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Comparative Study of Selenium-Enriched Conditions from *Ganoderma lucidum* and *Yeasts*

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Abstract: This paper compared the conditions of selenium-enriched from *Ganoderma lucidum* and *yeasts*. The optimal conditions for shake flask were carried out by a single factor test and orthogonal test $L_9(3^4)$, giving a *comparative study on the ability of* biotransformation of inorganic selenium between *Ganoderma lucidum* and yeasts. The orthogonal experimental results showed that the optimal conditions for shake flask growth of *Ganoderma lucidum* were as bellow: culture medium final concentration of Na_2SeO_3 (Se^{4+}) was 20mg/L, 15% inoculum, incubation time 42h, medium volume 60ml/250ml. The *total content of selenium in fermentation of G. lucidum* was 1540.34±19.21μg/g, and *the growth capacity of mycelium of G. lucidum* was 51.56±0.065g/L, respectively. The optimal conditions for shake flask cell growth of yeasts were as bellow: culture medium final concentration of Na_2SeO_3 (Se^{4+}) was 25mg/L, 10% inoculum, incubation time 48h, medium volume 50ml/250ml. The *total content of selenium in fermentation of yeasts* was 2454.86±29.82μg/g, and *the growth capacity of cell of yeasts* was 6.44±0.023g/L, respectively.

Keywords: *Ganoderma lucidum* (Fr.) Karst, Saccharomyces Cerevisiae, Selenium-Enriched, Single-Factor Test, Orthogonal Experiment

1. Introduction

Selenium is an essential trace element in human body which strengthens immune system, enhances antioxidant and anti-cancer effects, plays a physiological role in the prevention and treatment of Keshan disease, *etc*. The total accumulation of selenium in the human body is about 14~21mg, which maintains dynamic balance in ingestion and excretion. By microbial fermentation, biotransformation of inorganic selenium can reduce the toxicity of inorganic selenium, improve the absorption and utilization, which can be used as a safe source of selenium. It is an ideal functional food ingredient. This thesis compared optimized conditions for culturing selenium-enriched gloss *G. lucidum* and Yeast through single-factor test and orthogonal experiments L_9 (3^4).

2. Instrument and Materials

2.1. Instrument

Tableconcentrator (Sartorius AG). superclean Benches (Shanghai Bo Xun Industrial Co., Ltd. Medical Equipment Factory). Vertical Pressure Steam Autoclave (Shanghai Bo Xun Industrial Co., Ltd. Medical Equipment Factory). Digital stainless steel electric incubator (Shanghai Bo Xun Industrial Co., Ltd. Medical Equipment Factory). 721 spectrophotometer (Shanghai Bo Xun Industrial Co., Ltd. Medical Equipment Factory).

2.2. Materials

Ganoderma lucidum (Fr.) Karst，provided by College of Life Science, Anhui University and identified by Professor

Shen Ye-shou). Brewer's yeast (Saccharomyces cerevisiae, preserved in our laboratory). Na_2SeO_3 (purity of 99.99%, Anhui Star New Materials Technology Co., Ltd.). Edetate disodium (EDTA-2Na), 3, 3- diaminobenzidine (DAB) and so were of analytical reagent.

3. Experimental Method

3.1. Digestive Juice, Standard Stock Solution and Standard Working Solution Preparation

Digestive juice preparation: measureaccurately and mix ulfuric acid, hydrogen peroxide, perchlorate solution according to the ration 1: 3: 3, then store the mixture in a brown light-shading bottle at 4°C for backup.

Selenite standard stock solution (1mg/ml) preparation: weigh 0.0500g Na_2SeO_3; after nitric acid solution (nitric: ultrapure water =1: 30, V/V) was dissolved, reserve the volume (50ml volumetric flask) and formulate as a 1mg / ml standard stock solution, then pour into flask, 121°C, and autoclave it for 20min. After cooling, the solution was aseptically transferred to bottle with blue cap to saved up for use at 4°C.

Na_2SeO_3 working standard solution (10μg/ml) formulation: exact selenite stock standard solution Pipette 1ml, with a solution of nitric acid (nitrate: ultrapure water =1: 30, V/V) volume to 100ml; store for use at 4°C.

3.2. Single-Factor Test

3.2.1. Plus Selenium

Liquid volume 90ml/250ml, shaking speed 150r·min^{-1}, 28°C, culture time 56h. When the shaker has cultured for 8h, aseptic solution was added to Na_2SeO_3 *G. lucidum and* yeast broth so that the final concentration of the culture medium Na_2SeO_3 was 10mg/L, 15mg/L, 20mg/L, 25mg/L, 30mg/L; then culture it after mixing in the shaker.

3.2.2. Inoculum

Set five inoculum levels at 5%, 10%, 15%, 20% and 25%, respectively remove with a pipette the corresponding bacterial suspension volume of fungus and yeast cell; add it to marked Erlenmeyer flask containing the corresponding culture medium; set the rotation speed at 150r·min^{-1}, at 28°C; the culture time is 56h; when the shake flask has cultured for 8h, with the aseptic technique the Na_2SeO_3 solution was added to the *G. lucidum and* yeast broth to end the culture medium at a Na_2SeO_3 concentration of 20mg/L.

3.2.3. Culture Time

Shaking speed 150r·min^{-1}, 28°C, time and amount of adding selenium is the same as in 2.2.2. when shaking culture time of the *G. lucidum and* yeast is 30h, 36h, 42h, 48h, 52h and 56h, take out flasks respectively with the corresponding number, put in 5000 r·min^{-1} centrifugal for 15min, and discard the supernatant and wash with deionized water; break fast the fermentation product on the mixers; let stand for 12h, then remove the supernatant after centrifugation again; repeat the above operation twice. The resulting fermentation was placed in 60°C oven, drying to a constant weight, and weighed.

3.2.4. Liquid Volume

Setting liquid volume to 50ml/250ml, 60ml/250ml, 70ml/250ml, 80ml/250ml and 90ml/250ml respectively, with a pipett each flask was added the corresponding amount of broth, then add it to the activated *G. lucidum* mycelia and yeast suspension; shaking speed 150r·min^{-1}, 28°C; shaking culture for 54h, time and acceleration of adding selenium is the same as in 2.2.2.

3.3. Orthogonal Experiment

The orthogonal test conditions at the phase of shake flask cultures of *G. lucidum* and yeast were designed on the basis of single factor experiments, select four factors, the amount of selenium, inoculation, incubation time and the medium volume using L_9 (3^4) orthogonal optimization test. Table head design as shown in Table 1.

Table 1. *G. lucidum and yeast orthogonal experiments [L_9 (3^4)] factor level coding table.*

levels	factors				
	A selenium amount (mg / L)		B inoculation (%)	C incubation time (h)	D liquid volume (ml/250ml)
	G. lucidum	yeast			
1	10	15	10	42	50
2	15	20	15	48	60
3	20	25	20	52	70

Rotation speed 150r·min^{-1}, the culture temperature 28°C, added Na_2SeO_3 2 times, respectively, added after the first shake flask cultures at 8h and 13h.

3.4. Determination of Selenium Content

3.4.1. Selenium (Se^{4+}) Standard Curve

Exact amount of Na_2SeO_3 standard working solution 0.0ml, 2.0ml, 4.0ml, 6.0ml, 8.0ml, 10.0ml, sequentially apply to six clean separating funnel (125ml); add the appropriate amount of deionized water; adjust to neutral pH with 5% sodium hydroxide solution, plus 3ml formic acid solution (2mol/L), then add water to 35ml (at this point pH of the solution is about 2 to 3); shake, and then add 5ml 0.5% of DAB to each; shake and put in the dark to react for 30mins. After the reaction completed, adjust it to neutral pH with a 5% sodium hydroxide solution; add 10ml toluene, shaking extraction for 2mins; let it stand to be stratified, put the toluene layer filtrate in the cuvette; absorbance was measured at a wavelength of 420nm. Standard selenium (Se^{4+}) work concentration as abscissa, absorbance ordinate to draw selenium standard curve.

3.4.2. Sample Preparation

I. fermentation separated and dried

G. lucidum and yeast fermentation broth were poured into the appropriate number of centrifuge tube, 5000r·min^{-1} centrifugal 15min; the supernatant was removed, and the precipitate was broken by the ultra-pure water dip 2-3 times in rapid mixers; the supernatant was removed by centrifugation and dried to a constant weight, then respectively Se

Ganoderma mycelium and yeast powder were harvested.

II. sample digest

Accurately weighed mycelia and yeast that were dried to constant weight in 150ml beaker, add a small amount of deionized water wet first sample, and then add the appropriate amount of digestive juices, respectively, shake until the bubbles subside, then stamped surface dish, put in the ventilating kitchen, with electric stove digested to end (ie heating to fuming, until the solution becomes pale yellow, reheat 2~3min, then the solution becomes colorless). If not completely digested, after cooling a bit, add hydrogen peroxide and heating until it digested completely. After the beaker cooled, rinse glass and the beaker wall with 1ml deionized water; plus 2ml 5% EDTA-2Na solution and shake; pH was adjusted to neutral with 40% NaOH in a cold water bath, then set the volume to 100ml, use the same method to make blank solution.

3.4.3. For Sample Preparation and Measurement

Put the precise amount of the after-digestion juice and an equal amount of blank digestion solution in separating 125ml funnels; add water; adjust to neutral pH with 5% NaOH solution; plus 3ml formic acid solution (2mol / L), then add water to 35ml, shake, then plus 5ml 0.5% DAB solution to each, and shake, then set in the dark to react for 30mins, then adjust pH to 6.5~7.0 with 5% NaOH solution, adding precise 10ml toluene, shaking extraction for1min; let it stand to be stratified; put the toluene layer filtrate in the cuvette; absorbance is measured at a wavelength of 420nm. Calculate the selenium content in the sample with the comparison to the standard curve.

4. Results and Discussion

4.1. Selenium (Se^{+4}) Standard Curve

Linear equation of Selenium (Se^{+4}) standard curve is y = 0.0094x + 0.0214. R^2=0.998. Significant test result shows P<0.0001 (n=6, n is the concentration gradient of the selenium standard sample number). Selenium (Se^{+4}) standard curve working range is 0μg/ml~100μg/ml.

4.2. Single-Factor Test Results and Analysis

4.2.1. Plus Selenium

Test results of shake flask culture with different concentrations of Na_2SeO_3 are shown in Figure 1. The results show: added amount selenium at 10~15mg/L *G. lucidum* has higher growth, followed by *G. lucidum* of lower growth with increasing amount of selenium. When added Selenium is 15~20mg/L, *G. lucidum* has higher content of selenium; when Selenium is added at 15~20mg/L, yeast cells have higher growth, followed by lower growth of yeast with increasing amount of selenium. When Selenium is added at 20~25mg/L, yeast cells have high content of selenium.

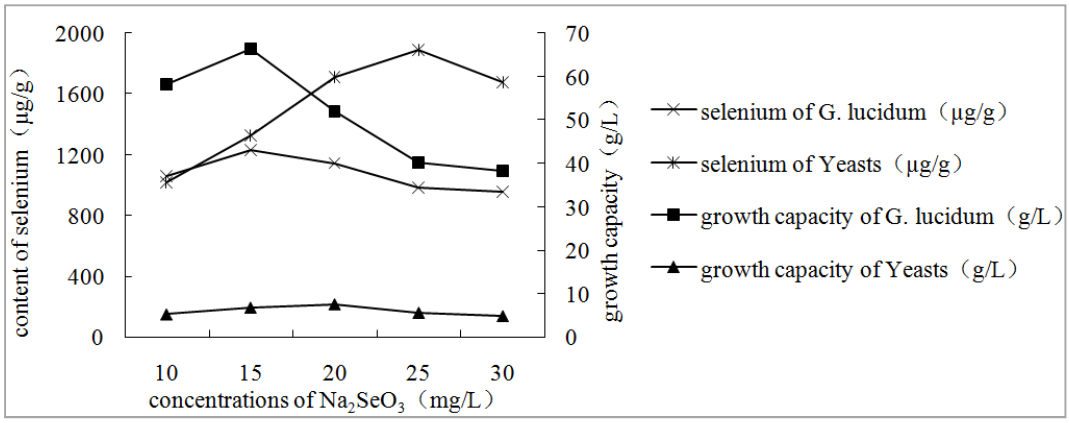

Figure 1. *Selenium yeast growth rate on G. lucidum and influence diagrams.*

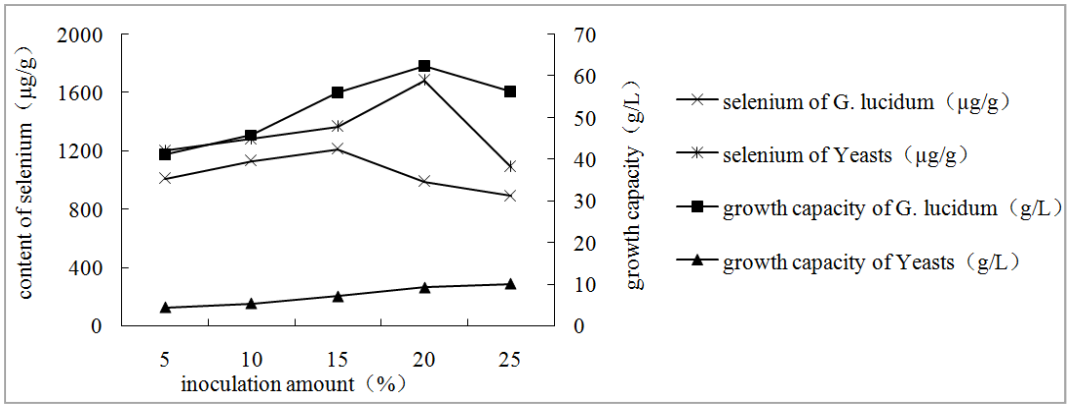

Figure 2. *Affect the amount of inoculum of G. lucidum and yeast growth.*

4.2.2. Inoculation Amount

Figure 2 is the test results showing how different inoculum affects selenium content and growth of *G. lucidum* and yeast. The results showed that, when inoculation was 20%, *G. lucidum* has higher growth, followed by a slight decline, while the growth of yeast has been an upward trend with the increasing inoculation amount. When inoculation amount is at 10% to 15%, the fungus selenium content is at a high level. When inoculation is greater than 15%, the selenium content in fungus begins to decrease. When inoculum concentration is greater than 20%, selenium content in the yeast began to decline.

4.2.3. Culture Time

Figure 3 is the test results showing how different culture time affects selenium content and growth of *G. lucidum* and yeast. The results showed that when the culture time is 48h~52h, selenium content in both fungus and yeast are of the maximum range; if cultured 52h, selenium content in *G. lucidum* and yeast declines.

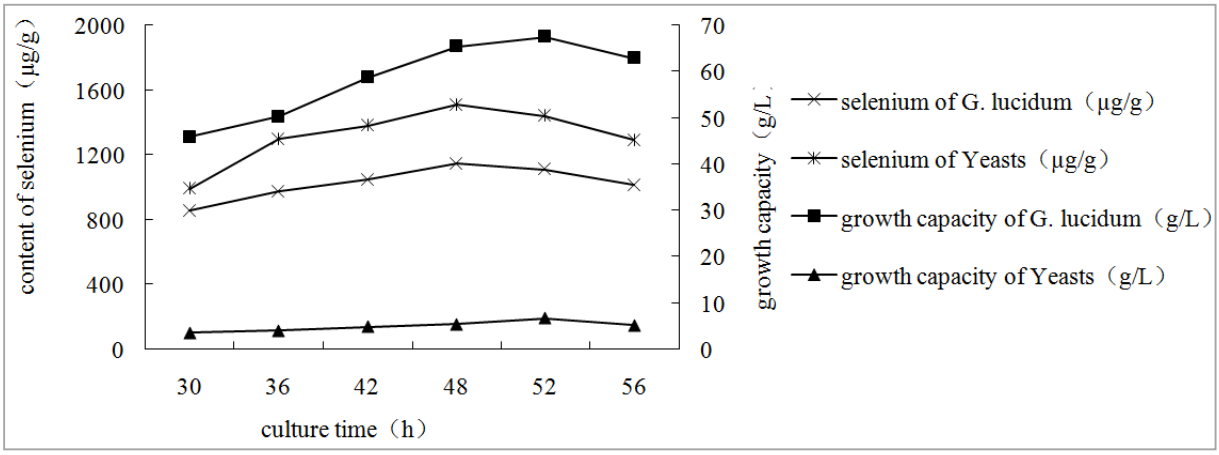

Figure 3. *Affect the cultivation time of G. lucidum and yeast growth.*

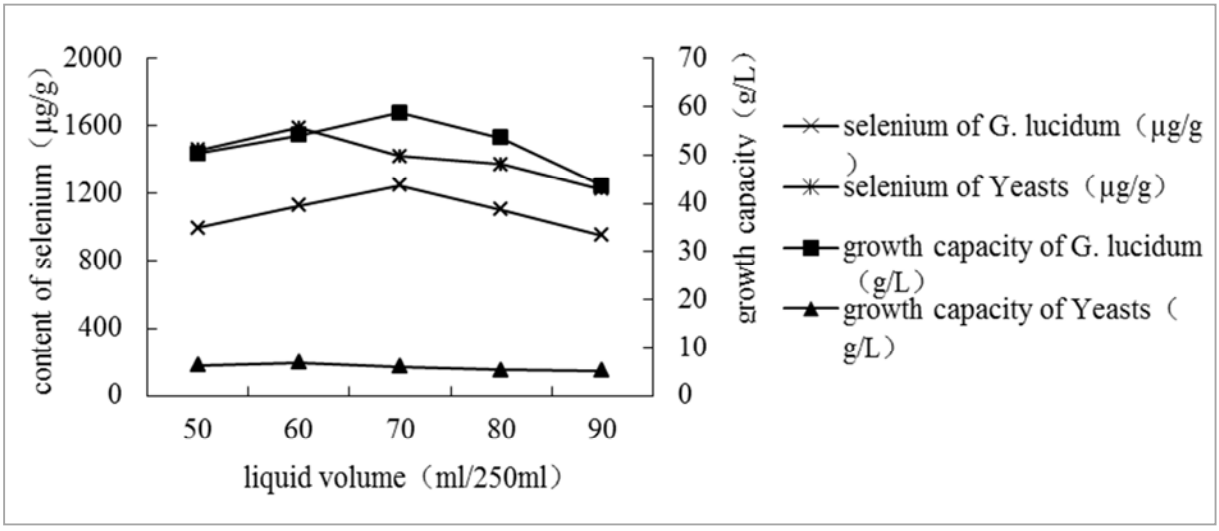

Figure 4. *Affect the liquid volume of G. lucidum and yeast growth.*

4.2.4. Liquid Volume

Figure 4 is the test results showing how different liquid volume affects selenium content and growth of *G. lucidum* and yeast. The results show that liquid volume is at 60~70ml/250ml, selenium content volume and growth of *G. lucidum* appear higher. After the value reaches the highest it begins to decline. When the liquid volume is at 50~60ml/250ml, yeast growth is slightly higher than the other groups. after liquid volume reaching the highest value, with the increase of liquid volume, the selenium content in yeast shows a more obvious downward trend.

4.3. Orthogonal Test Results of the Discussions

Based on the single factor test results, design 4 factors culturing *G. lucidum* and yeast including selenium amount, inoculation, incubation time and the medium volume and 3 levels orthogonal experiment L_9 (3^4).

According to the results of the orthogonal experiment, factors that affect the content of selenium in *Ganoderma lucidum* were as bellow: selenium amount>liquid

volume>inoculation>incubation time. Referring to Table 1, the culture conditions of fungus in the test No. 8 ($A_3B_2C_1D_2$) ie optimized culture conditions of *G. lucidum* conversion to inorganic selenium (ie, Se optimal conditions): final concentration of *G. lucidum* broth added Na_2SeO_3 was 20mg/L; inoculation amount was 15%, culture time was 42h; liquid volume was 60ml/250ml; maximum content of selenium of *G. lucidum* appears at 1540μg/g; culture conditions of *G. lucidum* in Test No. 3 ($A_1B_3C_1D_1$), ie conditions for *G. lucidum* growth of cell culture can be optimized as follows: final concentration of *G. lucidum* fermentative inorganic selenium Na_2SeO_3 is 10mg/L; inoculation amount was 20%; culture time was 42h; liquid volume was 50ml/250ml; the emergence of the maximum growth of *G. lucidum* was at 74.12g/L. Factors affecting the content of selenium yeast were: selenium amount>inoculation>culture time>liquid volume. Analysis referring to Table 1, yeast in the test number 7 ($A_3B_1C_2D_1$) culture conditions, i.e., optimized culture conditions for yeast cell's biotransformation inorganic selenium (i.e., conditions optimal Se): final concentration of yeast fermentation liquid with added Na_2SeO_3 was at 25mg/L; inoculation amount was 10%; culture time was 48h; liquid volume was 50ml/250ml; yeast cells showed a maximum content of selenium at 2454μg/g; culture conditions of yeast in Test No. 3 ($A_1B_3C_1D_1$), ie optimized culture conditions of growth of yeast cells; inorganic final concentration of selenium yeast fermentation broth with added Na_2SeO_3 was 15mg/L; inoculation amount was 20%, culture time was 42h; liquid volume was 50ml/250ml; yeast growth of maximum amount occurs at 11.84g/L.

5. Conclusion

The optimal conditions for shake flask were carried out by a single factor test and orthogonal test L_9 (3^4), giving a comparative study on the ability of biotransformation of inorganic selenium between *G. lucidum* and yeasts. According to the results of the orthogonal experiment, factors that affect the content of selenium in *G. lucidum* were: selenium amount>liquid volume>inoculation>incubation time. The orthogonal experimental results showed that the optimal conditions for shake flask growth of *G. lucidum* were as bellow: culture medium final concentration of Na_2SeO_3 (Se^{4+}) was 20mg/L, 15% inoculum, incubation time 42h, medium volume 60ml/250ml. The total content of selenium in fermentation of *G. lucidum* was 1540.34±19.21μg/g, and the growth capacity of mycelium of *G. lucidum* was 51.56±0.065g/L, respectively. The optimal conditions for shake flask cell growth of yeasts were as bellow: culture medium final concentration of Na_2SeO_3 (Se^{4+}) was 25mg/L, 10% inoculum, incubation time 48h, medium volume 50ml/250ml. The total content of selenium in fermentation of yeasts was 2454.86±29.82μg/g, and the growth capacity of cell of yeasts was 6.44±0.023g/L, respectively.

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Research Direction

Basic chemical analysis and food processing.

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