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Aspergillus awamori B-2의 균사체 고정화 의한 Melanoidin 탈색

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Melanoidin decolorization by immobilized cells of *Aspergillus awamori*, B-2

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Abstract

Aspergillus awamori, **B-2 which has a high ability to decolorize melanoidin was selected among various fungi.**

Aspergillus awamori, **B-2 showed the highest decolorization activity when it was cultivated in a melanoidin medium containing 3.0 % glucose, 0.5 % yeast extract, 0.1 % $KH_2PO_4$and 0.05 % $MgSO_4 \cdot 7H_2O$ at an initial pH 7.0 at 37℃ for 5 days.**

Mycelia immobilized system with Ca-alginate was more effective on melanoidin decolorization activity showed approximately 70 % in 10 days under the optimal conditions.

Continuous decolorzation of melanoidin using reuse of immobilized mycelia showed an almost constant decolorization of abort 60-70 % for 15 days.

INTRODUCTION

Melanoidin is formed by carbonyl-amino acid reaction through the so called Maillard reaction. Melanoidin occured not only in various food, but also in wastes of alcohol fermentation and refinery sugar by molasses. Melanoidin are involved the decrease in food qualities such as color, taste and loss of nutrients. Therefore, removal or inhibition of the formation of the colored substances, is one of the most important problem in the food industry at present. The microbial decolorization of melanoidin using mycelia of *Basidomycetes*[1]and *Aspergillus*[2]were examined as one of methods for the biological decolorization. Ohmomo et al.[3]were found melanoidin decolorizing enzymes in some strains of *Basidomycetes* and purified the two main enzymes showing melanoidin decolorizing activity. *Aspergillus fumigatus* G-2-6 were attempted the continuous decolorization of the melanoidin solution by using mycelia of thermophilic strain[4]. Furthermore, Ohmomo et al[5]attempted continuously to decolorize molasses waste by using immobilized mycelia of *Basidomycetes*. To develop a biological treatment for the decolorization of brown color, especially the microbial decolorization of melanoidin, we performed screening to find fungus having the ability to decolorize melanoidin. In this paper, we were confirmed the optimal conditions of fungus having melanoidin

decolorization and then were carried out continuous decolorization of melanoidin by using immobilization of *Aspergillus awamori*, B-2

MATERIALS AND METHODS

Microorganism and cultivation

Eleven strains of fungi studied were from stock cultures of our laboratory. Stock cultures of fungi used in these experiment were isolated from makuli factory of Sansung in pusan area(Table 1). The fungi were maintained by subculturing on potato dextrose agar.

Table 1. Melanoidin decolorixing activity of the various strains.

Fungus	Decolorization yield(%)
Aspergillus oryzae R-2	40
Aspergillus oryzae R-4	20
Aspergillus niger H-10	0
Aspergillus niger H-15	0
Aspergillus awamori B-2	65
Aspergillus awamoirl B-5	35
Aspergillus inui A-13	52
Aspergllus sp. A-30	45
Penicillium sp. P-3	50
Fusarium sp. F-20	0
Mucor hiemalis M-5	23

The reaction mixture consist of melanoidin mixture consist of melanoidin medium which diluted with 0.1M-acetate buffer, pH 5.0 and its color intensty at 475nm was adjusted to 3.5 by addition of melanoidin solution. The decolorization reaction was incubated at 30℃ for 5 days.

Media

Decolorization medium [6](basal medium) consisted of 3.0 % glucose, 0.2 %$NaNo_3$, 0.1 % KH_2PO_4 and 0.05 % $MgSO_4 \cdot 7H_2O$ at an inital pH of 6.0 and was adjusted to an optical density of 3.5 at 475 nm by addition of melanoidin solution.

Preparation of mefanoidim

90 g glucose, 37.5 g glycine and 26.5 g sodium carbonate were dissolved in 500ml of water and reflux in oil bath at 180℃ for 1h.

Immobilization of mycelia

Immobilization of *Aspergillus awamori*, B-2 mycelia was carried out by a slightly modified method of Kiersten and Bucke[7]. For immobilization, the mycelia were mixed with 3.0 % sodium alginate and added as dropwise of 0.1M $CaCl_2$ by using 18-20 gauge injector. The diameter of the resulted from sodium alginate gel bead was 2-3mm and the beads were stored at 4℃ for stability before their use.

Determination of melanoidin decolorizing activity

Melanoidin decolorization activity was determined by measurement of the decrease in color intensity as the absorbance at 475 nm. The reaction mixture consist of melanoidin medium which diluted with 3.5ml of 0.1M-acetate buffer, pH 5.0 and its color density at 475 nm was adjusted to 3.5 by the addition of a amount of synthetic melanoidin solution.

The decolorization reaction was allowed to proceed to proceed in an L-type tube at various temperatures. The decolorized yield was expressed as the degree of the decrease at 475nm toward the initial absorbance at same wave length[8].

Determination of dry weight of mycelia

The dry weight of mycelia was determined after drying at 105℃ for 24 hr.

RESULTS AND DISCUSSION

Screening of fungi for melanoidin decolorizing activity

Melanoidin decolorizing (MD) activity were determined in various fungi to select a strain which shows the highest activity as shown Table

1. These strains were detected in the method. *Aspergillus awamori*, B-2 showed the highest MD activity among those of the strains studied. *Aspergillus awamori*, B-2 removed about 68.0% of the melanoidin after 5 days of cultivation. Thus, *Aspergillus awamori*, B-2 was chosen as the reference mold having the MD activity for further investigation.

Cultural conditions of *Aspergillus awamori*, B-2 for melanoidin decolorizing activity.

Temperature: *Aspergillus awamori*, B-2 were grown actively at 37℃, moderately at 30℃ and pooly at 50℃. The maximum MD activity of this strain was found in the cultivation at 37℃ for 5 days.(Fig.1)

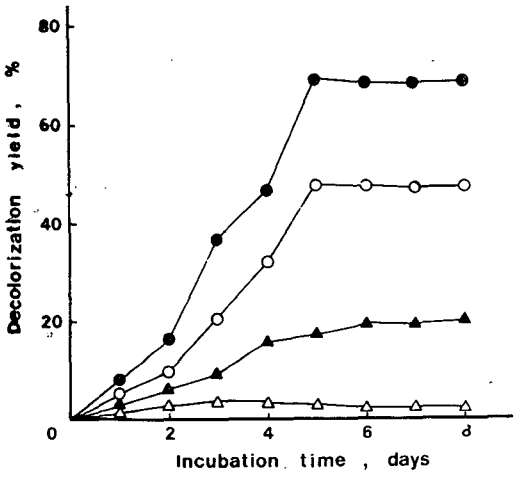

Fig. 1. Effect of incubation temperature on the decolorization of melanoidin by *Asperillus awamori*, B-2. ○: 30℃, ●: 37℃, ▲: 45℃, △: 50℃

Effect of the concentration of nitrogen and sugar substrates: The optimum nitrogen source for MD activity was examined in the various nitrogen sources. Organic nitrogen sources were more effective supporting both of MD activity and cell growth than inorganic nitrogen sources.(Table2)

Table 2. Effects of nitrogen sources on the decolorization of melanoidin by *Aspergillus awamori*, B-2.

Nitrogen sources	Decolorization yield(%)	D.W.M (g/100ml)
Corn steep liquor	66.5	1.90
Peptone	67.0	2.40
Meat extract	55.6	1.80
Yeast extract	68.9	3.06
$(NH_4)_2SO_4$	69.1	2.58
$NaNO_3$	68.0	2.44
NH_4NO_3	62.4	1.92
Urea	48.3	0.83
None	38.5	0.40

D.W.M. : Dry weight of mycelia

The fungus was cultivation at 37℃ for 5 days with the basal medium described in the Materials & Methods expect for the addition of the nitrogen sources. The concentration of organic nitroen sources were 0.5% and 0.2% inorganic nitrogen sources.

Although inorganic nitrogen sources were not shown observed desirable high level of MD activity. Ammonium sulfate supported the MD activity in a high level. On the other hand, MD activity of *Asperillus awamori*, B-2 were tested on media containing various sugars as the carbon source. All of the sugars tested were effective on decolorization and growth of mycelia than the control which contained no sugar. (Table3)

Among the carbon sources, glucose, galatose and fructose were shown to support high yield of MD activity. Ohmomo et al.[2] were suggested that glcose and peptone was the most optimum carbon and nitrogen sources for the decolorization of melanoidin, respectively.

Aoshima et al.[8]however, indicated that the addition of yeast extract and glucose were most efficient for the decolorization. Thus our results are in good agreement with the above results.[2,8]

Table 3. Effect of carbon sources on the decolorixation of melanoidin by *Aspergillus awamori*, B-2.

Carbon sources	Decolorization yield(%)	D.W.M. (g / 100ml)
Glucose	68.4	2.80
Galactose	67.3	2.51
Fructose	67.1	2.82
Maltos	64.0	2.71
Sucrose	64.1	2.70
Xylose	50.2	0.90
Arabinose	49.2	0.88
Ribose	55.4	0.95
None	40.3	0.47

D.W.M. : Dry weight of mycelia

The fungus was cultivated at 37℃ for 5 days with basal medium described in the Materials & Methods expect for the addition of carbon sources. The concentration of carbon sources added was 3.0%.

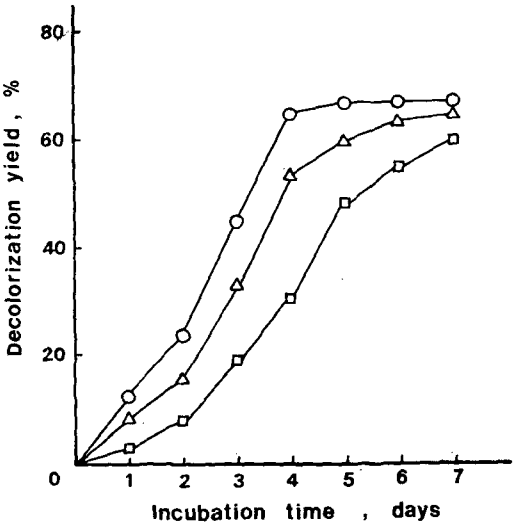

Fig. 2. Effect of inoculum size at 37℃ on the decolorization of melanoidin by *Asperillus awamori*, B-2. ○: 10^8, △: 10^6, □: 10^4

Inoculum size for melanoidin decolorizing activity : Inoculum size is one of important factors influencing the MD activity and decoloriaztion yield. The reationship between the inocuulum size and melanoidin decolorization is shown in fig.2. *Aspergillus awamori*, B-2 decolorized 65% - 67% of the melanoidin when mycelia was inoculated as size of 10^8 / 100ml at 37℃ for 5 days. Increase in the inoculum size caused an increase in decolorization yield.

Time course of the typical decolorization : The decolorization of melanoidin depended on incubation period and growth of *Aspergillus awamori*, B-2.(Fig.3)

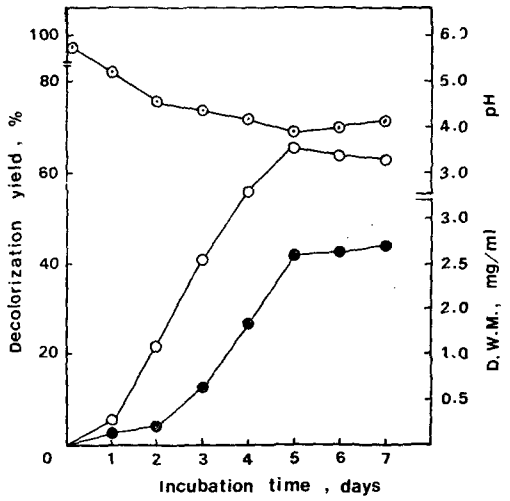

Fig. 3. Time course of melanoidin decolorization by *Asperillus awamori*, B-2. The fungus was cultivated under the optimum conditions as described in the text.
⊙: pH, ○: decolorization yield, ●: dry weight of mycelia(D.W.M)

The decolorization by this strain was almost in coincide with the growith of mycelia. Maximal decolorization yield (70%) was reached after 5

days of incubation and dry weight of mycelia was similar with same pattern during 5 days of incubation. After 5 day of cultivation, the decolorization was not increased, but mycelia were grown slightly for 2 days more. Decolorization was gradually reduced after 5 days in the same pattern of *Mycelia Sterilia* D 90[6], or *Coriolus versicolor* Ps 4a[8].

Improvement of the decolorization by immobilized system

Asperillus awamori, B-2 decolorized 65%-67% of the melanoidin when mycelia was inoculated as size of 10^8 / 100ml at 37℃ for 5 days. Increase in the incoulum size caused an increase in decolorization yield.

Immobilized mycelia system was compared with free mycelia in the system. Immobilized mycelia system was more effective on decoloration then that of free mycelia.(Fig 4)

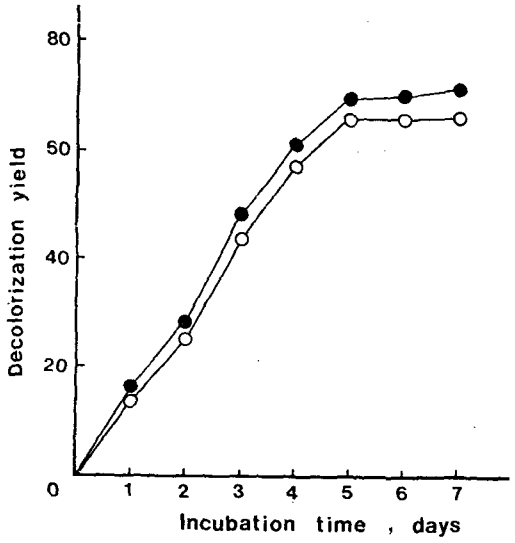

Fig. 4. Melanoidin decolorization of *Aspergillus awamori*, B-2. ●: immobilized mycelia, ○: free mycelia

Aspergillus awamori, B-2 decolorized about 70 % of melanoidin solution, when it was cultivated at 37℃ for 10 days reusing immobilized mycelia. (Fig. 5)

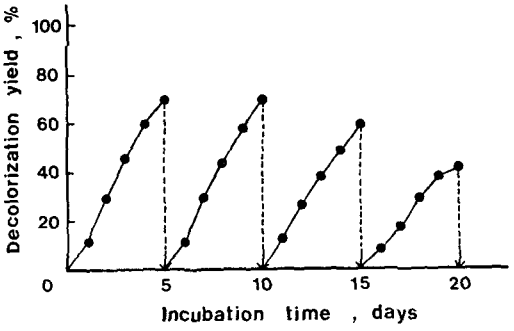

Fig. 5. Succesive decolorization by reusing immobilized mycelia(●).
"↓" shows the replacement of the reaction medium.

After cltivtion of 15 days, melanoidin decolorization yield showed about 60% under the same conditions. Decolorization level continued for 20 days were still maintained to constant and sufficient decolorization activity. This strain showed MD activity during four times replacement off substrate under optimal condition. There is no significant decoloriation activity decreased slightly on the 20 days.

Decrases of decolorization caused by lead of bead gel[9]. The MD activity in *Basidomycetes* was already reported by Watanabe[1]and Aoshima et al.[8]Nevertheless anybody hardly reported a microbial decolorization of melanoidin by fungi exceppt *Basidomycetes*. We have isolated a fungi having a high MD activity. To decolorize the melanoidin effectively, a continuous decolorization using immobilized mycelia was performed successively. The MD activity resulted from *Aspergillus awamori*, B-2 was superior to that by *Basidomycetes*[1], and the present experiments suggested the possibility of the application of fungi to the biological treatment of browning color in food. In addition, the reuse of immobilized mycel-

ia is more effective and sucessive technique of decolorization for a long time. This studies are now under way to thesemechanism in more detail.

요 약

곰팡중에서 melanoidin의 탈색력이 있는 *Aspergillus awamori*, B-2을 선별하여 탈색력을 실험하였다. *Aspergillus awamori*, B-2는 3.0 % glucose, 0.5 % yeast extract, 0.1 % KH_2PO_4 및 0.05 % $MgSO_4 \cdot 7H_2O$을 함유하는 melanoidin 용액(pH 7.0) 에서 5일 동안 37℃에서 배양했을때 가장 높은 탈색력을 나타내었다. 탈색력을 효과적으로 하기 위하여 Ca-alginate로 고정화하여 최적조건에서 계속적으로 탈색하였을때 mycelia 단독보다는 더욱 효과적이었다. Melanoidin 탈색력은 최적 조건에서 10일 동안 반응하였을때 약 70 %을 나타내었다. 고정화한 mycelia의 재사용에 의한 계속적인 탈색을 시도하였던바 15일 동안 연속적으로 반응시켜도 약 60-70 %의 탈색력을 계속 유지하였다.

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