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Purification and gene cloning of α-Amylase of *Neurospora crassa*

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*Neurospora crassa*에서 알파아밀라제의 정제 및 유전자의 클로닝

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ABSTRACT: α-Amylase (EC.3.2.1.1) of *Neurospora crassa* (ATCC 9279) was cloned in *E. coli* HB101 using shotgun method, and the enzymes isolated from both *N. crassa* and *E. coli* were compared.

Chromosomal DNA isolated from the spores of *N. crassa* was partially digested with PstI restriction endonuclease and rejoined to pBR322 which had been digested with the same enzyme. The resulting recombinant DNA were introduced into *E. coli* HB101 which had competancy by treating with $CaCl_2$. As the result, about 8000 colonies which showed tetracycline resistance were selected and two of the colonies which had 13.5 Kb recombinant plasmid exhibit starch degrading activity on starch-containing plate when treated with D-cycloserine.

α-Amylases from both *N. crassa* and *E. coli* were isolated by using ammonium sulfate precipitation, DEAE-cellulose ion exchange column chromatography and Bio-Gel P150 gel filtration column. As the result, about 81.3 fold and 5.6 fold purifications in specific activities were obtained respectively, and specific activities of the gel filtrates were 6.1 u/mg and 85 u/mg respectively.

The properties of both enzymes were compared and they showed quite the similar patterns in optimal temperature, optimal pH and had same molecular weight about 100,000 daltons on gel filtration method. Optimal temperatures for both enzymes were 70°C and optimal pH were about 6 and 10.

KEY WORDS□*N. crassa*, α-amylase

α-Amylase (EC.3.2.1.1), which can hydrolyze starches, has numerous biotechnical applications, especially in food-processing factories. It can hydrolyze α-1-4-glucosidic linkage of starch in endo fashion to yield glucose, maltose and a series of branched α-limitted dextrins but cannot break α-1-6 linkage in general (W.M. Forgaty, 1983).

The distribution of α-amylase is very wide, from prokaryotic and fungal species to mammalian cells. The properties of this enzyme from various species were studied extensively and the molecular cloning of this enzyme has also been reported from many laboratories. α-Amylase encoded by *B. licheniformis* in *Bacillus subtilis* was reported by Ortlepp *et al.* (Ortlepp *et al.*, 1983). From *B. stearothermophillus* in *E. coli* and *Bacillus subtilis* by Ryu & Yang and Mielenz independently (Ryu and Yang, 1985; Mielenz 1983).

In this study, the α-amylase gene encoded by *Neurospora crassa* was cloned in *E. coli* and its successful expression was observed. Also enzymes were isolated from both *N. crassa* and *E. coli* and the properties of these enzymes were compared.

MATERIALS AND METHODS

A. Cloning of alpha-amylase in *E. coli*

Materials: *N. crassa* (ATCC 9279) were obtained

from American Type Culture Collections. All dehydrated culture media were purchased from Difco Laboratories and all restriction enzymes were purchased from Betheda Research Laboratories and Bio-Rad. D-cycloserine and tetracycline were purchased from Sigma Chemical Co.

Induction and collection of *N. crassa* spores: Spores of *N. crassa* were spreaded over sporulation agar plates (1.0 gr yeast extracts, 1.0 gr beef extracts, 2.0 gr tryptose, trace amount of $FeSO_4$, 10.0 gr glucose, 15.0 gr agar, pH 7.2, make 1 litter by distilled water), and incubated at 27 °C without light for 4-6 days and then further incubated at 30 °C with illuminations for 2-3 days. The resulting orange-colored spores were resuspended in distilled water and then collected by centrifugation. (Sorvall GSA rotor, 6,000 rpm. 20 min.)

Isolation of chromosomal DNA from spores of *N.* crassa: Isolation of chromosomal DNA from *N. crassa* was performed according to the method described by Case *et al.* (Case *et al.*, 1979). The 5 gr wet weight of spores were resuspended in 200 m*l* of *N. crassa* culture media (5.0 gr yeast extracts, 5.0 gr proteose peptone No. 3, 40.0 gr maltose, make 1 litter by distilled water) and then incubated at 30 °C for 4 hrs to weaken the spore cell walls and collected by centrifugation. (Sorvall GSA rotor, 6,000 rpm. 20 min). It than resuspended in 100 m*l* of lytic buffer (25 mM Tris-Cl, 2 mM EDTA, 100 mM glucose, 1 mg/m*l* lysozyme, pH 8.0) and incubated at 37 °C for 2 hrs, SDS were added to final concentration 0.5% and further incubated at 40 °C for 30 min.

The resulting spore suspension was mixed with equal volume of phenol which was pre-equilibrated with lytic buffer and gently extracted to denaturate proteins. The two layers were separated by centrifugation (Sorvall GSA rotor, 3,000 rpm, 20 min.) Second phenol extraction was performed. The phenol-chloroform 1:1 mixture extraction and chloroform extraction were followed two times each. The resulting aqueous phase was finally extracted with water-saturated ether for two times to remove any rsidual organic solvents and two volumes of cold ethanol were addded and placed at -20 °C for 2 hrs and centrifuged to collect precipitated chromosomal DNA. (Sorvall SS-34 rotor, 12,000 rpm. 20 min.) After drying completely, the DNA precipitated was redissolved in 500 μl of TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0).

Construction of genomic library: Chromosomal DNA obtained as described was partially digested with retriction endonuclease Pst I. The amonut of enzyme used was so controlled to yield 7 Kb fragments as the major products. The reaction condition was as followes.

100 μl of chromosomal DNA (0.48 mg/m*l*)
15 μl of Bovine Serum Albumin (10 mg/m*l*)
15 μl of 10 x Reaction buffer (500 mM NaCl, 100 mM Tris-Cl, 10 mM dithiothreitol, 100 mM $CaCl_2$, pH 7.5) (Maniatis *et al*, 1982)
100 unit of PstI restriction endonuclease
double distilled water to 200 u*l*
Incubate at 37 °C for 2 hrs.

The resulting DNA fragments were heated at 65 °C for 10 min to inactivate the nuclease activity and rejoined to pBR322 which was also digested with the same enzyme. The ligation reaction was performed as described by Dugaiczyk *et al.* with a slight modifications (Dugaiczyk *et al*, 1975; Maniatis *et al.*, 1982). The reaction condition was as followes.

36.2 μg of chromosomal DNA fragments
2.8 μg of pBR 322
5.0 μl of reaction buffer (500 mM Tris-Cl, 100 mM $MgCl_2$, 200 mM dithiothreitol, 10 mM ATP, pH 7.8)
50 units of T4 DNA ligase
double distilled water to 420 μl
And incubated at 12.5 °C, overnight.

Transformation of *E. coli:* Transformation of *E. coli* HB 101 was performed as described by Mandel with some modifications (Mandel and higs, 1970; Cohen *et al.*, 1972; Maniatis *et al.*, 1982). To the 200 μl of competent cells, 100 μl of ligated DNA (50 ug/100 μl) was added. And after heat treatment at 42 °C for 2 min, it was added 1 m*l* of 1.3 x LB media and incubate at 37 °C for 45 min.

The resulting transformed cells were plated on LB plates containing 12.5 μg/m*l* tetracyclin and incubated at 37 °C for 2 days.

Screening for alpha-amylase activity: The screening of alpha-amylase bearing cells were performed as suggested by Mielenz (Mielenz, 1983). Cells which showed tetracyclin resistancy were transferred onto assay agar plates (0.3% KH_2PO_4, 0.6% Na_2HPO_4, 0.05% NaCl, 1% starch, 1% peptone, 0.1% NH_4Cl, 0.1% yeast extracts, 1.5% agar.) and incubated for 4 to 6 hrs and then assay agar containing 3 mg/5 m*l* of D-cycloserine were overlayed and further incubated at 37°C, overnight. The resulting plates are tested by pouring 2.5% I_2 in 10% KI on the surface of the plates.

Analysis of the amylase-coding vector: Vector that isolated from amylase producing *E. coli* was isolated by the method of Birnboim and Doly (Birnboim and Doly, 1979) and restriction map was made with restriction endonuclease PstI, EcoRI, Hind III, BamHI, KpnI, PvuII.

B. Purifications of amylase

Materials: DEAE-cellulose was obtained from Merck Co. and Bio-Gel P 150 was from Bio-Rad Co. All other chemicals were of highest quality available.

Culture conditions: The spores of *N. crassa* was inoculated in 3 liter of *N. crassa* culture media and incubated at 28°C for 7 days. The resulting mycelia of *N. crassa* was collected by filtration through four-fold gauge and frozen at -20°C untill used.

E. coli HB101 that containes pV32, amylase-coding vector, was inoculated in 2 litter of LB media that contains 12.5 mg/m*l* of tetracyclin and incubated at 37°C for 1 day and harvested by centrifugation (Sorvall GSA rotor, 6000 rpm, 20 min.).

Enzyme assay: In this study, two kinds of methods was used to assay amylase activity, the 'relative activity assay' and the 'absolute activity assay'.

I. Relative activity assay: This method was used to compare conveniently the relative activities of each fractions of column chromatography. Due to the fact that starch-iodine color is unstable and the deepness of the color is time and temperature dependent, the measurement of absolute activity of the enzyme requires much works and cares but is not demanded in fractionation of the active effluents of the column, so this method was used to fractionate the effluents of column chromatography.

The measured amounts of starch solutions were prepared in each test tubes and also the measured portion of fractions were mixed. Then after incubated at 37°C for some proper times, color developer (0.025% I_2 in 0.1% KI) was added and after some color developing time had passed, the absorbance at 617 nm was read. The less the solution absorbe the light, the stronger the activity of the enzyme.

The exact condition for this assay was varied according to the purification steps and mentioned at each steps.

II. Absolute Activity Assay: This method was used to determine the absolute activity of each purification steps and operated on the basis of the measurement of the starch degraded in specified time and conditions

One unit of enzyme activity was defined as the amount that decompose 1 mg of starch at 37°C for 1 hrs, pH 6.8.

The amount of starch decomposed was determined by spectrophotometric way. First the standard curve for starch concentration was determined as follows.

Starch concentrations varying from 0.0 to 0.1% (1 mg/m*l*) were mixed with one-tenth volume of color developer and after 5 min, the absorbances at 617 nm were read. The correlation coefficient of the result was 0.996 by least square method and showed fairly good linearity.

Absolute activity was measured exactly the same condition as described above. Enzyme samples were mixed with substrate to make final starch concentration 0.1% then after incubate at 37°C for 1 hr, the absorbance at 617 nm was read after one-tenth volume of color developer was added.

These methods were the modification of Pfueller and Eliott (Pfueller and Eliott, 1969).

Protein assay: The assay for the amount of protein was performed according to Bradford's method with a slight modification (Bradford, 1976; Scopes, 1982). Standard protein solution was

made using BSA (bovine serum albumin).

Preparation of crude extracts: The crude extract of *N. crassa* was prepared by the following procedures.

About 60 gr of frozen mycelia was resuspended in 150 m*l* of buffer A (0.005 M Tris-maleate, pH 7.0) and sonicated at 0 °C (Heat System-Ultrasonic, Inc., Model W-225R) for 1 hr at the power range about 100 and centrifuged (Sorvall GSA rotor, 10,000 rpm, 20 min). The resulting 140 m*l* brown-colored and somewhat turbid supernatant was designated as 'crude extract of *N. crassa*' and further purification was performed using this crude extract.

The crude extract of *E. coli* was prepared by the similar method. 5 gr of *E. coli* cells were resuspended in 70 m*l* of buffer A and sonicated for 30 min at the power range about 100 and centrifuged (Sorvall GSA rotor, 10,000 rpm, 20 min). The resulting yellow-colored supernatant was designated as 'crude extract of *E. coli*'.

Ammonium sulfate precipitations: The purification steps for both *N. crassa* and *E. coli* were nearly the same. Some differences between the two will be noted separately.

To fractionate amylase solubilized in the crude extracts, 100% saturated solution of ammonium sulfate was added to make final ammonium sulfate 40% saturations and after standing on ice for 2 hrs, the precipitates were collected by centrifugations (Sorvall GSA rotor, 10,000 rpm, 20 min) and resuspended in 40 m*l* and 30 m*l* of buffer A, respectively then dialyzed against the same buffer to remove ammonium sulfate.

DEAE-cellulose column chromatography: The products partially purified by ammonium sulfate precipitation were loaded on DEAE-cellulose columns which were pre-equilibrated with buffer A and eluted with increasing NaCl concentration. The dimension of the columns were 3.7 × 18 cm and flow rates were 89 m*l*/hr. After washing the column with 2 bed-volume of buffer A, NaCl gradients were made from 0.0 to 0.6 N and effluents were franctioned by 6 m*l* volumns.

The fractions collected was assayed by relative activity assay method. Assay condition was as followed.

1. Assay for *N. crassa*-origin amylase
   2 m*l* of 0.1% starch in buffer C (0.01 M K^+ phosphate, pH 6.8)
   100 μl of fractionations

Incubated at 37 °C for 1 hr, then 200 u*l* of color developer was added and after 30 min, the absorbances at 617 nm were read.

2. Assay for *E. coli*-origin amylase
   1 m*l* of 0.1% starch in buffer C
   50 μl of fractionations

Incubate at 37 °C for 1 hr, then absorbances at 617 nm were read 30 min. after 100 μl of color developer was added.

The active fractions were pooled and concentrated with ultrafiltration technique.

Gel chromatography: Bio-Gel P150 (exclusion limit 150,000 daltons), which was pre-equilibrated with buffer B (0.001 M acetate buffer, pH 5.2) was used. The column dimensions were 2.5 × 30 cm and the void volumes estimated by Blue-Dextran were 51.5 m*l*. The flow rate for this column was 25 m*l* per an hour.

1. Amylase of *N. crassa* was concentrated to 4 m*l* nd loaded on gel-filtration column and fractions were collected by 5 m*l* for each test tube. Activity assas was performed by the followings.
   200 μl of 0.05% starch in buffer C
   100 μl of fractions

Incubate at 37 °C for 2 hrs, then absorbances at 617 nm were read after 200 u*l* of color developers were added.

2. Amylase of *E. coli* was concentrated to 8 m*l* and 5 m*l* of this was loaded. 6 m*l* fractions were collected and activity assay was done by the following way.
   2 m*l* of 0.1% starch in buffer C
   50 μl portions of fractions

Incubate at 37 °C for 40 min, then 200 μl of color developers were added and after 1 hr, the absorbances at 617 nm were read.

The active fractions were collected and concentrated through ultrafiltrations.

Measurement of optimal temperatures: Optimal remperatures for the enzymes were tested as followings.

1 m*l* of buffer C

100 μ*l* of 1% starch in buffer C for *E. coli* origin or 0.1% in buffer C for *N. crassa* origin

10 μ*l* of enzyme solutions

Incubated at 30-80 °C with 10 °C increments for 30 min. the 100 μ*l* of color developer were added and after 30 min, the absorbances at 617 nm were read.

Measurement of optimal pH: Optimal pH for the two enzymes were tested by the following procedures.

2 m*l* of buffers of various pH

200 μ*l* of 1% starch in 0.01 M Tris-Cl, pH 8.0

20 μ*l* of enzyme solutions for *E.coli* origin or 200 μ*l* of enzyme solution for *N. crassa* origin

Incubated at 37 °C for 30 min. then absorbances at 617 nm were read after 100 μ*l* of color developer were added. The compositions of each reaction buffer were as followes.

pH 4.0 and 5.0: acetate buffer

pH 6.0, 7.0 and 8.0: phosphate buffer

pH 9.0 and 10.0: carbonate buffer

The activity comparisons for above two experiments were performed with control which contained no proteins, and the absorbance ratios between sample and control, $A_{617}(S)/A_{617}(C)$ were used for calculations.

Molecular weight estimation: Molecular weights for both enzymes were estimated by gel filtration methods. Bio-Gel P150 (1.5 x 25 cm) column was used. The bed volume was 44 m*l* and the void volume was 14 m*l* when estimated by Blue-Dextran. The flow rate of this column was adjusted to 9.3 m*l*/hr.

The standard markers were as followes.

Lysozyme (mw 14,300), chymotrypsinogen (mw 25,700), BSA (mw 67,000) and gamma-globulin (mw 160,000).

RESULTS

A. The cloning of α-amylase

The amount of chromosomal DNA isolated by the method described before was 0.25 mg from 5

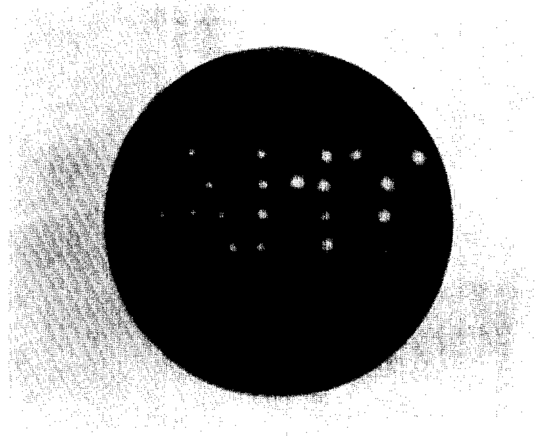

Fig. 1. *α-Amylase assay on plate.*
Clear Zone has appeared because starches are degraded and no starch-iodine color has developed in colonies which has amylase activities.

gr wet weight of spores and the size was as estimated by 0.8% agarose gel electrophoresis was about 40 Kb or greater. The constructed genomic library of *N. crassa* genes, partially digested by PstI were mixed with pBR322 vector DNA cut with the same enzyme and ligation reaction was performed using T4 DNA ligase. As the result about 50% of vectors had the inserted foreign DNA fragments.

Transformation efficiency was 1.5×10^{-6} transformed cells/cell and among about 8,000 colonies which showed tetracyclinresistant and ampicillin-sensitive phenotype, 2 colonies exhibitted starch degrading activity on starch containing plates treated with cycloserine. The example of activity assay on starch-containing plate is presented on figure 1.

The two isolated vectors bearing amylase activity were compared in terms of size and restriction map and the two were concluded as the identical one and named as pV32. The size of pV32, amylase-coding vector, was 10.2 Kb on 1% agarose gel electrophoresis and the restriction map is presented in figure 2. and one of the PstI site was inactivated due to some unknown reason.

B. Purification of α-amylase

Enzymes from *N. crassa* and *E. coli* were partially purified and properties were studied. En-

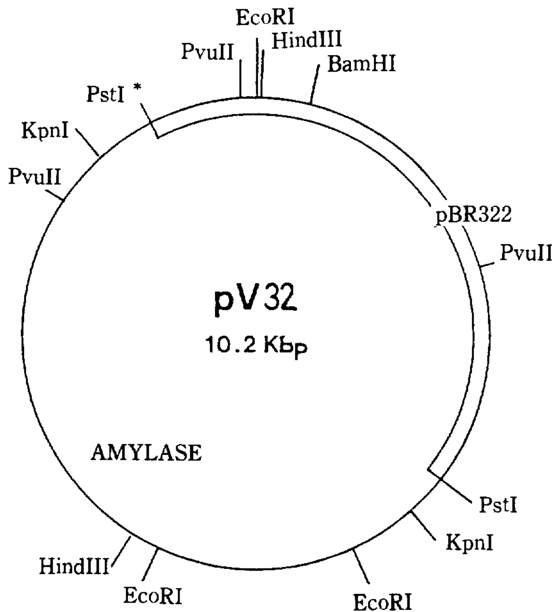

Fig. 2. *The Restriction Map of pV32.*
E: EcoRI site, P: PstI site, B: Bam HI site, H: HindIII site, K: KpnI site, P: PvuII site
* stand for inactivated PstI site.

zymes precipitated by ammonium sulfate were further purified through ion exchange chromatography with DEAE-cellulose and the peaks of activity were eluted at 0.4N NaCl for both enzymes. The elution profiles are presented in figure 3 and

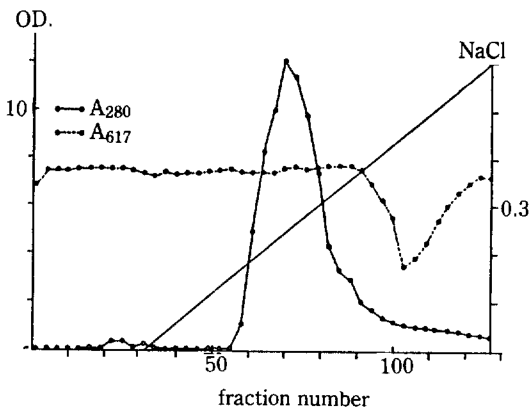

Fig. 3. *DEAE-cellulose elution profile of N. crassa-origin α-amylase.*
Column was equilibrated with Tris-maleate buffer, pH 7.0 and eluted with increasing NaCl concentration from 0.0 to 0.6N. Solid line stands for the protein amount monitored by absorbance at 280 nm and dashed line stand for amylase activity. The lower the absorbance, the stronger the activity. Peak of activity was eluted at NaCl concentration of 0.4N.

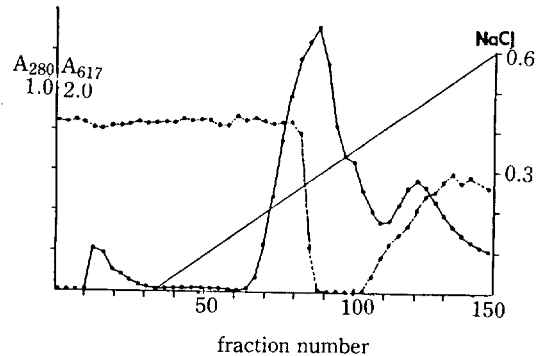

Fig. 4. *DEAE-cellulose elution profile of E. coli-origin α-amylase.*
Column was equilibrated with Tris-maleate buffer, pH 7.0 and eluted with increasing NaCl concentrations. As can be seen, the activity was so strong that the exact peak of activity could not determined but agreed with the result of *N. crassa*.

4.

The active fraction of DEAE-cellulose were collected and purified by gel filtrations. As the result 5.6 and 81.3 fold purifications were achieved for *E. coli* and *N. crassa* origin enzymes, respectively. The active peaks of the enzyme were well agreed with the protein peaks monitored by absorption at 280 nm. The elution profiles for these are presented in figure 5 and 6.

Total purification result is presented in purifi-

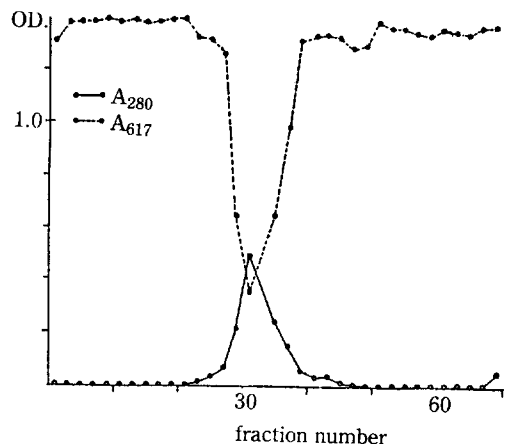

Fig. 5. *Gel filtration profile for N. crassa-origin α-amylase.*
Bio-Gel P150(2.5 x 30 cm) was used and the void volume was 51.5 m*l*. Used buffer was 0.001 M acetate buffer, pH 5.2 and the flow rate was 25 m*l*/hr.

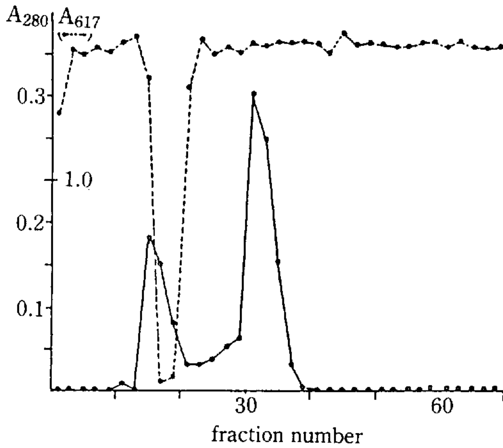

Fig. 6. *Gel-filtration profile of E. coli-origin α-amylase.* Bio-Gel P150(2.5 × 30 cm) was used and flow rate was 25 ml/hr.

cation table. One unit of enzyme is defined as the amount that decompose 1 mg of starch at 37 °C for 1 hr, pH 6.8. As can be seen in the table 1, the specific activities of crude extracts of *N. crassa* and *E. coli* were 0.91 and 15 units/mg protein and that of gel-filtrated were 64.1 and 89 units/mg protein, respectively. As seen in figure 7, optimal temperatures for both *N. crassa* and *E. coli* origin enzyme were found to be 70 °C. And optimal pHs were 6 and the second pH optima were observed at pH 10 for both. (Figure 8)

Molecular weights for both enzymes were estimated by gel filtration method. The enzymes were eluted at 29.3 ml and the corresponding molecular weight were 100,000 daltons. (Figure 9)

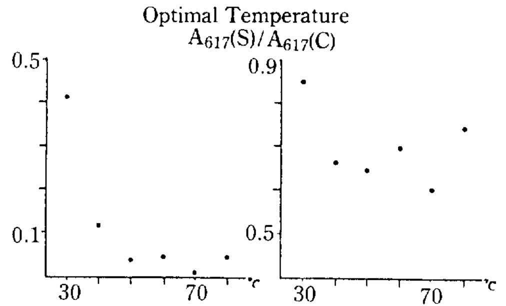

Fig. 7. *Optimal temperature of the enzymes.* The absorbance ratio between sample and control was used to measure optimal temperature. Figure left was measured for *E. coli* origin enzyme and right was for *N. crassa*. The two figures were very similar in patterns and optimal temperature was 70 °C.

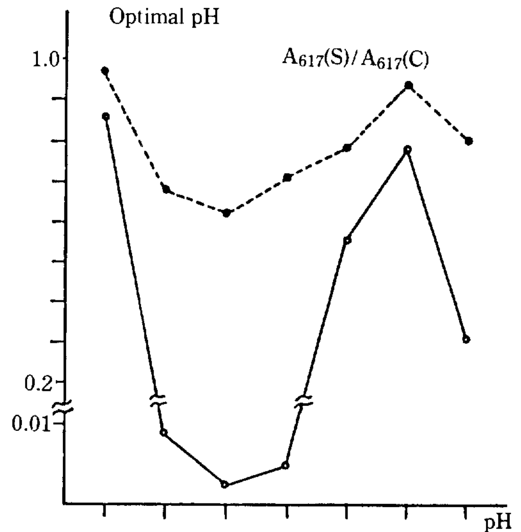

Fig. 8. *Optimal pH of the enzyme.* This measurement was performed with controls that contained no enzyme. Straight line was for *E. coli*-origin enzyme and dashed line for *N. crassa*-origin. They showed similar patterns and seemed to have two pH optima, pH 6 and 10.

DISCUSSIONS

From the result of molecular cloning of α-amylase of *N. crassa* and its successful expression in *E.*

Purification Table

	N. crassa			E. coli		
	Specific Activity (u/mg)	Total Activity (unit)	Yield (%)	Specific Activity (u/mg)	Total Activity (unit)	Yield (%)
crude extract	0.78	128.5	100.0	15	2324	100.0
$(NH_4)_2SO_4$	0.24	7.2	5.60	10.4	660	28.4
DEAE	8.1	4.68	3.64	40	375	16.1
Gel Filtration	64.1	2.94	2.29	89	140	6.02

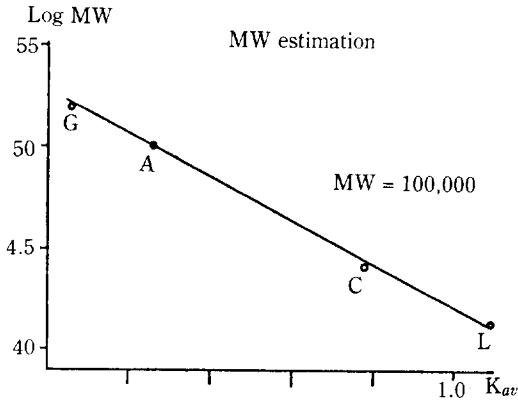

Fig. 9. *Molecular weight estimation.*
Bio-Gel P150(1.5 × 25 cm) was used and void volume was 14 m*l*. The enzyme activity was eluted at 29.3 and molecular weight was calculated as 100,000 daltons from the calibration curve.
G: gamma-glovulin, C: chymotrypsino gen A, L: lysozyme, A: -amylase.

coli, it is proved that certain eukaryotic gene without introns can be correctly transcribed and translated in prokaryotic systems. This was agreed with the result reported by Kushner group, who observed the correct expression of quinic acid metabolizing gene cluster of *N. crassa* in *E. coli* (Vapnek *et al.,* 1977; Schweizer *et al.,* 1981).

The vector pV32, which encodes for α-amylase gene lose one of its PstI recognition site. The exact reason for this is not found yet, but supposed to be as followes.

1. Due to the star-effect of restriction endonuclease when digesting chromosomal DNA, which results cohesive ends with the same base sequences but does not have the correct recognition sites. If this DNA fragments were rejoined with vector DNA, the resulting recombinant DNA would have one or two incorrect recognition sites in it and so appeared as to loss its recognition sites.

2. Some contaminant nucleae activity during the digestion or ligation reaction which might remove one or two bases from the cohesive ends of DNA fragments.

First the star effect would not seemed to be the answer, because there are no reports on the star-effects of PstI. Second, the nuclease contamination may give the answer. If one of the cohesive end of chromosomal DNA or vector DNA would be attacked by contaminant nuclease, the ligation reaction would yield gapped DNA, but when this gapped DNA is transduced in *E. coli* and the gap is sealed, the vector could be replicated, and so the expression of enzyme is possible.

But still now, there are not critical evidences for both suggestions, only restriction map of pV31 could be made and the inactivated PstI site is found. Considering the restriction map of pBR322, the mother DNA of pV32, the inactivated site can be found easily, and as the result, the inactivated site lies nearer to the EcoRI site of pBR322. The size of inserted DNA is about 5.9 Kb, and the size of α-amylase gene, when calculated from the size of α-amylase enzyme, is about 1.3 Kb, so there is further possibility to minimize the size of vector pV31.

As the result of comparisons for the characteristics of both enzymes isolated from *N. crassa* and *E. coli,* the two show identical behaviors in optimal temperature and optimal pH and molecular weights. Optimal temperature of this enzyme is shown to be 70 °C and this enzyme is very stable at room temperature. After 3-4 weeks at room temperature, there is no serious loss of activity of this enzyme. This enzyme has two pH optima, pH 6 and 10. But it is not certain that pH 10 is optimal pH, because at this pH, starch had been hydrolyzed without enzymes due to high OH^- concentrations. This result may come from improper assay method, which is described in 'Materials and Methods'.

The enzyme isolated from *E. coli* should be heavier than that isolated from *N. crassa* in molecular weight, because the original enzyme is extracellular and so should have signal peptides which would be spliced in *N. crassa* but not spliced in *E. coli* due to the different splicing system. But the result shows the two are about the same, about 100,000 daltons. This is because the portions of signal peptides in total proteins is small enough not to be detected by the technique adopted in this experiments.

One of the purpose of gene cloning, the over production and easy recovery of proteins is achieved through this study. The specific activity of

the crude extracts of *N. crassa* was 0.78 units/mg proteins, while that of *E. coli* was 15 units/mg proteins. This menas about 19 fold amplifications in specific activity. And if we compare the enzyme units per weights of used microorganisms, *N. crassa* origin enzyme is 2.94 units was obtained from 60 gr of mycelia, while for *E. coli* 140 units were obtained from about 3 gr of organisms. This means about 950 fold increase in activities per unit weight of microorganisms. And because *E. coli* grows rapidly and has cell wall which could be easily broken compared with that of *N. crassa* which requires 7 days to express the amylase activity fully, the isolation of enzyme would be more facilitated.

적 요

N. crassa (ATCC 9279)의 α-아밀라아제(EC. 3. 2. 1. 1)를 shotgun 방법에 의해 대장균안에서 클로닝하였으며 N. crassa와 대장균 양쪽으로부터 분리된 효소의 특성들을 비교하였다.

곰팡이 포자로부터 분리된 DNA를 Pst I 제한 효소를 이용하여 부분 절단시키고 역시 같은 효소에 의해 잘려진 pBR 322 DNA에 접합시켰다. 이리하여 얻어진 재조합염색체를 염화칼슘 방법을 사용해서 대장균의 형질로 전환시켰다. 결과로서 테트라사이클린에 내성을 보이는 8000여개의 콜로니가 얻어졌으며 이들 중 10.2 Kb의 재조합 염색체를 포함한 두 개의 대장균 클로니가 녹말을 분해하는 역가를 보여 주었다.

*N. crassa*와 대장균으로부터 α-아밀라아제를 암모니움 설페이트 침전법, DEAE-셀룰로오즈 이온교환 크로마토그래피법 및 Bio-Gel P 150 젤 크로마토그래피법을 이용하여 분리하였다. 결과로서 *N. crassa*와 대장균에 대하여 각각 81.3배와 5.6배의 정제가 얻어졌다. 양쪽으로부터 얻어진 효소들의 특성을 최적 활동온도, 최적활동 pH, 분자량의 면에서 비교하였더니, 분자량은 10만달톤이고, 최적활동 온도는 70℃, 최적활동pH는 6과 10으로, 이 두가지 효소는 완전히 같은 성질을 보여주었다.

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