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Studies on the β-Galactosidase Activity of Whole Cell *Aspergillus phoenicis*

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Aspergillus phoenicis Whole Cell의 β-Galactosidase 活性에 관한 硏究

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Abstract: β-Galactosidase activity of *Aspergillus phoenicis* was studied using ONPG and lactose as substrate. It increased monotonically during the exponential growth phase and dropped rapidly at the beginning of the stationary one. It exhibited high tolerable temperature and acidic optimal pH which provides certain advantages from the industrial view point. Enzyme of β-galactosidase had more subsrate affinity for ONPG than for lactose and its apparent maximum activity was also higher with the former as substrate. Activity of this enzyme depended upon the conditions of immobilization. Optimum crosslinking reaction was occurred at pH 7.2 and 0.35 vol. % of glutaraldehyde concentration.

Keywords: *Aspergillus phoenicis*, β-Galactosidase, Immobilization of whole cells, Lactose hydrolysis.

Enzyme of β-galactosidase hydrolyze lactose into glucose and galactose. This transformation is principally utilized not only to prepare the aliments for lactose intolerant populations, but also to treat the milk industrial wastes, the disposal of which provokes severe pollution problems.

β-Galactosidase can be isolated from plant, animal and microbial sources. The microbes (bacteria, fungi and yeast) however, offer high yields for commercial production.

β-Galactosidase preparations from different sources are not identical with respect to their size, structure and the optimum conditions for lactose hydrolysis.

The trend toward large-scale production of soluble β-galactosidase has already set in, as evidenced by the growing number of patented production processes. However, soluble enzyme is generally seldom stable and expensive. Moreover it is very difficult to recover the soluble enzyme after the reaction. Immobilization of enzyme is one of the most efficient solutions to these problems.

The number of active site in β-galactosidase per molecule (M.W.=700,000) is temperature dependent (at 4~6°C, 1.0; at 20~22°C, 4.7), suggesting that at a low temperature the molecular conformation is such that not all active sites are available (Shukla 1975). The high reaction rate at higher temperature may be attributable, at least in part, to this type of effect. However, the reaction temperature should not be so high that the denaturation of enzyme proceeds.

β-Galactosidase from different sources have been inmobitized by a variety of methods and, naturally, with varying degree of success. Major procedures

used are as follows.

Physical methods

① adsorption

② inclusion within polymer gel

③ microencapsulation

Chemical methods

① immobilization by adsorption, followed by reticulation.

② immobilization by covalently fixation directly on a support.

③ immobilization by cross linking between inactive protein and enzyme.

Most of the methods involving simple adsorption and entrapment suffer from leaking, desorption and problems of diffusional resistance. The covalent binding on inorganic surface such as porous glass causes enzyme denaturation and an accompanying loss of activity.

Immobilization by reticulation, which is developped recently by Broun *et al.* (1975) is one of the most efficient method to fix biocatalysts.

Cross linking occurs between inactive protein and enzyme, protecting its three dimensional structure. Larretagarde (1980) used this method of reticulation to immobilize membranes of photosynthetic bacteria.

Whole cell immobilization present certain advantages.

This permits—elimination of extraction and purification steps of enzymes
—stabilization of enzyme within its natural environment.
—possibility of regeneration of cofactors if necessary.

Reaction kinetics of immobilized β-galactosidase is reported to be well represented by Michaelis-Menten equation, the kinetic constants of which being replaced by apparent ones owing to the diffusional resistances.

In this study, we investigated β-galactosidase activity of *Aspergillus phoenicis* and effects of immobilization upon it.

Materials and Methods

Culture of Strain

A spore suspension of *Aspergillus phoenicis* was prepared by adding 10 ml of sterilized water to the slant culture, and by rubbing gently the surface with a sterilized wire loop.

One ml of the spore suspension was inoculated into 500ml flask each containing 100ml of medium with following composition: lactose 40g, peptone 10g, yeast extract 5g and q.s.p. 1*l* of water.

The incubation was carried out at 28~30°C for 46 hours in a rotary shaker at a speed of 180rpm.

After incubation, the mixture was filtered leaving filterate, which was collected for further enzymatic assays.

Measurement of β-Galactosidase Activity

The measurement of β-galactosidase activity was effected by the method of Lederberg (1950) and Sánchez *et al.* (1979) using ONPG (O-nitrophenyl-β-D-galactopyranoside) as substrate.

This enzyme transforms ONPG into ONP and galactose. The yellow color of the ONP produced permits to measure easily the degree of completion of the reaction.

For assays of lactose hydrolyzing activity, the amounts of glucose liberated during hydrolysis were measured by a glucose oxidase-peroxidase chromogen procedure.

Cell Immobilization

Aspergillus phoenicis was immobilized in cross-linked albumin foam structure by mixing, 3ml of 0.02M pH7.2 phophate buffer, 1.875ml of a 20% bovine serum albumin solution, 1.5ml glutaraldehyde solution and 2g of cells (wet weight). The mixture was frozen at −20°C for several hours and then slowly thawed at 4°C and rinsed.

Results and Discussion

The logarithmic growth curve of *Aspergillus phoenicis* is shown in Fig. 1. Fig. 2 represents β-galactosidase activity of this strain at different steps of growth.

The growth rate of *A. phoenicis* seems to be first order with respect to the cell mass, and its growth rate constant is about $0.08h^{-1}$ under the culture

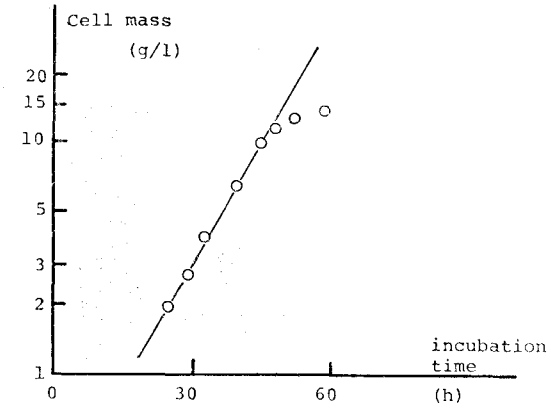

Fig. 1. Variation of cell mass of *Aspergillus phoenicis* with incubation time.

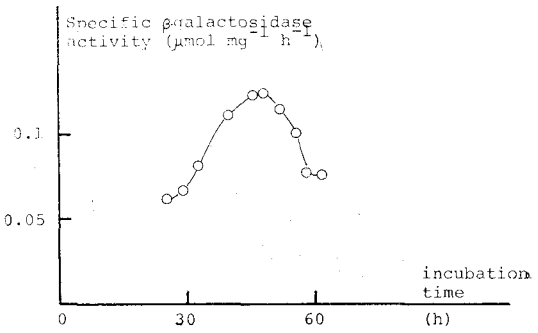

Fig. 2. Specific β-galactosidase activity of whole cell *Aspergillus phoenicis* as function of incubation time.

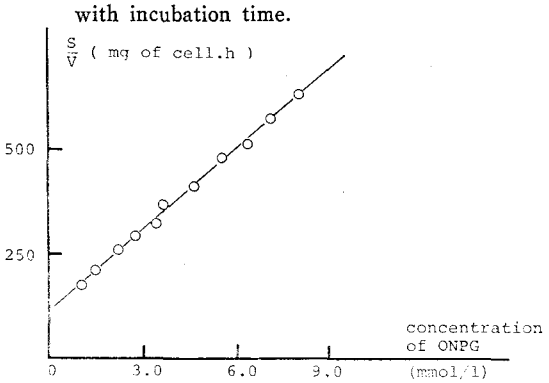

Fig. 3. Determination of kinetic parameters of ONPG hydrolyzing reaction by whole cell *Aspergillus phoenicis*. Hanes' linear transformation

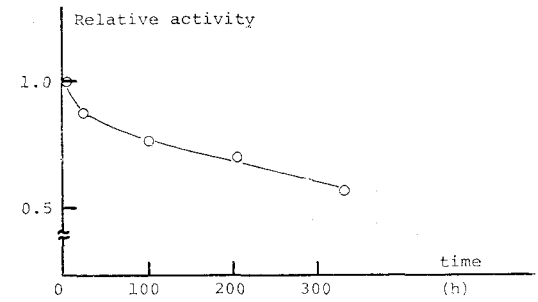

Fig. 4. Time course for relative activity of β-Galactosidase of whole cell *Aspergillus phoenicis* incubated at 45°C.

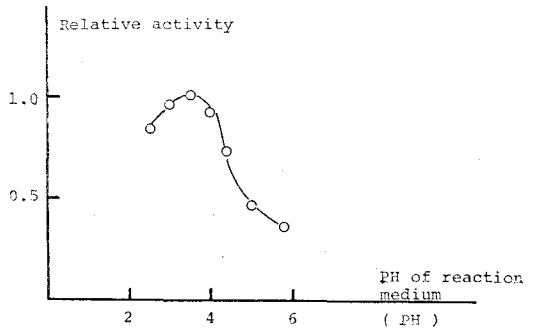

Fig. 5. pH dependence of relative activity of β-galactosidase of whole cell *Aspergillus phoenicis.*

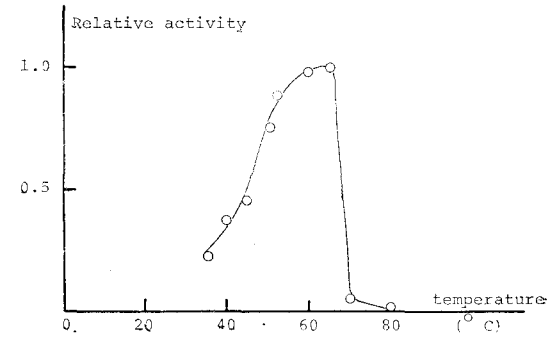

Fig. 6. Temperature dependence of β-galactosidase activity of whole cell *Aspergillus phoenicis.*

conditions previously cited. β-Galactosidase activity augments during the exponential growth phase and drops rapidly at the beginning of the stationary one.

Park *et al.* (1979) observed that β-galactosidase activity increased continuously even in the stationary phase. However, they used purified β-galactosidase of *A. oryzae* and the liberation of β-galactosidase of the autolyzed cells can be more rapid than that of living cells.

Fig. 3. showing the linear transformation of the kinetic data into Hanes plot, permits to calculate the following enzymatic apparent kinetic parameters. Kmapp.=1.86mM, Vmapp.=$1.62\times10^{-2}\mu$mol·ml^{-1}·h^{-1}·mg^{-1} of cell.

Fig.4 shows the time dependence of the β-galactosidase activity at 45°C relative to that of zero time. Variation of activity measured at 30°C as function of pH of the reaction medium is represented in Fig. 5.

At optimal pH, the author measured the variation of β-galactosidase activity as function of reaction temperature and the results are reported in Fig. 6.

It can be found that the maximum tolerable temperature for β-galactosidase of *A. phoenicis* situates around 65°C.

The stability at acid pH and thermostability of the enzyme have very important advantages from the industrial view point. Not only can the contamination of reactor by other microorganisms be limited, but also can the activation of another enzymes in the cell and parasitic transformation of sugars produced be avoided.

The lactose hydrolysis by *A. phoenicis* was also studied. Apparent maximum activity and apparent Michaelis-Menten constant of the enzyme, determined from the Hanes plot, were $9.06\times10^{-3}\mu$ mol·ml^{-1}·h^{-1}·mg^{-1} of cell and 13.3mM respectively (Fig. 7.). Reaction was carried out at 65°C with pH 3.0 citric acid-phosp hate buffer.

With these results, it can be said that enzymatic affinity for substrate as well as optimal pH changes with the subsrate used. The β-galactosidase of *A. phoenicis* has more affinity for ONPG than for lactose, the latter giving higher value of apparent Michaelis-

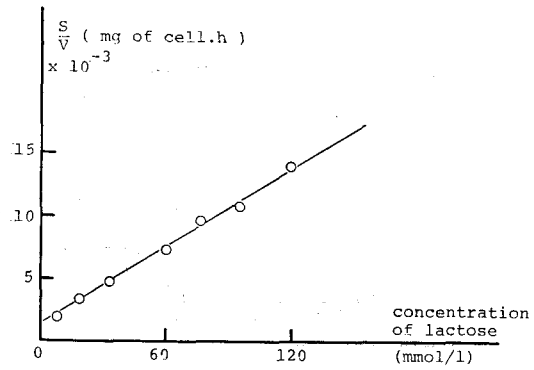

Fig. 7. Determination of kinetic parameters of lactose hydrolyzing reaction by whole cell *Aspergillus phoenicis*. Hanes' linear transformation

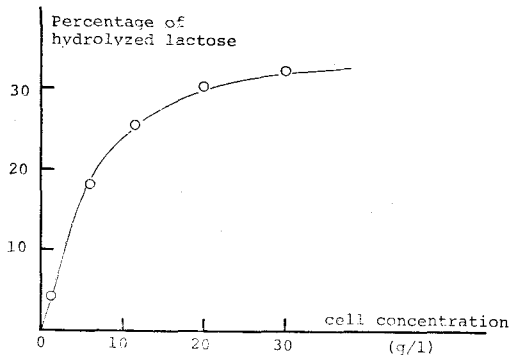

Fig. 8. Cell quantity dependence of the percentage of lactose hydrolyzed by *Aspergillus phoenicis* whole cell incubated at 65°C for 5 hours.

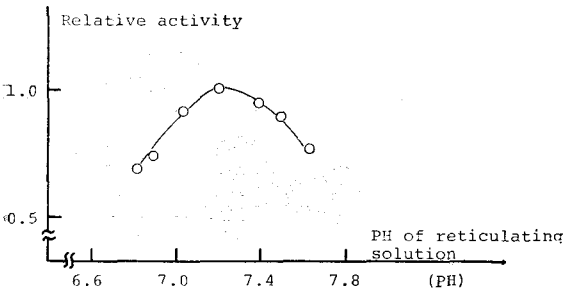

Fig. 9. Dependence of the β-galactosidase activity of whole cell *Aspergillus phoenicis* on the pH of reticulating solution.

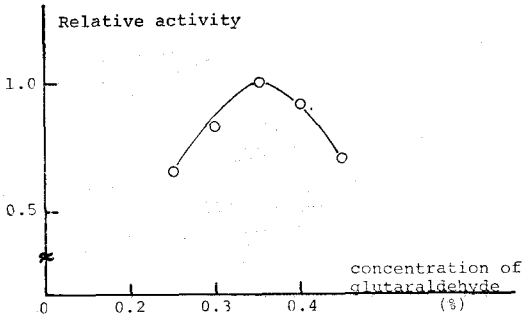

Fig. 10. Dependence of the β-galactosidase activity of whole cell *Aspergillus phoenicis* on the concentration of glutaraldehyde of reticulating solution.

Menten constant than the former.

Variation of the percentage of hydrolized lactose after 5 hours of incubation with cell quantity is shown in Fig. 8.

A. phoenicis showed same order of magnitude of lactose hydrolyzing activity as another strains studied by other authors.

A. phoenicis hydrolyzed 1.7% of 4% lactose solution, while Ohmiya *et al.* (1977) obtained 1.6% with *Neurospora crassa* with comparable operation conditions.

Wierzbicki and Kosikowski (1972) obtained 7.5% of hydrolysis of 2% lactose solution with *Aspergillus niger*, while *A. phoenicis* achieved 9% of hydrolysis with 1.33% lactose solution.

Among many methods of immobilization of enzyme, reticulation is known to be one of the most efficient ones. However, enzyme activity depends upon the methods and conditions of reticulation.

The variation of β-galactosidase activity with pH of the solution during the reticulation is represented in Fig. 9. The test of activity was carried out at 45°C with pH 7.0 phosphate buffer. The pH of the reticulation solution was adjusted by varing the pH of the buffer containing glutaraldehyde, the pH of bovine serum albumin solution being fixed at 6.8. Fig. 9 shows that the optimal pH of reticulation is around pH 7.2. Besides, the mechanical properties of the polymer were satisfying between pH 6.8 and 7.6 for immobilization.

β-Galactosidase activity as function of glutaraldehyde concentration is determined leaving the other factors constant. The results are reported in Fig. 10. The reaction was carried out at 45°C with pH 7.0 phosphate buffer. A. phoenicis immobilized at pH 7.2 and at 0.35 vol. % of glutaraldehyde gave maximum β-galactosidase activity, while mechanical properties of the reticulated polymer were acceptable within 0.23~0.47% concentration range of glutaraldehyde.

Cocquempot *et al.* (1980) reported that the catalytic stability of enzyme increased with glutaraldehyde concentration, but that the enzymatic activity decreased considerably.

摘 要

*Aspergillus phoenicis*의 β-galactosidase 활성을 ONPG와 lactose를 기질로서 사용하여 조사하였다. 이 효소 활성은 성장의 대수기 동안은 서서히 증가하였으나 정지기가 시작되면서 급격하게 감소하였다. 또한 이 효소는 높은 온도에서도 좋은 효소 활성을 유지하였으며, 산성 pH에서 최고의 효소활성을 나타내었다. β-galactosidase는 lactose보다 ONPG에 대한 기질의 친화력이 더 좋았으며. 효소 활성도 ONPG의 경우가 더 높았다.

Lactose의 가수분해율은 반응액중의 lactose의 농도가 낮을 수록 높았으며, 사용균의 무게에 따라 초기에는 증가하다가 어느 수준 이상에서 부터는 점근값을 나타내었다.

효소의 활성은 효소의 고정 방법 및 조건에 영향을 받았으며, matrix의 가교가 pH 7.2 및 0.35 vol. %의 glutaraldehyde 농도에서 행하여졌을 때, 가장 높은 효소 활성을 보였다.

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