3	48	4	110
3	35	15	3	48	6	110
4	35	30	3	24	4	130
5	35	30	9	24	6	130
6	25	30	3	24	6	110
7	25	30	9	48	4	130
8	35	15	3	48	4	130
9	35	15	9	24	4	110
10	25	15	9	48	6	130
11	35	30	9	48	6	110
12	25	15	9	24	4	110

Fermentation Conditions

All the fermentation conditions determined by JMP software, *S. cerevisiae* was cultivated to produce the Se-enriched yeast biomass. Different concentration of Se i.e. 15µg and 30µg were added at different hours after incubation on adjusted 4 and 6 pH for 24, 48 hours in 100mL of media within Erlenmeyer flasks and shaken in incubator at speed of 110-130 rpm on altered temperatures of 25-35°C. Two factors (inoculum size and selenium source) were kept constant in all the experiments i.e. 40gm/liter and Sodium selenite (Na_2SeO_3) and Se concentrations in the media was 15µg/ml and 30µg/ml. In the course of cultivation, yeast growth of biomass was determined by measuring the density of the growing cells.

Media composition

The media composition for cultivation of Se yeast biomass to optimize various parameters for selenium enriched yeast.

Yeast Extract Peptone Dextrose Medium

S.No	Ingredients	Per liter composition
1	Bacteriological peptone	20gm
2	Yeast extract	10gm
3	Dextrose	20gm

Sugarcane molasses based Medium

S.No	Ingredients	Per liter composition
1	Molasses substrate	10%
2	Ammonium nitrate	0.5%
3	Zinc chloride	1.33gm
4	Magnesium chloride pantothenate	0.025gm
5	Sodium citrate	15gm
6	Sodium glutamate	10gm

Apple waste based Medium

S.No	Ingredients	Per liter composition
1	Apple waste substrate	10%
2	Glucose	2.7%
3	Yeast extract	01%
4	Ammonium nitrate	0.5%

Harvesting and drying of final Se-enriched yeast

The incubation period of biomass subsequently Se-enriched yeast cells were separated and collected from the solution through the process of centrifugation at 3500 rpm for 15 min. The washing and vacuum filtration through sterile filter paper with 0.45µm pore size, thebiomass was transferred to pre-weighed aluminum pan and dried to a constant weight in hot air oven at 60°C for 30-35 min.

The collected dry mass was transformed to powder form by using a sterilized mortar and pestle. The dried mass was weight using an electronic balance.

Determination of Se Content in Se enriched Yeast

The determination of selenium content(0.1 g) of selenium enriched yeast was digested in 4ml HNO_3 (65%) under the following conditions (Michalska-Kacymirow *et al*., 2014).

I.500 W/10min.
II.1000 W/15min.
III.Cooling/5min.

The digested samples were filtered through sterile syringe driven 0.45µm nylon membrane filter. Transparent solutions were transferred to plastic tubes filled with 20mL de-ionized water and were stored in a refrigerator below 4°C before measurements. Selenium content was determined by inductively coupled plasma mass spectrometry (ICP MS). Calibration was performed with the standard solutions containing selenium (Kurek *et al*., 2016).

Statistical analysis

The statistical analysis of the data was performed on statistical software JMP predicted variables for optimization. The data of variables cover a wide range of values which have not been studied before for fermentation was built-in to the response variables to specific yeast biomass.

Results and discussion

Morphological characteristics such as colonial morphology (colony shape, colour, and surface appearance) and ascospore formation are presented in table 3. Despite of its detrimental effect of the genera yeast, they are carotenoid biosynthetic yeasts easily identifiable by distinctive cream (milky) coloured, round large colonies (Krinsky, 2001).

The biochemical identification needs carbon assimilation pattern for the yeast isolates as shown on table 4.

Carbon assimilation is necessary for identification of yeasts which depends on organic carbon sources for their energy supply and growth. Glucose isonly carbon source for the medium, however when it is not available, the ability of the yeast cells to assimilate galactose (plant source) indicated the expression of the GAL genes (Yun *et al*, 2001).

Furthermore, yeast strains that metabolized sucrose, lactose, cellibiose express genes that activate the synthesis of invertase, beta-galactosidase and beta-glucosidase which eventually hydrolyse the substrates to glucose and fructose, glucose and galactose; and two glucose units respectively (Ogawa, 2000).

Table 3. Morphological identification of *Saccharomyces cerevisiae*.

Size	Color	Appearance	Nature	Elevation	Margin	Gram Stain	Wet mount slide
Large colonies	Milky	Round	Smooth and shiny	Convex	Entire	Gram positive	Yeast buds

Table 4. Biochemical characterization (carbon assimilation pattern) of *Saccharomyces cerevisiae.*

Fructose	Sucrose	Maltose	Glucose	Galactose	Lactose	H_2O_2	Urease
+ive	+ive	+ive	+ive	+ive	–ive	+ive	–ive

+ive = Positive, –ive = Negative

Molecular identification of *Saccharomyces cerevisiae* is shown in Fig. 1. The effects of various factors upon the yeast biomass and Se accumulation were determined.

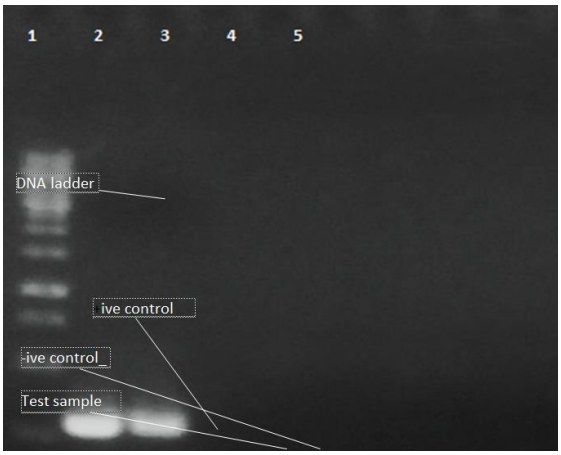

Fig. 1. The PCR product of reference *S. cerevisiae* strain and *S. cerevisiae* isolate. M: 100 bp plus marker, line 1: DNA ladder, line 2: Positive control, line 3: test sample, and line 4: negative control.

The optimized conditions for selenium enriched yeast production were presented in table (5).The result showed that tested factors were dependent on each other; the value of one factor is affected by the other. The content of Se accumulated in yeast associated positively with total biomass yield. The addition of Se after 9 hrs of incubation at 25°C temp, 4 pH , shaking speed was 130 rpm, total incubation time was 48 hrs and selenium concentration was 30 µg, total biomass yield and selenium accumulation were increased i.e. 65.59g/L and 46.23mg/kg respectively. Similar results had been reported by other researchers (Oraby *et al.*, 2015). The incubation period had significant role in total yeast biomass and Se accumulation. Similarly the addition of (Na_2SeO_3) in growth phase with incubation of9 hrs enhances Se accumulation as well as total biomass production. The shaking speed and pH of medium elevated Se enriched yeast production. The optimized level of pH (4.0) increased biomass yield and consequently enhanced incorporation of Se pH variation in optimized value can affect cellular morphology, structure and biomass production. Our results are in line to (Salari and Salari, 2017) they investigated the effect of pH on the *S. Cerevisiae* growth and confirmed that 4 pH is the optimum value for best growth. The increased pH level can degrade yeast proteins and arrest cellular cycle (Pena *et al.*, 2015). The results of shaking speed 130 rpm obtained maximum growth. Similar to our results (Amiri *et al.*, 2017) reported 137.7 rpm as an optimized shaking speed for *S. Cerevisiae* growth. Selenium concentration in medium is the most critical factor, it can affect *S.Cerevisiae* growth and Se accumulation. This study confirmed that 30µg/ml Selenium addition to the media can achieve highest growth with more Se accumulation of yeast cells. Our results are supported by (Suhajda *et al.*, 2000) they also reported the same selenium amount for incorporating into the yeast cells.

Table 5. Results of the six process variable factors along with total biomass yield and organic Se formation.

S.No	Temperature (°C)	Concentration of selenium added (µg/ml)	Selenium adding time (Hrs)	Incubation time (Hrs)	pH	Shaking speed (rpm)	Total biomass yield (g/L)	Organic selenium accumulation (mg/kg)
1	25	15	3	24	6	130	20.50	15.82
2	25	30	3	48	4	110	43.20	20.64
3	35	15	3	48	6	110	36.16	25.22
4	35	30	3	24	4	130	40.80	25.10
5	35	30	9	24	6	130	32.62	20.35
6	25	30	3	24	6	110	34.10	15.91
7	25	30	9	48	4	130	65.59	46.23
8	35	15	3	48	4	130	45.90	25.82
9	35	15	9	24	4	110	40.21	23.74
10	25	15	9	48	6	130	54.01	30.45
11	35	30	9	48	6	110	33.95	15.52
12	25	15	9	24	4	110	42.20	25.30

Furthermore, the impact of six variables in table (5), the temperature, Selenium concentration, Selenium adding time, incubation time, pH and Shaking speed) on the *S.Cerevisiae* biomass production and assimilating of Se into yeast cells. The results revealed that these have considerable impact on both biomass production and Se bioaccumulation into yeast cells. Though high pH and selenium concentration has a negative impact on cell growth of yeast as reported by other researchers that higher selenium concentration slow down yeast growth due to oxidative stresses (Kieliszek *et al*., 2019). Moreover in the present study total biomass yielded and organic selenium accumulation are directly interlinked as higher yield gives more bioaccumulation of selenium. Similar to our findings (Zare *et al*., 2018) reported same pattern. However, Selenium enriched yeast production for the assimilation of Se into yeast cell (Alidee *et al*. 2001; Esmaeili, 2012) used "The Plackett-Burman design" (PBD) and "Response Surface Methodology" (RSM) for screening different variables while in the current study JMP statistical software was used to design values for optimization.

Conclusion

Selenium enriched yeast biomass production could be easily attained by designing statistically based experiments for the optimization of various parameters. In the present study optimization of various factors i.e. Temperature, pH, Se concentration, Time of Se addition, Shaking speed, and incubation time resulted in considerably increased productivity and Se bioaccumulation. The optimized values were Temp 25°C, pH 4, Se conc. 30µg/ml, addition of Se after 9hr of incubation, shaking speed 130rpm and incubation time 48 hrs. Further, it is suggested that bacterial or fungal species can be exploited for the mass production with amalgamation of essential nutrient on medium of different agro-industrial waste.

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Conflict

No conflict of interest.

References

Aggarwal A, Upadhyay R. 2013. Heat stress and immune function. In: Heat stress and animal productivity: Springer p. 113-136.

Alidee T, Habbal H, Tohla M. 2016. Optimization of selenium enriched Saccharomyces cerevisiae by Response Surface Methodology (RSM). International Journal of ChemTech Research **9,** 221-226.

Amirimm, Fazeli MR, Amini M, Roodbari NH, Samadi N. 2017. Optimization of Culture Conditions for Enrichment of Saccharomyces cerevisiae with Dl-α-Tocopherol by Response Surface Methodology. Iranian Journal of Pharmaceutical Research: IJPR **16,** 1546.

Avery JC, Hoffmann PR. 2018. Selenium, selenoproteins, and immunity. Nutrients **10,** 1203.

Briens M, Mercier Y, Rouffineau F, Vacchina V, Geraert PA. 2013. Comparative study of a new organic selenium source v. seleno-yeast and mineral selenium sources on muscle selenium enrichment and selenium digestibility in broiler chickens. British Journal of Nutrition **110,** 617-624.

Diarra SS, Tabuaciri P. 2014. Feeding management of poultry in high environmental temperatures. International journal of poultry science **13,** 657-661.

Esmaeili S, Khosravi-Darani K, Pourahmad R, Komeili R. 2012. An experimental design for production of selenium-enriched yeast. World Appl Sci **19,** 31-37.

Gupta M, Gupta S. 2017. An overview of selenium uptake, metabolism, and toxicity in plants. Frontiers in Plant Science **7,** 2074.

Krinsky NI. 2001. Carotenoid antioxidants. Journal of Nutrition, **17,** 815-7.

Khan FA. 2017. Replacement of protein source in the existing poultry feed with single cell microbial protein. In: University of Peshawar.

Kieliszek M, Błażejak S, Bzducha-Wrobel A, Kot AM. 2019. Effect of selenium on growth and antioxidative system of yeast cells. Molecular biology reports **46,** 1797-1808.

Kurek E, Ruszczynska A, Wojciechowski M, Luciuk A, Michalska-Kacymirow ME. 2016. Bio-transformation of selenium in Se-enriched bacterial strains of Lactobacillus casei. Roczniki Państwowego Zakładu Higieny **67.**

Kurtzman C, Fell JW, Boekhout T. 2011. The yeasts: a taxonomic study: Elsevier.

McDonald V. 1963. Direct microscopic technique to detect viable yeast cells in pasteurized orange drink. Journal of Food Science 28, 135-139.

Michalska-Kacymirow M, Kurek E, Smolis A, Wierzbicka M, Bulska E. 2014. Biological and chemical investigation of Allium cepa L. response to selenium inorganic compounds. Analytical and bioanalytical chemistry **406,** 3717-3722.

Nasir A, Rahman SS, Hossainmm, Choudhury N. 2017. Isolation of Saccharomyces cerevisiae from pineapple and orange and study of metal's effectiveness on ethanol production. European Journal of Microbiology and Immunology **7,** 76-91.

Ogawa Y, Nitta A, Uchiyama H, Imamura T, Shimoi HI, to K. 2000. Tolerance mechanism of the ethanoltolerant mutant of sake yeast. Journal of Bioscience and Bioengineering. **90,** 313-20

Oliveira VA, Vicente MA, de Oliveira Santos J, Araújo LD. 2008. Biochemical and molecular characterization of Saccharomyces cerevisiae strains obtained from sugar-cane juice fermentations and their impact in cachaça production. Appl. Environ. Microbiol **74,** 693-701.

Orabymm, Allababidy T, Ramadan E. 2015. The bioavailability of selenium in Saccharomyces cerevisiae. Annals of Agricultural Sciences **60,** 307-315.

Pena A, Sanchez NS, Alvarez H, Calahorra M, Ramírez J. 2015. Effects of high medium pH on growth, metabolism and transport in Saccharomyces cerevisiae. FEMS yeast research **15.**

Salari R, Salari R. 2017. Investigation of the best Saccharomyces cerevisiae growth condition. Electronic physician **9,** 3592.

Suhajda A, Hegoczki J, Janzso B, Pais I, Vereczkey G. 2000. Preparation of selenium yeasts I. Preparation of selenium-enriched Saccharomyces cerevisiae. Journal of Trace Elements in Medicine and Biology **14,** 43-47.

Surai P. 2002. Selenium in poultry nutrition, Antioxidant properties, deficiency and toxicity. World's poultry science journal **58,** 333-347.

Yun CW, Bauler M, Moore RE, Klebba PE, Philpott CC. 2001. The role of the FRE family of plasma membrane reductases in the uptake of siderophore iron in Saccharomyces cerevisiae. Journal of Biol.Chem. **276,** 10218-23.

Zare H, Owlia P, Vahidi H, Khujin MH. 2018. Simultaneous Optimization of the Production of Organic Selenium and Cell Biomass in Saccharomyces Cerevisiae by Plackett-Burman and Box-Behnken Design. Iranian Journal Of Pharmaceutical Research: IJPR **17,** 1081.