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Studies on the Fermentation of Lupinseed

(Part 1) Determination of the Growth Rate of *Aspergillus oryzae* on Beans.

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루우핀 콩의 발효에 관한 연구

(제 1 보) 콩培地에서 *Aspergillus oryzae* 成長速度의 측정

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Abstract

The methods determining the growth rate of mold on beans were investigated in order to compare the growth of *Aspergillus oryzae* on lupinseed to that on soybean. The growth of *A. oryzae* on cooked whole or paste form of bean substrates was evaluated by the measurements of colony diameter and hyphae length of the mold. The mold showed characteristic lag times to form the colony on different types of substrate. The growth of colony diameter was coincided with the increase in α-amino nitrogen content of the substrate when the moisture level of the substrates was similar each other. The colony diameter and the cultivation time after the lag period showed a straight line relationship, from which the growth rate was estimated.

In general, lupinseed paste allowed faster growth of *A. oryzae* than soybean paste at the initial growth phase. The lag time to form the colony was 24.0 hrs on lupinseed paste and 44.4 hrs on soybean paste. The growth rate after colony formation was, however, 7.05 mm/day for lupinseed paste and 8.83mm/day for soybean paste, which indicated that the growth rate after the lag period was faster on soybean compared to lupinseed. The sporulation time of the mold was related to the lag time for the colony formation.

The measurement of hyphae length on whole beans could be used as a simple and rapid method of estimating the growth property of mold on different substrates. It showed that the growth of *A. oryzae* was partly hindered by the thick hull of the lupinseed.

Introduction

Lupins have been grown as a pulse crop for human consumption in Mediterranean countries and the highlands of South America for several thousand years. The seeds are valued for their high protein content, which varies from 30 to more than 40% on a dry matter basis.

The primitive varieties grown are extremely bitter because of an alkaloid content of 1.0 to 2.5% in the dry

seeds, but this is reduced to about 0.1% by boiling and prolonged steeping in brine before eating. During the period 1928-1935, German scientiests selected true breeding mutant strains of several lupin species, having natural alkaloid contents of only 0.02%.

The German "sweet" selections formed the genetic basis of all the improved sweet lupin varieties. The sweet white lupin seed of the species *Lupinus angustifolius* grown in Australia is considered to have great potential for being a new protein food resource.[1] The appearance of the seed is similar to that of soybean. It does not contain noticeable amounts of antinutritional components such as trypsin inhibitor and hemagglutinins, and has very low level of unpleasant flavor, compared to soybean, but it has slight bitter taste.

One of the possible use of lupinseed is for the substitution of soybean in making fermented soybean products. A series of research work on lupinseed fermentation has been undertaken at the authors' Laboratory. In order to compare the fermentation property of lupinseed to that of soybean in a system similar to the preparation of traditional Meju, the fermentation starter for Korean soysauce and soybean paste making, a simple method of estimating the growth rate of molds on the solid medium was needed.

Several methods of estimating the growth rate of molds have been reported in the literature. The measurements of mycelial weight in Koji, the Japanese style fermenttion starter, have been suggested.

Sakaguchi et al.[2] reported Asbestos Koji method, in which molds are grown on a piece of asbestos containing a culture medium. The amount of molds grown on asbestos is estimated from the ashed weight increase of the asbestos. Yamamoto[3]suggested Nylon-paste method, in which molds are grown on a nylon sheet covered on soybean paste. The weight of mycelia grown on the nylon bean paste. The weight of mycelia grown on the nylon sheet is determined, but the separation of nylon sheet from the paste without the adherent substrate is greatly difficult.

Both Taka-diastase method[4] and Glucosamine content method [5] are not applicable to soybean substrate, because of the high contents of diastase insoluble matter and glucosamine in soybean itself.

This paper compares the colony diameter of *A. oryzae* grown on the cooked and mashed beans to the increment of α-amino nitrogen content of the substrate. The growth rate of molds was estimated from the changes in colony diameter, and the sporulation time was measured. The growth of hyphae length on the surface of whole beans was also observed as a simple and rapid method determing the growth parameter.

Materials and Methods

Materials

Lupinseeds (*Lupinus angustifolius*) harvested at the Western Australia in 1981 was used. Soybean was purchased at the market in Seoul.

Preparation of seed cultures

A culture of *A. oryzae* was obtained from the Fermentation Laboratory of Korea University. It was grown on Czapeck agar plates[6] at 25°C for 4 days. The conidiospores formed were harvested by flooding the surface of plates with phosphate—Tween buffer (0.1M, ph 7.0, 0.01% Tween 80) and collecting the spore suspension asceptically. The spore suspension was stored at 4°C until use.

Preparation of Substrates

Fresh soybean and lupinseed were treated separately to make three different types of substrates from each bean. Type I; the cooked beans were mashed in a mortar into paste and spreaded on the petridish, as shown in Fig. 1. Type II; in order to test the influence of moisture con-

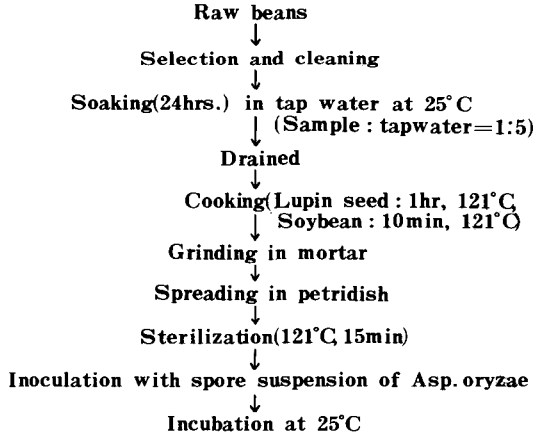

Fig. 1. Schematic Diagram of Sample Preparation.

tent on the growth of mold, cooked beans were dried at 60°C for 24 hours and ground with cutting mill. The powder passing through the 40 mesh sieve were mixed with certain amount of water to adjust the moisture content of the substrate. Type III; the cooked whole beans were used as the substrate. All samples in the petridish were sterilized before inoculation.

Inoculation

The spore suspension was vortexed every time when the inoculum was taken. The paste form of substrate was divided into four parts by drawing a cross line in a petridish. A single touch (ca. 0.01 ml of inoculum) of a platinum needle was applied at the center of the quater area of petridish. When colonies were developed the average diameter measured. In case of whole bean substrates, four grains of cooked whole beans were put in a petridish. The inoculation was made in two different ways; surface inoculation and subsurface inoculation. The surface inoculation was made by touching an inoculum of platinum needle on the surface of a cooked bean. The subsurface inoculation was made by thrusting the platinum needle 1 mm depth into the surface of cooked beans. The inoculated beans were incubated at 25°C.

Measurements

The colony diameter on the paste substrates was measured periodically by a calipers, and recorded in mm. The growth of hyphae length on whole beans was measured by using a magnifying glass (x5) and recorded in mm. The α-amino nitrogen content in the water extracts of the samples was determined. Two grams of sample were put into a 50 ml beaker and 18 ml of cold distilled water was added, and extracted for 15 minutes. It was stirred for 3-4 min prior to centrifugation at 1080 x g for 10 minutes. The supernatant was taken for the determination of α-amino nitrogen content by Sorensen's method.[7]

Results and Discussion

Colony Diameter and α-amino N Content.

The moisture content of cooked lupinseed paste was 67.5% and that of cooked soybean paste was 64.6%. Fig. 2 shows the changes in colony diameter and α-amino

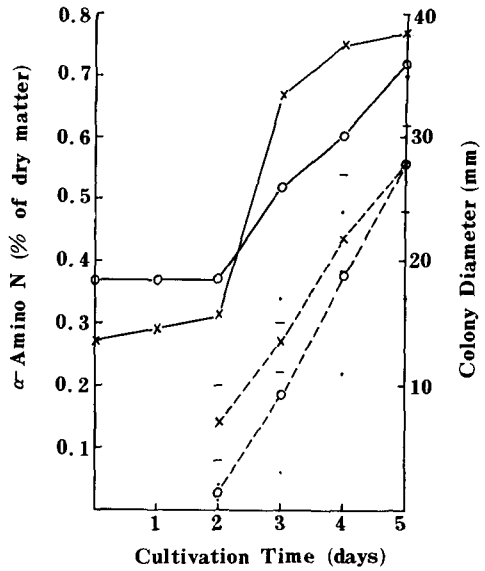

Fig 2. The Changes in Colony Diameter and α-Amino N Content of Cooked Paste of L upinseed and Soybean Grown with A.p. *oryzae*.

nitrogen content of cooked pastes of lupinseed and soybean on which *A. oryzae* was grown. The α-amino nitrogen content of soybean paste did not change for 2 days of incubation, while lupinseed showed a slight increase in α-amino N for the same period. A rapid increase in α-amino nitrogen content was observed from the third day of the incubation in both lupinseed and soybean. Lupinseed substrate yielded larger amount of α-amino nitrogen compared to soybean. It was well coincided with the increase of colony diameter of *A. oryzae* grown on the substrates. Fig. 3 and 4 show the changes in the colony diameter and α-amino N content of cooked lupinseed and soybean pastes of which moisture content were adjusted to 75% and 60%, respectively.

The colony diameter of *A. oryzae* was well correlated to the α-amino nitrogen content of the substrate when the moisture content was similar. The correlation coefficients between the colony diameter and the α-amino nitrogen content of the substrates were 0.93 ($P<0.01$) for cooked bean paste, and 0.90 ($P<0.01$) and 0.66 (not significant) for the pastes of which moisture contents

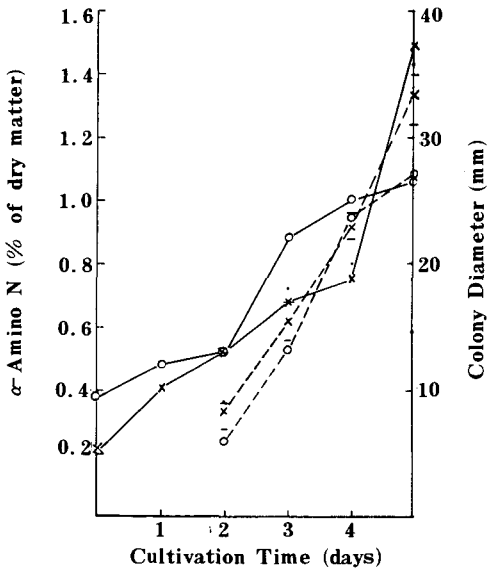

Fig 3. The Changes in Colony Diameter and α-Amino N Content of Cooked Lupinseed and Soybean Powder, of Which Moisture Content was Adjusted to 75% (Legends same as in Fig. 2)

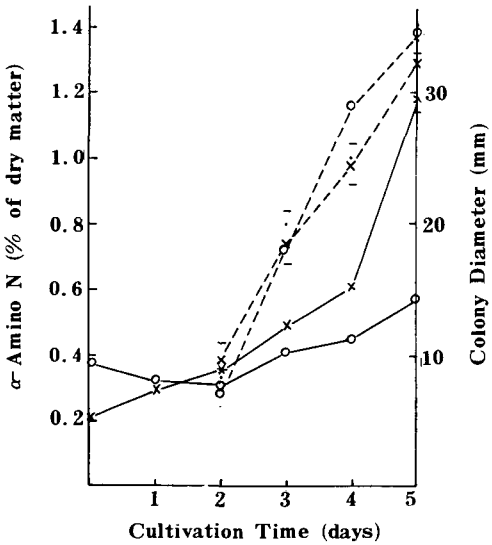

Fig. 4. The Changes in Colony Diameter and α-Amino N Content of Cooked Lupinseed and Soybean Powder, of Which Moisture Content was Adjusted to 60%. (Legends same as in Fig. 2)

were adjusted to 75% and 60%, respectively. However, the colony diameter was inversely related to the α-amino N content when the moisture content of the substrate varied from 60% to 85%, as shown in Fig. 5. This inverse correlation was more apparent in soybean compared to lupinseed. The increasing α-amino N content with the increasing moisture content of the substrate, despite of the smaller colony diameter, may indicate the effect of moisture content on the proteolytic activity of the molds. This fact dictates that the α-amino N content is not suitable parameter indicating the growth of mold per se, especially when the moisture content of the substrates varies widely.

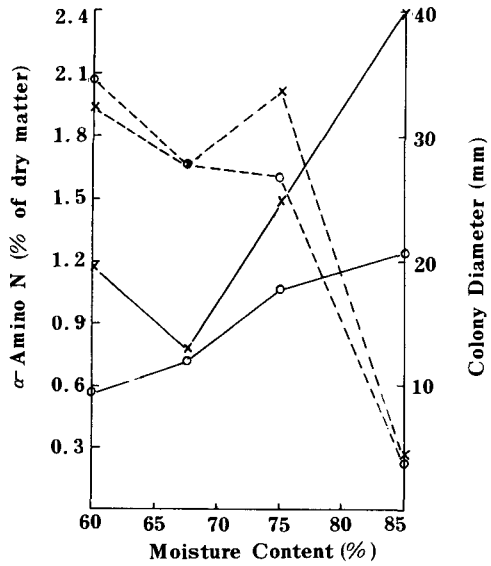

Fig 5. Effect of Moisture Content on the Colony Diameter and α-Amino N Content of Lupinseed and Soybean Grown with *A. oryzae* for 5 days.

In general, lupinseed paste grew *A. oryzae* faster than soybean paste at the initial growth phase. The colonies of *A. oryzae* were not visible for two days of incubation. The curves for colony diameter vs. cultivation time approximated a straight line. By extrapolating the colony growth curve the lag time to form the colony of *A. oryzae* on lupinseed was estimated to be 24 hrs, as shown in Fig. 6. The lag times to form the colony on lupinseed were shorter than those on soybean, regardless of the moisture content of the substrate, as shown in Table 1. The slope

Table 1. The Lag time and Growth Rate of *A. oryzae* Grown on Beans With Different Treatment.

Treatments	*Lag time (hrs)*		*Growth rate* (mm/day)	
	Lupin seed	*Soybean*	*Lupin seed*	*Soybean*
Cooked paste	24.0	44.4	7.05	8.83
Adjusted moisture content				
75% Water	25.2	28.8	8.25	7.35
60% Water	24.6	30.0	7.35	9.25

of the curve, representing the growth rate of the mold after the lag period, was 7.05 mm/day for lupinseed paste, and 8.83 mm/day for soybean paste, as shown in Table 1. It indicates that the growth rate after the lag period is faster on soybean compared to lupinseed. However, this trend was affected by the moisture content of the substrate.

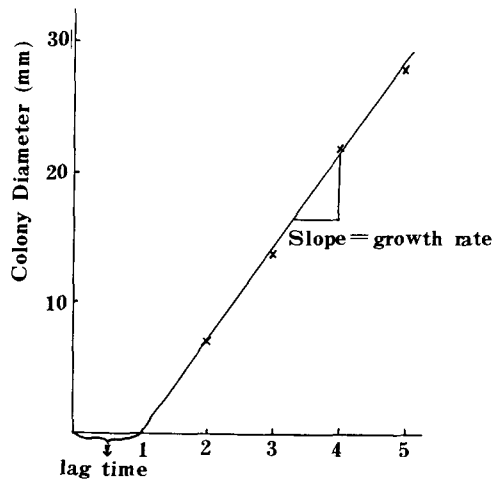

Fig 6. Example for the Calculation of Lag Time for the Formation of Colony and the Growth Rate after Lag Time.

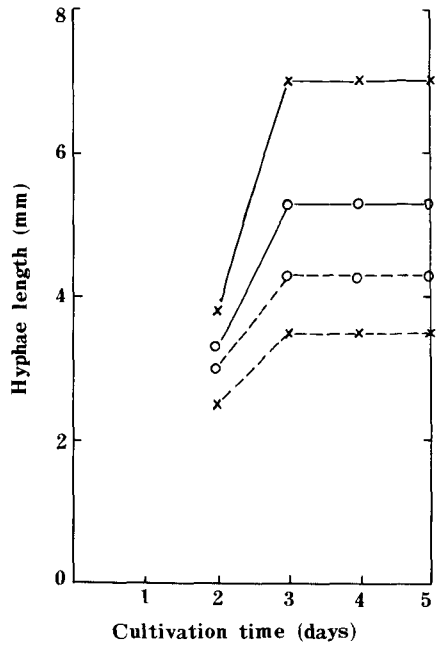

Fig 7. The Growth of Hyphae Length of *A oryzae* Inoculated on the Surface or Subsurface of Cooked Lupinseed and Soybean.

× — × Lupinseed ——— Subsurface inoulation
○ — ○ Soybean ····· Surface inoculation

Hyphae length

Fig. 7 shows the growth of hyphae of *A. oryzae* on the surface of cooked whole beans. The subsurface inoculation showed in general faster hyphae growth than surface inoculation. In subsurface inoculation, lupinseed allowed faster growth of *A. oryzae* than soybean did. On the other hand, *A. oryzae* grew faster on the surface of soybean compared to lupinseed. This fact illustrates the effect of the hull of beans on the growth of the mold. The hull of lupinseed is thicker and harder than that of soybean, and the thick hull inhibites the growth of *A. oryzae* on the surface of lupinseed.

Sporulation time

Table 2 shows the sporulation time of *A. oryzae* on different substrates. In general, the sporulation time is shorter on lupinseed compared to soybean. The lag time for the formation of colony was appeared to be related to

Table 2. The Sporulation time of *A. oryzae* Grown on beans with Different Treatments. (hrs)

Treatments	Lupin seed	Soyean
Cooked paste	36	60
Adjusted moisture content		
85% water	48	120
75% water	40	60
60% water	40	48
Subsurface inoculation	36	36
Surface inoculation	36	40

the sporulation time of the mold. The sporulation time tended to increase by the increasing moisture content of the substrates.

Conclusion

The measurement of colony diameter was applicable in comparing the substrate characteristics of lupinseed to that of soybean for the growth of *A. oryzae*. The colony diameter was well correlated to the increase in α-amino N content of the substrates when the moisture content of the substrate was similar. The colony diameter increased almost linearly with the cultivation time after the lag time for the colony formation. The lag time and the slope of the curve for colony diameter versus cultivation time could be used as the parameters characterizing the growth of mold on bean substrates.

The sporulation time coas related to the lag time for the colony formation of the mold. The measurement of hyphae length could be used as a Simple and rapid method of estimating the growth property of the mold.

In case of cooked whole bean, the growth of mold was partly hindered by the thick hull of the lupinseed. This fact indicated the importance of hull treatment for the fermentation of lupinseed.

요 약

삶은 콩을 이용한 고체 배지상에서 *Aspergillus oryzae*의 성장 속도를 측정하는 방법에 대하여 연구하고 이들 방법을 대두와 루우핀콩 배지에서의 균 성장 특성을 비교하는데 적용하였다.

여러가지 배지 조건에서 배양 기간에 따른 균체 colony의 형성시간, 크기변화, α-아미노 질소함량의 변화, 포자형성 시간 및 균사길이의 변화등을 측정 비교하였다.

배지의 종류에 따라 Aspergillus oryzae는 특정한 균체 colony형성 시간을 나타내었으며 colony 형성후 부터는 colony 직경의 크기는 배양 시간에 따라 직선적으로 증가 하였다. 균체 colony 직경은 배지중의 α-아미노 질소함량과 높은 직접적인 상관관계를 나타내었으나 배지의 수분함량이 변하면 이러한 상관관계가 성립되지 않았다. 오히려 배지의 수분함량이 증가 하면 (60%-85%) α-아미노 질소함량은 증가하나 colony 직경은 감소하는 역 관계를 나타내었다.

일반적으로 *Aspergillus oryzae*는 삶은 루우핀콩 페이스트에서 최초 성장 속도가 대두보다 빨랐으며, 루우핀콩 배지에서의 colony 형성시간이 24시간인데 반해 대두에서는 44.4 시간이 걸렸다. 그러나 colony 형성이후의 성장 속도는 대두에서 더 빠르게 나타나 대두의 경우 8.83 mm/day 인데 반하여 루우핀 콩에서는 7.05mm/day이었다. spore 형성 시간은 colony 형성 시간과 깊은 상관관계를 나타내었으며 일반적으로 루우핀콩에서 spore 형성 시간이 짧았다.

콩의 표면에서 균사길이를 측정하는 방법은 간단하고 빨리 균체의 성장 속도를 비교할수 있었으며 특히 콩의 구조와 형태가 균체 성장에 미치는 영향을 조사하는데 편리하였다. 본 실험에서는 이 방법으로 루우핀콩의 두꺼운 외피가 균체 성장을 저해하고 있음을 발견하였다.

References

1) Lee, C.H., *Food Technology (Korean)*, **23(1)**, 3. (1982).
2) Sakaguchi, K., H., Okazaki and M., Takeuchi: *J. Agr. Chem. Soc. Japan*, **29**, 349. (1955).
3) Yamamoto, K., *Bull. Agr. Chem. Soc. Japan*, **21(5)**, 308. (1957).
4) Terui, G. and T., Morimoto: *J. Ferm. Tech. Japan*, **34**, 575. (1956).
5) Arima, K., and T., Vozumi: *Agr. Biol. Chem.* **31(1)** 199. (1967).
6) *BBL Manual of Products and Laboratory Procedures*, Division of Becton, Dickinson and company, 102. (1973).
7) *AOAC Methods of Analysis*, 13th edition. (1980).