THE MEDIA OPTIMIZATION OF THE GROWTH OF CAULIFLOWER MUSHROOM (*Sparassis crispa*) at Dongseo University, Busan, South Korea

PRACTICAL TRAINING REPORT

This practical training report is submitted for the partial requirement for Bachelor Degree

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PREFACE

By completing the practical training report titled "The Media Optimization of The Growth of Cauliflower Mushroom (*Sparassis crispa*)", the author would like to praise the Lord because author believe that author could have done all these things through His Grace who strengthens. This report is a complete accountability from the research which was held from 15th January to 26th February 2018 at Department of Biotechnology Dongseo University, Busan, South Korea.

The author have been surrounded by many kind-hearted people. There are so many people to thank for their help and support during the research and arrangement of this report. The author could never have accomplished the heights or explored the depths without them. The author would like to send the warmest thanks to:

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Finally, the author sends prays for all of the kind-hearted people. The practical training report still have many shortcomings. The author hopes that this report may be useful for all readers wherever you are.

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Josephine Permatasari Handoyo

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1. INTRODUCTION

1.1. Background of Pratical Training

Nowadays, the development of science and technology is boundless. Researchers always try to discovery something that is proven by the theoretical knowledge, especially in food aspects. Every products in the market needs to get permission from the country that the quality of product is guaranteed. The process of producing products through many steps that always related to knowledge. The widespread knowledge become the main reason for holding the practical training for students from Food Technology Department, Soegijapranata Catholic University, Semarang, Indonesia. Besides, the students can learn about how to make relation with the new people and environment in abroad. They can also learn about food tradition in the other country. Moreover, the soft skill and also hard skill will be sharpened during the practical training.

1.2. Purpose of Pratical Training

The purpose of pratical training are:

a. To give opportunity to students about exploring and doing research in the new environment.
b. To get more knowledge and experience.
c. To widen the society relation with the lecturer or new friends in different country.

1.3. Dongseo University

Dongseo Educational Foundation has vision to spread education based on the teachings of Jesus Christ in 1965. Dongseo University was established in 1992 by Dr. Sung Man Chang ("Min Soek") that only have 8 major such as Mathematics, Electronic Technology, Computer Technology, Industrial Technology, Environmental Technology, Chemical Engineering, Food Science, and Industrial Design. The first generation consisted of 400 students in the whole major. In 1993, the name of university was changed to Dongseo University of Technology. But in 1995, when Dr. Sung Man Chang was the president of

university, the name was changed again to Dongseo Universirty. Dr. Sung Man Chang was elected First Chairman of the Busan Christian Organization and had to leave his position in Dongseo University. In 1999, Dr. Dong Soon Park, who had ever been the Executive Director of Kyoungnam College of Information and Technology, became the fourth president of Dongseo University. He supplied the remarkable development and global expansion in the university. Dr. Jekuk Chang replaced Dr. Park as the eight president of Dongseo University. The commitment of Dongseo University is also to create graduate that has faith, hope, and love in the spirit of Jesus Christ. It is hoped that the graduates can serve the others in that spirit. Logo of Dongseo University of Korea at Figure 1.

Figure 1. Logo of Dongseo University of Korea

1.4. Department of Biotechnology

Department of Biotechnology at Dongseo University is headed with Prof. Man Ghi Cho as the head of department. The vision of department is producing graduates that prepared the future challenges with cutting-edge science. The students not only get the theory knowledge but also the practical skills. Department of Biotechnology cooperates with institute in Technical University of Berlin. The three main objectives of department are preparing graduates to work in the wide environment, offering them to explore their knowledge through faculty's degree programs, and helping graduates to get financial scholarships.

1.5. Time and Place of Pratical Training

The practical training is executed in the Faculty of Biotechnology, Dongseo University, Busan, South Korea from 15th January to 26th February 2018.

2. BACKGROUND OF RESEARCH

To date, the development of science and technology is extraordinary which the amount of research on the health and food aspects increases every time. Food has an important role because its functional properties to several aspects of life. As well as *Sparassis crispa*, which is known as cauliflower mushroom or Ggotsongyi in Korean. It is an edible mushroom that can be found in the forest around Korea and Japan. *Sparassis crispa* is usually famous in Chinese medicine factory because of its medical function based on its content, especially high 6-branched 1,3-β-D glucan content. Naturally the bacteria or plants may have β-glucan contents in their cell walls which present with different conformational complexity (Pengkumsri *et al*., 2017). It can be differ by considering the length and amount of branches. Japan food research laboratories reported that the fruit body of *Sparassis crispa* contains around 43,6% β-glucan which is detected using enzyme method (Farooq *et al*., 2014).

Research by Kimura (2013) showed that the component of β-glucan in *Sparassis crispa* can be used as medical function because of its antitumor activity that has been proven in mice. Moreover, *Sparassis crispa* also have anticancer activities, anti metastatic, anti angiogenic, and immune stimulation effects. β-glucan of *Sparassis crispa* also have a role in various biological effects such as increase the hematopoietic response in cyclopghos-phamide-induced leukopenic mice and response to human cell of peripheral blood monocular which induces cyctokines production (Tada *et al*., 2007). The mechanism of β-glucan in medical aspects is depend on its level of complexity (Chan *et al*., 2009).

The growth of culture is influenced by many factors which is one of them is the kind of medium. In the present study, we desire to find the suitable concentration medium, Luria Broth, that will lead to the optimum growth of *Sparassis crispa*. The culture will be measured using freeze-drying methods and microplate methods. The growth rate of *Sparassis crispa* is also predicted by calculating using Monod equation.

3. RESEARCH METODOLOGY

3.1. Materials

The main material used in this research is fresh cauliflower mushroom (*Sparassis crispa*) that has purchased by the Biotechnology Department of Dongseo University (Figure 2). The other materials are Difco™ Lactobacilli MRS Broth (Becton, Dickinson and Company Sparks, USA) and agar bacteriological (MB Cell products) as the medium for growing fresh cauliflower mushroom (*Sparassis crispa*) to get a host culture. Luria Broth (Sigma Aldrich products, St. Louis, USA) used as the choosen medium for inoculation of host culture to get know which concentration has the optimum growth od *Sparassis crispa*.

Figure 2. Fresh cauliflower mushroom (*Sparassis crispa*)

Table 1. The composition of Luria Broth

Composition	Content (g/l)
Tryptone	10
Yeast Extract	5
Sodium Chloride	0.5

Table 2. The composition of deMan Rogosa Sharpe Agar (MRSA)

Composition	Content (g/l)
Protease peptone	10
Beef extract	10
Yeast extract	5
Dextrose	20
Polysorbate 80	1
Ammonium citrate	2
Sodium citrate	5
Magnesium sulphate	0.1
Manganese sulphate	0.05
Dipotassium phosphate	2
Agar	15

3.2. Equipments

The equipments used include incubator, autoclave, shaking incubator, freeze dryer machine (Figure 3), microplate reader (Figure 3), microscope, and clean bench.

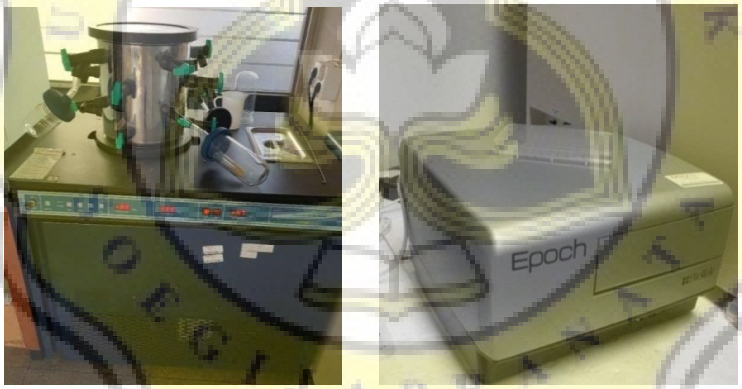


Figure 3. The equipments used in the research such as freeze-dryer machine (left) and microplate reader (right)

3.3. Methods

3.3.1. Inoculation Fresh Mushroom (*Sparassis crispa*)

To inoculate the mushroom, deMan Rogosa Sharpe (MRS) Agar medium were used. MRS Agar medium was prepared then divided into two sides. The small body parts of

Sparassis crispa were put in the both sides. The culture is incubated for 2 days until 3 days until the zone around its body parts was shown (Farooq *et al*., 2014). After there was a zone around *Sparassis crispa*'s body parts, it had to make sure that the growth culture was *Sparassis crispa*. The small body parts of *Sparassis crispa* and the growth culture in MRS Agar was observed using microscope and compared. If the appearance of both is similar, it can be stated that the growth culture is *Sparassis crispa*. Streaking out was done on the new deMan Rogosa Sharpe Agar (MRSA). Streaking out can separate single colony from the colonies. Single colony that was gained would be used as the host for the cultivation in the selected medium. The single culture in the medium was transferred in deMan Rogosa Sharpe Broth (MRSB). The stock culture in deMan Rogosa Sharpe Broth was incubated and used for the measurements.

3.3.2. Measuring the Growth of Culture using Freeze-drying Methods

Freeze-drying method was used for measuring *Sparassis crispa* in deMan Rogosa Sharpe Broth (MRSB). The aim of this method is to make sure that *Sparassis crispa* grows in the medium by measuring its dry weight during 5 days. The method that was done for freeze-drying methods can be seen in the flowchart (Figure 4).

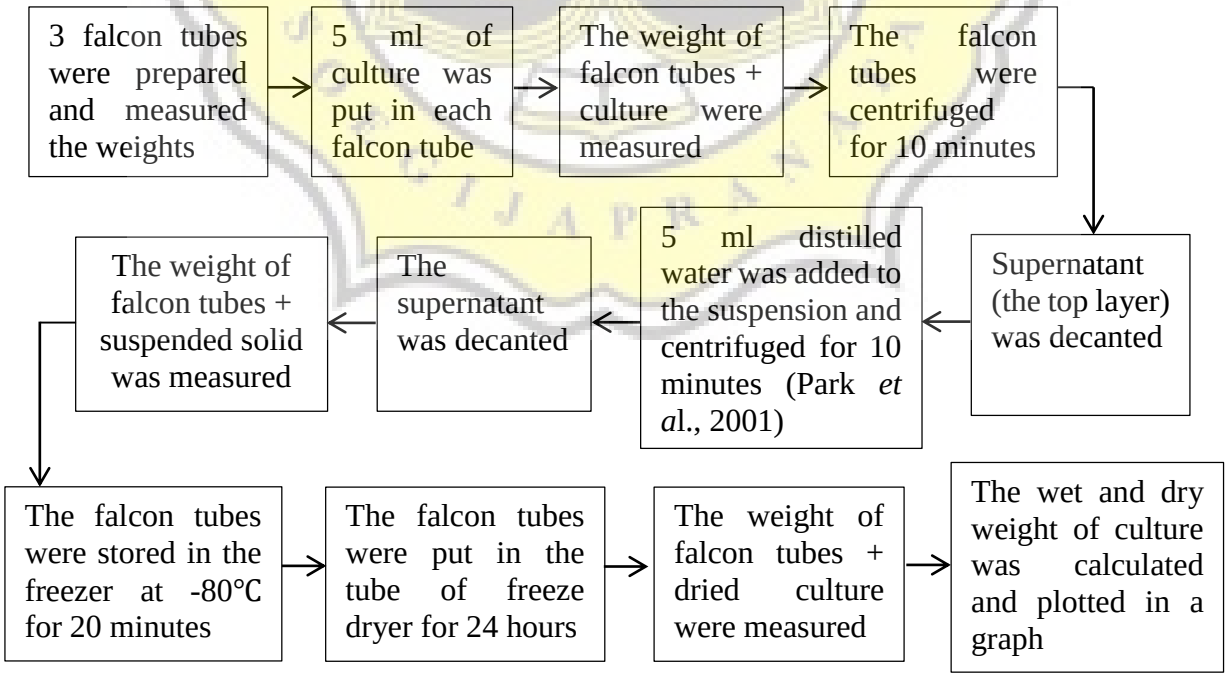


Figure 4. Freeze-drying Methods

3.3.3. Microplate Measurement Method

Method of microplates that is used for measuring the growth of *Sparassis crispa* can be seen in the flowchart (Figure 5).

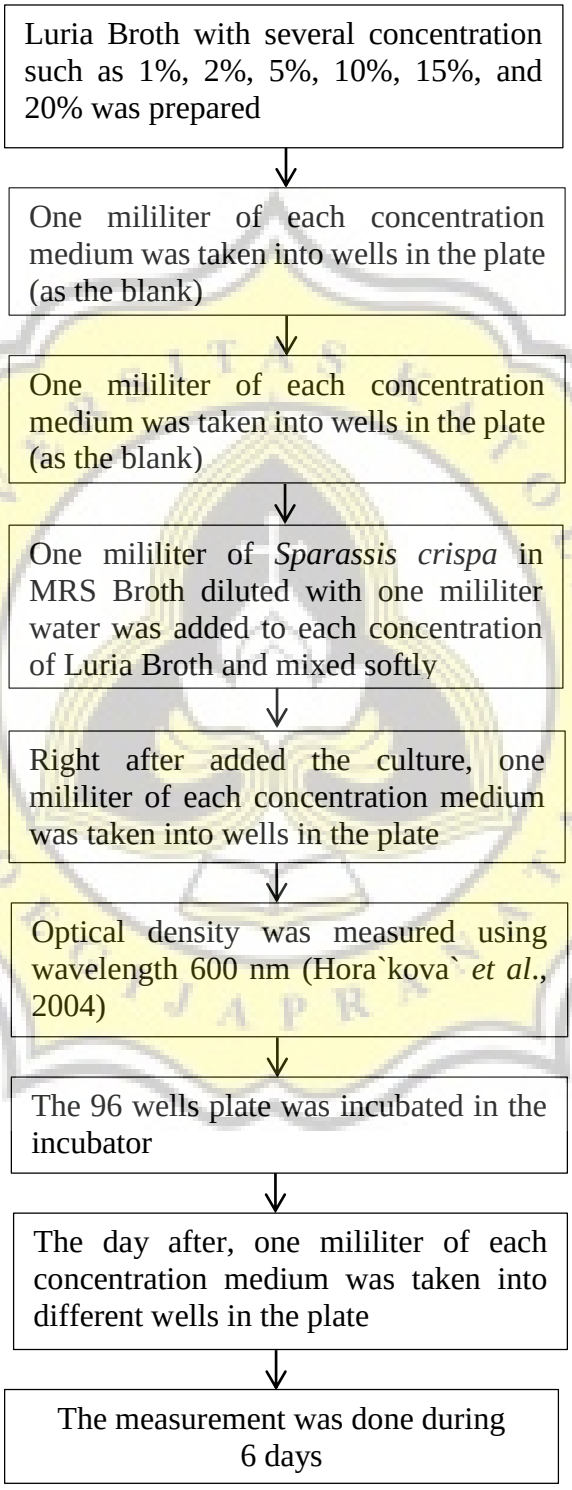


Figure 5. Microplate Measurement Methods

4. RESULT AND DISCUSSION

Sparassis crispa is an edible mushroom which is mostly found in the forest around Korea and Japan. *Sparassis crispa* has medical function, therefore, it is usually used as the components of traditional Chinese medicine. It had been proven since 1923 that *Sparassis crispa* take a part of controlling the health of human (Igarashi & Takeuchi, 1985). The higher components of *Sparassis crispa* that contribute its medical function is β-glucan. Vannucci *et al*. (2013) reported that many types of mushroom has β-glucan content naturally in their fruit body, as well as *Sparassis crispa* that is familiar with the highest β-glucan content around 43,6%. Because of its important function, in this research, the growth of *Sparassis crispa* is monitored in the different concentration of medium. The aim of this research is to gain the higher production of *Sparassis crispa* which will also increase the higher β-glucan content as used in traditional Chinese medicine. The main factor that affects the productivity of *Sparassis crispa* is the kind of medium which contains different materials that will determine the growth of the culture (Ryu *et al*., 2009).

In this research, three kind of medium has been used such as deMan Rogosa Sharpe Agar (MRSA), deMan Rogosa Sharpe Broth (MRSB), and Luria Broth. DeMan Rogosa Sharpe Agar (MRSA) used as the medium for growing the culture until get the host culture. The host culture is transferred in deMan Rogosa Sharpe Broth (MRSB) to make easier while doing the measurements. According to Lee *et al*. (2013), the existence of citrate will take a part as toxic in the blood bacteria. Thus, deMan Rogosa Sharpe Agar (MRSA) and deMan Rogosa Sharpe Broth (MRSB) were used for inoculation to reduce the risk of contamination caused by bacteria so the host culture that will be used is free from contamination. The growth of culture is affected by several factors, one of them is the medium or environment where the culture live. According to Yadav & Ram (2014), mushroom will grow by absorbing food from the medium they grow. Luria Broth as the chosen media for growing the host culture because it is much cheaper than deMan Rogosa Sharpe (MRS). If comparing the amount of substrate in both medium, Luria Broth has lower amount of substrate thus the results may show the *Sparassis crispa*'s growing

ability in Luria Broth. Several concentration certainly has different concentration of substrate which may affect the growth of *Sparassis crispa*.

Inoculation firstly did by taking the body part of *Sparassis crispa* in both sides of medium, deMan Rogosa Sharpe Agar (MRSA). The growth of *Sparassis crispa* may produce an appeared zone around its body part which means the greater diameter zone will lead to greater amount of culture. But it can not be easily said that the growth culture is certainly *Sparassis crispa* because its growth can not be seen physically. The main way to prove it by comparing the fresh *Sparassis crispa* and colonies that is shown with appeared zone in deMan Rogosa Sharpe Agar (MRSA) using microscope (Figure 6). Both of them has similar appearance such as the hyphae although the fresh *Sparassis crispa* has thicker hyphae. In accordance with the statement of Yadav & Ram (2014), the mycelium helps the hyphae in nutrient absorption by producing enzyme that may digest complex components.

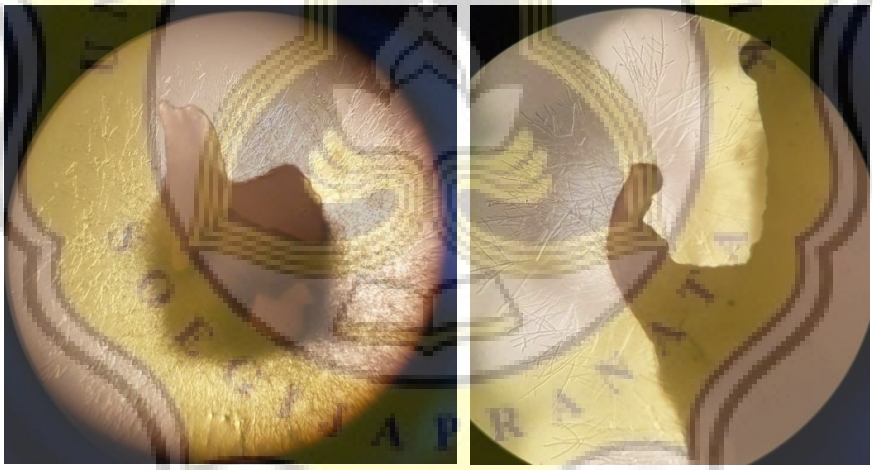


Figure 6. The appearance of fresh *Sparassis crispa* (left) and the appeared zone in deMan Rogosa Sharpe Agar (MRSA) (right) using microscope

The appeared zone in deMan Rogosa Sharpe Agar (MRSA) contains many colonies of *Sparassis crispa* (Figure 6). The single colony used as the host culture for inoculation thus the streaking out method is needed. Streaking out method was used to get discrete colonies which can be easier to identify the culture and also reduce the rist of contamination (Babu *et al*., 2013). It is the best method to get single colony where the amount of culture is spread out over a wide area. Single colony that is shown as single

dots (Figure 7) will be transfered in deMan Rogosa Sharpe Broth (MRSB) because it is easier to have a stock culture in liquid medium.

Figure 7. The appeared colonies after streaking out method

The growth of *Sparassis crispa* was measured using two methods, freeze drying and microplate methods. The freeze-drying method used for measuring the stock culture to ensure that *Sparasiss crispa* grows well in the medium. It is important because if *Sparassis crispa* does not grow, it will affect the other measurements. By using freeze-drying methods, the dry weight of culture can be measured every day. Freeze-drying is a preservation method that works with principle of removing water content well as reducing the water activity. Borgognoni *et al*. (2012) stated that freeze-drying needs longer time than the other methods of drying. If it compares with oven drying method, freeze-drying is much better because oven drying method has risk of evaporation of culture. While in freeze-drying, before starting the drying process the sample should be in the frozen state. When the culture is kept in the low temperatures the culture will be kept in a dormant state, a condition where the culture is incapable to grows. Thus the accuracy of the measurement can be maintained.

The dry weight of *Sparassis crispa* increases during 5 days although the rate of increase is not high (Figure 8). The growth of *Sparassis crispa* shows its growth ability in Luria Broth that only contains tryptone, yeast extract, and sodium chloride (Table 2). The low rate of increase may happen because *Sparassis crispa* shocked when it is transferred from solid medium to liquid medium thus it grows slowly while trying to adapt in the new medium. It can also due to the method of separating deMan Rogosa Sharpe Broth (MRSB)

and the culture where the culture may still contains in the medium or distilled water that is decanted. Freeze-drying method may reduce the risk of evaporation but it also has side effects such as dagame of cells that decrease vialibility of cells which can affect the result of measurements (Leslie *et al*., 1995). The freeze drying method can be optimized by adding cryoprotectans during the process. Research by Siaterlis *et al*. (2009) shows that the survival of microbial is affected by the kind of cryoprotectans and its concentration. Freeze-drying will cause stress to the culture during freezing and drying process thus the cryoprotectant can be used to increase the stability during the process.

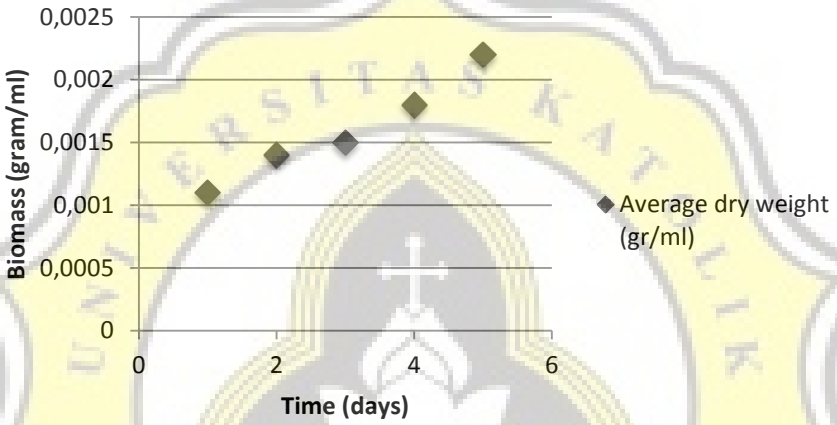
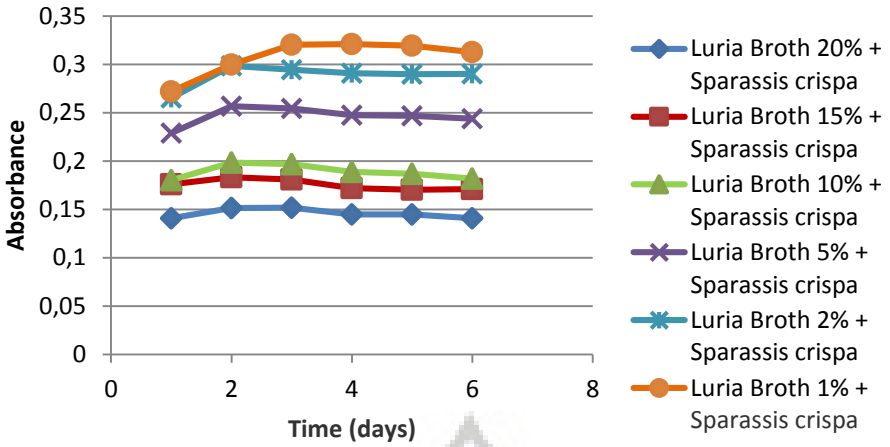


Figure 8. The dry weight of *Sparassis crispa* using freeze-drying method during 5 days.

The result of freeze-drying shows that *Sparassis crispa* grows in deMan Rogosa Sharpe Agar (MRSA). After 5 days of measurements, Sparassis crispa is added to Luria Broth with several concentration. Microplate method used for measuring their growth by reading the optical density of its concentration medium. Basically, the optical density is depend on the power of solution to absorp and scatter the light. The higher optical density will lead to higher concentration of culture in the medium (Dominguez *et al*., 2001). The wavelength 600 nm is usually used in several research, according to Stubbings *et al*. (2004), the moronecidin, antimicrobial peptide, and the antimicrobial properties can be read in this wavelengeth. Microplate methods used in the research that related with microorganism because it has several benefits such as only need few time to analyze, high accuracy, and required less chemical reagents (Hora'kova' *et al*., 2004).



Figu density

of *Sparassis crispa* in different concentration of Luria Broth during 6 days.

Based on the data in Figure 9, there is a slightly increase of absorbance in the second day for each culture but it cannot be a consideration. It is expected that the higher concentration of Luria Broth will lead to the higher growth rate of culture. The data of dry weight Sparassis crispa shows that the concentration is still low. In the low concentration condition, the sensitivity of spectrophotometer to measure the optical density is often adequate that may affect the data. While measuring the optical density, the spectrophotometer will measure between the culture and others that may absorb at the wavelength. If there are impurities in the culture, it will be read and calculated in optical density. According to Scott & Hwa (2011), the growth of bacteria can be divided into 4 phases, such as lag phase, exponential (log) phase, stationary phase, and death phase. The phase of microbial growth is expected to be seen in the data. In fact, the data only shows a flat graph that may be caused of the short time of measurement. The culture is transferred from deMan Rogosa Sharpe Broth (MRSB) to Luria Broth that has lower amount of substrate, thus the culture needs longer time to adapt in the new environment.

The optical density of *Sparassis crispa* can not show clearly the growth of culture in several concentration of Luria Broth. Thus, monod equation is calculated to predict the growth rate of *Sparassis crispa*. Monod equation is usually used for measuring the growth rate of culture in limited nutrient condition or bacth system. Monod equation is given as follows:

$\mu = \frac{\mu \max \times S}{S + K_s}$ (Owens & Legan, 1987)

μ = specific growth rate (day^{-1})

μ max = maximum specific growth rate

Ks = substrate saturation constant (g. l^{-1})

S = concentration of the growth rate-limiting nutrient (g. l^{-1})

By using the Lineweaver-Burk equation, the maximum specific growth rate (μ max) and half-saturation constant (K_s) will be known. According to Owens & Legan (1987), Lineweaver-Burk equation is widely used in the enzyme research projects. Lineweaver-Burk equation plot 1/ μ against 1/S that will get the equation to calculate the Km and μ max. In 1 gram of Luria Broth contains 0.65 gram tryptone, 0.33 gram yeast extract, and 0.03 gram sodium chloride. Substrate of Luria Broth are tryptone and yeast extract thus the substrate concentration of each concentration Luria Broth [S] can be calculated (Table 3). The optical density of *Sparassis crispa* in each concentration medium is inputed in a graph then will get a linear equation that can be written as y = ax + b that means y = μx + b. After the specific growth rate of each concentration Luria Broth and its substrate concentration are known, plot the datas in a graph (Figure 10).

Table 3. The subtrate concentration of Luria Broth in several concentration

Concentration of Luria Broth	Amount of tryptone (gram)	Amount of Yeast Extract (gram)	Subtrate Concentration (mg/ml)
1%	0.65	0.33	9.8
2%	1.3	0.66	19.6
5%	3.25	1.65	49
10%	6.5	3.3	98
15%	9.75	4.95	147
20%	13	6.6	196

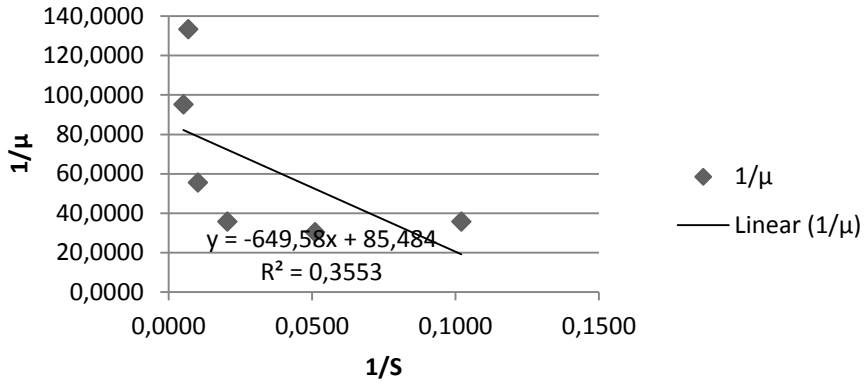


Figure 10. The Lineweaver-Burk equation that plot of 1/μ against 1/S

The Lineweaver-Burk equation such as y = - 649,58x + 85,484 which means y=Ks/μmax x + 1/μmax thus get Ks and μmax in the amount of 7.598849 and 0.011698. By knowing the Ks and μmax, the specific growth rate of monod equation can be calculated (Table 4).

Table 4. The specific growth rate of *Sparassis crispa* in several concentration of Luria Broth using Monod Equation

Concentration Luria Broth (S) (%)	Subtrate Concentration [S] (mg/ml)	Growth rate specific using monod equation
1	9.8	0.0066
2	19.6	0.0084
5	49	0.0101
10	98	0.0109
15	147	0.0111
20	196	0.0113

The specific growth rate of Luria Broth in several concentration Luria Broth is various with the range of 0.0066 until 0.0113. It may indicate that the growth rate of *Sparassis crispa* is slow, especially in Luria Broth. The differences of specific growth rate in each concentration of Luria Broth is not significant. Thus it can not be conclude which concentration has the optimum growth of *Sparassis crispa*. However, in the range concentration of Luria Broth from 1% until 20%, the highest specific growth rate is achieved in the 20% Luria Broth. It may be caused of highest nutrient content that will support the growth of *Sparassis crispa*.

5. CONCLUSION AND SUGGESTION

5.1. Conclusion

Sparassis crispa may grow in Luria Broth with several concentration such as 1%, 2%, 5%, 10%, 15%, and 20% but the highest specific growth rate is detected in 20% Luria Broth. The growth of *Sparassis crispa* can be measured using freeze-drying and microplate methods. Freeze-drying may reduce the risk of evaporation but it has side-effect such as damage cells that will decrese vialibility. Microplate methods will measure the optical density of culture that is related with its concentration. The specific growth rate of *Sparassis crispa* can be predicted using Monod Equation that is primar affected by the substrate concentration.

5.2. Suggestion

For further research needs to evaluate the the growth of *Sparassis crispa* by changing the characteristic of medium such as pH, nitrogen sources, and carbon sources. It also should observe the important effect of using cryoprotectans in freeze-drying methods.

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7. APPENDIX

Appendix 1. Institute Member

The administrators of the department are as follow:

a. Head of Department: Prof. Man Ghi Cho
b. Prof. Jae-Ha Shin
c. Prof. Justing Fendos
d. Prof. Ulf Stahl
e. Prof. Christoph Lindenberger
f. Dr. Alexander Jahn
g. Dr. Giovanni Luzi
h. Prof. Jeong In Yeong

Appendix 2. Place of Practical Training

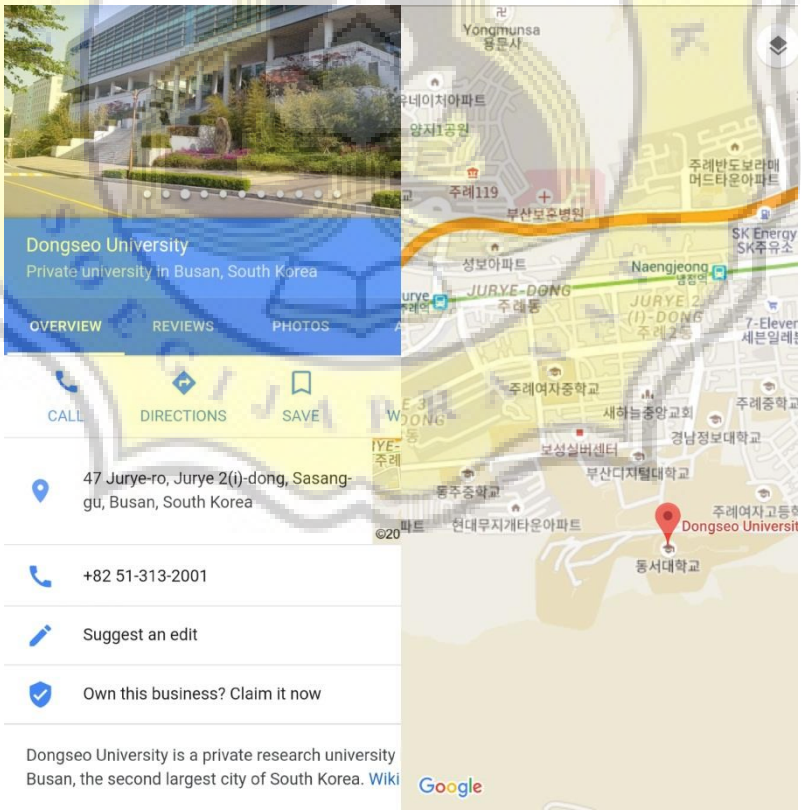

Figure 11. The map of Dongseo University

The practical training is in the Department of Biotechnology Dongseo University, Busan, South Korea. Dongseo university is located in 47 Jurye-ro Jurye 2(i)-dong, Sasanggu, Busan, South Korea.