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Production of Auxin and Abscisic Acid by *Phanerochaete chrysosporium* ME446 Immobilized on Polyurethane Foam

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Abstract: The fungus *Phanerochaete chrysosporium* ME446 was entrapped on polyurethane foam to synthesize auxin and abscisic acid (ABA). The maximum amounts of auxin produced on the 18th day in both free and immobilized cells were 55.91μg/mL and 76.07μg/mL, respectively. Maximum ABA concentrations on the 12th day in both free and immobilized cells were 6.87 μg/mL and 10.69 μg/mL, respectively. An immobilized system of auxin and ABA production resulted in a comparatively higher yield than free fungal mycelium.

Key Words: *Phanerochaete chrysosporium* ME446, immobilization, polyurethane foam, auxin, abscisic acid (ABA).

Poliüretan Köpük Üzerine Tutuklanmış *Phanerochaete chrysosporium* ME446'dan Oksin ve Absisik Asit Üretimi

Özet: *Phanerochaete chrysosporium* ME446 oksin (IAA) ve absisik asit (ABA) üretmek amacıyla poliüretan köpük üzerine tutuklandı. Maksimum IAA hem serbest hem de tutuklanmış hücrelerde 18. günde belirlendi (sırasıyla 55.91 μg/mL ve 76.07 μg/mL). Maksimum ABA hem serbest hem de tutuklanmış hücrelerde 12. günde belirlendi (sırasıyla 6.87 μg/mL ve 10.69 μg/mL). IAA ve ABA üretiminde tutuklanmış sistem serbest fungal miselyumdan daha yüksek verim sağladı.

Anahtar Sözcükler: *Phanerochaete chrysosporium* ME446, tutuklama, poliüretan köpük, oksin, absisik asit(ABA).

Introduction

Auxin (IAA) and abscisic acid (ABA) are known as plant growth regulators which have hormonal functions (1). They are mainly produced by plants (2,3), but they are also produced by fungi as primary or secondary metabolites (4).

Microorganisms are widely used in industries including food, chemistry, medicine and fermentation. These industries primarily used free organisms and later immobilized microorganisms have been used because they can be produced in various surfaces.

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Immobilized cells have received considerable interest in recent years and several novel features of such biocatalysts have been proposed (5). One of the reasons for these studies was to explore the possibility of extending the longevity of the secondary metabolite production phase, which is an important parameter in the industrial uses of such organisms (6).

Kumar and Lonsane (7) have reported that immobilized growing cells are known to offer advantages such as a) superior stability due to protection of cells by physico-chemical interactions between gels and cells, b) protection of growing cells against unfavourable environmental factors, c) changed permeability of cells towards high penetrations of substrate, d) faster removal of end products from fermentation sites, and e) renewable or self-generating or self proliferating nature of biocatalytic system.

Many industries use conventional methods to produce economical substances (enzymes, antibiotics, plant growth hormones, steroids and organic acids). In recent years, microorganisms have been immobilized in both laboratory and industrial processes. The production of patulin and penicilin, gibberellic acid, and bikaverin by immobilized fungi have been extensively examined (6, 8, 9). However production of IAA and ABA has not been examined yet.

In this paper, we describe production of IAA and ABA by *P. chrysosporium* ME446 immobilized on polyurethane foam.

Materials and Methods

Organisms

The microorganism used in this study was *P. chrysosporium* ME446. The culture was maintained on sabouroud dextrose agar (10). To study the control groups, the suspension of *P. chrysosporium* ME446 was aseptically added to 100 mL stock basal mineral (SBM). The culture medium was buffered to pH 5.0 in KH2P04 buffer.

Determination of dry weight mycelium

The dry weight of the mycelium was determined. The growth medium was filtered on weighed Whatman No.1 filter paper. The vegetative growth and the filter paper were dried at 70°C for 24 h to get a constant weight. The growth of the immobilized cells was measured after filtration on Whatman No.1 filter paper and dried for 24 h, at 70°C. An average predetermined foam cube dry weight was subtracted from the weight of foam plus mycelium.

Immobilization

A modification of the method of Capdevilla et al. was applied to entrap or immobilize *P. chrysosporium* ME446 (11). In this method, polyurethane foam (30 PPT) was used as the support medium. Three polyurethane foam cubes, each 1 cm x 1 cm x 0.5 cm, were placed in 250 mL Erlenmayer flasks and autoclaved at 120°C for 15 min. Incubation was carried out at 30°C without shaking, in Erlenmayer flasks containing 100 mL of medium. Experiments were repeated at least three times. After inoculations with mycelia, the cultures were flushed with 100 % oxygen for 2 min. Mycelia attached easily to the polyurethane foam and growth took place throughout the support.

Identification of auxin and ABA

The extraction, purification and identification of IAA and ABA were carried out according to the method by Ünyayar et.al. (5).

Results and Discussions

Our study showed that the yield of auxin, ABA and growth are significant in free and in immobilized *Phanerochaete chrysosporium* ME446 cells ($P<0.05$ t-test). A comparison of the growth and auxin production by free and immobilized cells of *P. chrysosporium* ME446 is shown in Figure 1. Maximum amounts of auxin produced on the 18th day in both free and

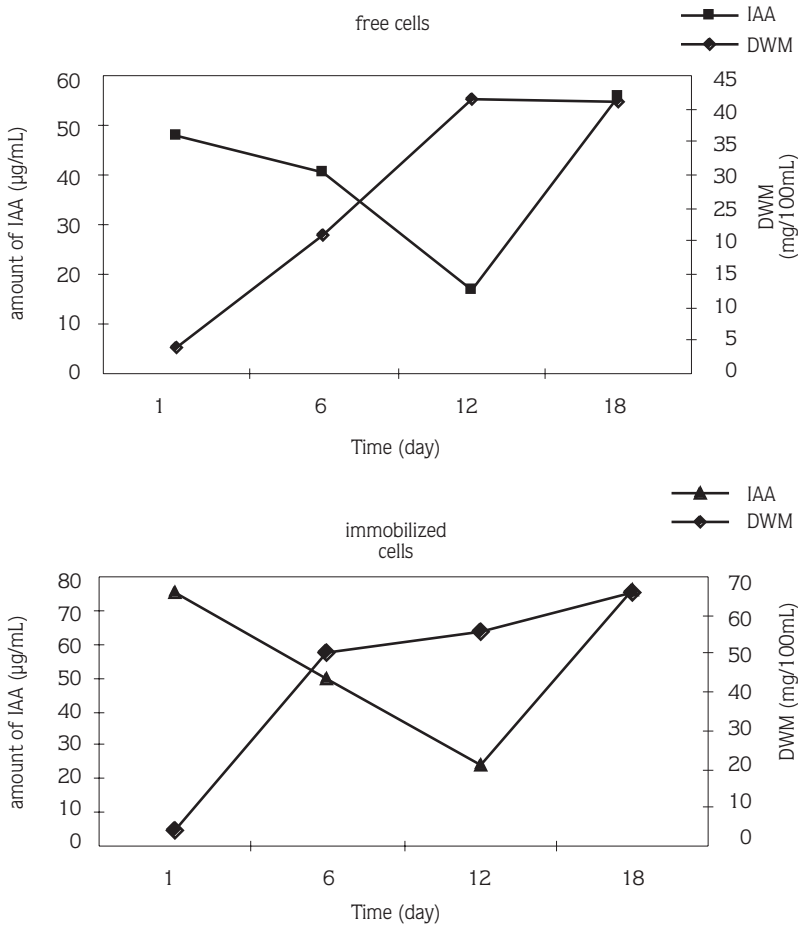

Figure 1. The growth and IAA production in free and immobilized *P. chrysosporium* ME446 cells.

immobilized cells were 55.91 μg/mL and 76.07 μg/mL, respectively. These values are comparable with the amount of auxin on the first day (47.99 μg/mL and 75.07 μg/mL, respectively).

A comparison of the growth and ABA production by free and immobilized cells of *P. chrysosporium* ME446 is shown in Figure 2. Maximum amounts of ABA produced on the 12th day in both free and immobilized cells were 6.87 μg/mL and 10.69 μg/mL, respectively. The levels of ABA in free and immobilized cells are significant ($P<0.05$ t-test). When maximum ABA

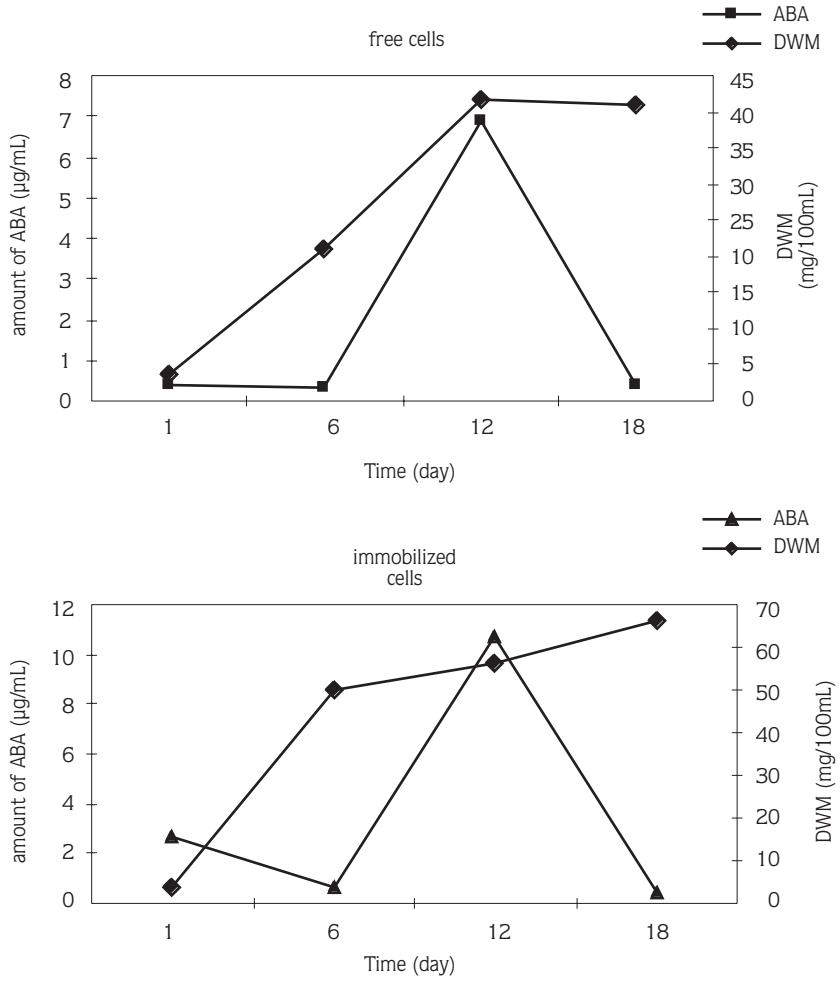

Figure 2. The growth and ABA production in free and immobilized *P. chrysosporium* ME446 cells.

was synthesized on the 12th day, auxin fell to a minimum. We can say that fungal growth was inhibited and fungus entered the secondary metabolic phase on the 12th day due to the fact that the ABA showed an inhibitory effect on IAA production.

Dry weight of mycelium (DWM) constantly increased up to the 18th day from the first day in both free and immobilized cells. But DWM was the same on the 12th and 18th days. DWM was in higher levels in immobilized cells than in free cells ($P<0.05$ t-test).

Immobilization has only been investigated to produce various industrial products and GA_3. But it has not been examined to produce IAA and ABA. Researchers, using polyurethane foam as an immobilization agent, reported that GA_3 and other secondary metabolites are in higher levels in immobilized cells than in free cells (11-13). They observed that the effect of polyurethane foam on GA_3 and other secondary metabolites are due to an increase in the mycelial surface exposed to the oxygen-rich atmosphere. These data correspond with our findings. However, Kumar and Lonsane (7) reported that GA_3 was produced in higher amounts in free cells than in immobilized cells. Kahlon and Malhotra (12) found that the levels of GA_3 increased due to increasing culture periods. They reported that the highest GA_3 level is 565 µg/mL, obtained on the 12th day. In our data, the amount of IAA decreased on the 12th day and remarkably increased on the 18th day. In contrast, the amount of ABA remarkably increased on the 12th day and decreased on the 18th day.

Our study shows that the advantage of using foam immobilized *P. chrysosporium* ME446 is to increase the production plant hormones and DWM. These results are in accordance with the literature (6, 12).

References

1. Iten, M., Hoffmann, T. and Grill, E., Receptors and signalling components of plant hormones, J. of Receptor & Signal Transduction Research, 1999, 19 (1-4), 41-58.
2. Wang, Y.Y., Zhou, R. and Zhou, X., Endogenous levels of ABA and cytokinins and their relation to stomatal behavior in dayflower (*Commellina communis* L.), J. Plant Physiol., 1994, 144, 45-48.
3. Blakesley, D., Weston, G.D. and Elliott, M.C., Endogenous levels of indole-3- acetic acid and abscisic acid during the rooting of *Cotinus coggygria* cuttings taken at different times of the year, Plant Growth Regulation, 1991, 10, 1-12.
4. Ünyayar, S., Topcuoğlu, Ş.F. and Ünyayar, A., A modified method for extraction and identification of indole-3-acetic acid (IAA), gibberellic acid (GA3), abscisic acid (ABA) and zeatin produced by *Phanerochaete chrysosporium ME446*, Plant Physiology and Biochemistry, 1996, Special issue, 307.
5. Moeira, M.T., Feijoo, G., Palma, C. and Lema, J.M., Continuous production of manganese peroxidase by *Phanerochaete chrysosporium* immobilized on polyurethane foam in a pulsed packed-bed bioreactor, Biotechnology and Bioengineering, 1997, 5,2, 130-137.
6. Jones, A. and Pharis, R.P., Production of gibberellins and bikaverin by cells of *Gibberella fujikuroi* immobilized in carrageenan, J. Ferment. Technol., 1987, 65, 6, 717-722.

7. Kumar, P.K.R. and Lonsane, B.K., Immobilized growing cells of *Gibberella fujikuroi P-3* for production of gibberellic acid and pigment in batch and semi- continuous cultures, Appl. Microbiol. Biotech., 1988, 28, 537-542.

8. Gaucher, G.B. and Behie, L.A., Cell immobilization in the production of patulin and penicillin by *Penicillium urticae* and *Penicillium chrysogenum*, Methods in Enzymology, 1987, 136, 329-343.

9. Saucedo, J.E.N., Barbotin, J. and Thomas, D., Continuous production of gibberellic acid in a fixed-bed reactor by immobilized mycelia of *Gibberella fujikuroi P-3* in calcium alginate beads, Appl. Microbiol. Biotechnol., 1989, 30, 226-233.

10. Ünyayar, A. and Kolankaya, N., Biotechnological approach for production biopulp, Doğa Turkish Journal of Biology, 1990, 14, 1, 51-58.

11. Capdevila, C., Corrieu, G. and Asther, M.A., A feedharvest culturing method to improve lignin peroxidase production by *Phanerochaete chrysosporium* INA12 immobilized on polyurethane foam, J. Ferment. Bioeng., 1989, 68, 1, 60-63.

12. Kahlon, S.S. and Malhotra, S., Production of gibberellic acid by fungal mycelium immobilized in sodium alginate, Enzyme Microb. Technol., 1986, 8, 613-616.

13. Cihangir, N. and Aksöz, N., Production of gibberellic acid by immobilized *Aspergillus niger*, Doğa Turkish Journal of Biology, 1993, 17, 175-180.