تبدیل زیستی دیادزین به ODI-′۳ توسط تیروزیناز تثبیت شده در سطح اسپور

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چکیده

مقدمه: کنیک نمایش در سطح اسپور باسیلوس سابتیلیس برای نمایش آنتی ژن‌ها و آنزیم‌ها برای اهداف صنعتی و پزشکی استفاده شده است. در این تکنیک، آنزیم به صورت ژنتیکی در سطح اسپور تثبیت می‌شود و یکی از کاربردهای آنزیم نمایش داده شده در سطح قابلیت استفاده مجدد می‌باشد. در این مطالعه تیروزیناز نمایش داده شده در سطح اسپور برای تبدیل زیستی دیدزین استخراج شده از دانه‌های سویا به ترکیب هیدروکسیله تر شده و ضد سرطان ODI-′۳ مورد استفاده قرار گرفت.

مواد و روش‌ها: باسیلوس سابتلیس (pSDJH-cotE-tyr DB104)، که در پژوهش قبلی ساخته شده بود به عنوان منبع آنزیم استفاده شد. واکنش در C°37 در ۱ ساعت انجام شد. برای مشاهده محصولات واکنش از کروماتوگرافی مایع با فشار بالا استفاده شد.

نتایج: نتایج نشان داد که mM 1 از دیدزین به حدود mM 1 از ODI-′۳ در طول ۶۰ دقیقه توسط ۱۰۸×۴ اسپور تبدیل شد. همچنین فعالیت مجدد تیروزیناز تثبیت شده در سطح اسپور پس از سه بار استفاده حدود ۵۸ درصد مشخص شد.

بحث و نتیجه‌گیری: آنزیم‌های بیان شده در سطح اسپور مسیر جدیدی به سمت اصلاح پایداری و قابلیت استفاده مجدد آنزیم باز کرده است. نتایج ما نشان داده که تیروزیناز فعال در سطح اسپور پتانسیل استفاده در شرایط صنعتی برای تولید ایزوفلاون ها با درجه هیدروکسیله بالاتر را دارد.

کلمات کلیدی: تبدیل زیستی، تیروزیناز، نمایش در سطح اسپور، دیدزین، ODI-′۳

واژه‌های کلیدی: کلمات کلیدی: تبدیل زیستی، تیروزیناز، نمایش در سطح اسپور، دیدزین، ODI-′۳

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Bioconversion of Daidzein to 3′-ODI by *Bacillus Subtilis* Spore Displayed Tyrosinase

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Abstract

Introduction: *Bacillus subtilis* spore surface display technique has long been used to display antigens and enzymes for medical and industrial proposes. In this technique, the enzyme is genetically immobilized on the spore surface and one of the capabilities of spore displayed enzyme is the reusability. In this study, spore displayed tyrosinase was used for the bioconversion of soybean extracted daidzein to more hydroxylated, anticancer compound, 3′-ODI.

Materials and methods: *Bacillus subtilis* DB104 (pSDJH-cotE-tyr) which was constructed in our previous study was used as an enzyme source. The reaction was done in 37˚C for 1 hour. To detect the product of the reaction, *high-performance liquid chromatography* was used.

Results: The results revealed that 1mM of daidzein was converted to about 1mM 3′-ODI during 60 min by $4*10^8$ spores. The retained activity of the spore displayed tyrosinase was also detected about 58% after three times usage.

Discussion and conclusion: Spore surface displayed enzymes have created a new way to improve enzyme stability and reusability. Our results showed that active tyrosinase on the surface of the spores has the potential to be used in industrial conditions to produce more hydroxylated isoflavones.

Key words: Bioconversion, Tyrosinase, Spore Surface Display, Daidzein, 3′-ODI

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Introduction

Daidzein (4′, 7-dihydroxyisoflavone) is a natural isoflavone and a bioactive compound isolated from soybean (1). The protective effects of daidzein against various diseases such as breast and prostate cancer, diabetes and cardiovascular diseases have been investigated. The pharmaceutical mechanism of daidzein is because of the structural similarities to mammalian estrogens. Daidzein has antiproliferative effects and it is an antiangiogenesis compound which plays a remarkable role in the inhibition of the migration of cancer cells to prevent metastasis (2). Phenolic compounds, especially ortho-dihydroxylated phenolic compounds, have the potential to prevent cancer (3). 3′-ODI (7, 3′, 4′-trihydroxyisoflavone) is one of the ortho-dihydroxylated isoflavones which is not present naturally (4). Anti-oxidative and anti-cancer effects of 3′-ODI have been investigated in several researches (5, 6). CytP450 and tyrosinase were used for the bioconversion of daidzein, to 7, 3′, 4′-trihydroxyisoflavone (3′-ODI) to increase the antioxidant effects (7). Cyt P450 can selectively introduce atomic oxygen into non-activated carbon-hydrogen bonds; however, the utilization of Cyt P450 in industry, because of their low efficiencies, low yields, and necessity for cofactor has several problems (8). Tyrosinase, a type III copper containing monoxygenase, is an applicable enzyme in cosmetic, drug development and pharmaceutical industries and also it has been used in variety of biotechnological applications such as bioremediation, biosensor, biomedicine, and biocatalysis (9, 10).

The most important reaction organized by tyrosinase is the bioconversion of L-tyrosine to L- DOPA (3,4-di hydroxy phenylalanine) which is a drug for Parkinson disease (11). Unlike the Cyt P450, tyrosinase is simply used in industry with high efficiencies and outputs (8). Due to the high utilization of the enzyme, it is necessary to immobilize the enzyme on appropriate support for the efficient usage (12). Spore surface display technique is a developmental genetic engineering method to express and immobilize proteins on the surface of *Bacillus subtilis* spores (13). According to the spore resistance to heat shocks, pH changes and UV radiation, genetically immobilized protein can be used in such extreme conditions. The spore surface display technique has been used to develop vaccines and biosensors successfully (14, 15).

In this study, we used the spore displayed tyrosinase as a stable and reusable enzyme for synthesis of 3′-ODI from daidzein.

Materials and Methods

Bacterial Strain and Culture Condition: *Bacillus subtilis* DB104 (pSDJH-cotE-tyr) which was constructed in our previous study was used as an enzyme source. To collect the spores, vegetative cells were inoculated into Difco sporolation medium (DSM) and cultivated for 24h at 37°C in a shaker incubator (200rpm). This medium contained 0.8% w/v nutrient broth, 0.1% KCl, 0.025% $MgSO_4.7H_2O$, 1mM $Ca(NO_3)_2$, 0.01mM $MnCl_2$, and 0.01mM $FeSO_4$ in 1L distillated water, pH 7. The spores were then purified using renografin (sodium diatrizoate, S-4506, Sigma) gradient method (16).

Bioconversion Assay of Diadzein to 3′-ODI: The purified spores ($4 * 10^8$) were used as an enzyme source for the bioconversion of daidzein to 3′-ODI. The reaction of 1mM daidzein (**D7802** Sigma) was done in 50mM tris- HCl buffer pH 8 with 5mM ascorbic acid and 10µM $CuSO_4$ in 37˚C for 1 hour. HPLC (YL9100, Young Lin instrument) was used to monitor the concentrations of substrates and products. The experiments were repeated in triplicate.

Reusability of the Enzyme: To assay the enzyme reusability, the spores were washed three times with Tris-HCl buffer (pH 8), and

the formation of the product was monitored by HPLC again. The HPLC graphs were analyzed by ChromNAV 2.0 HPLC software.

Results

Bioconversion of Daidzein to 3′-ODI by Spore Displayed Tyrosinase: Bioconversion of daidzein to 3′-ODI by spore displayed tyrosinase was monitored by HPLC. As it is shown in figure 1, after 60 min, almost all content of diadzein were converted to 3'-ODI. Tyrosinase transferred one hydroxyl group on daidzein to create 3′-ODI (Fig. 1).

Reusability of Spore Displayed Tyrosinase: The reusability of tyrosinase-displaying spores was also investigated by washing them repeatedly with a buffer. Retained activity of immobilized tyrosinase was about 58% after three times washing with Tris-HCl buffer, pH 8 (Fig. 2).

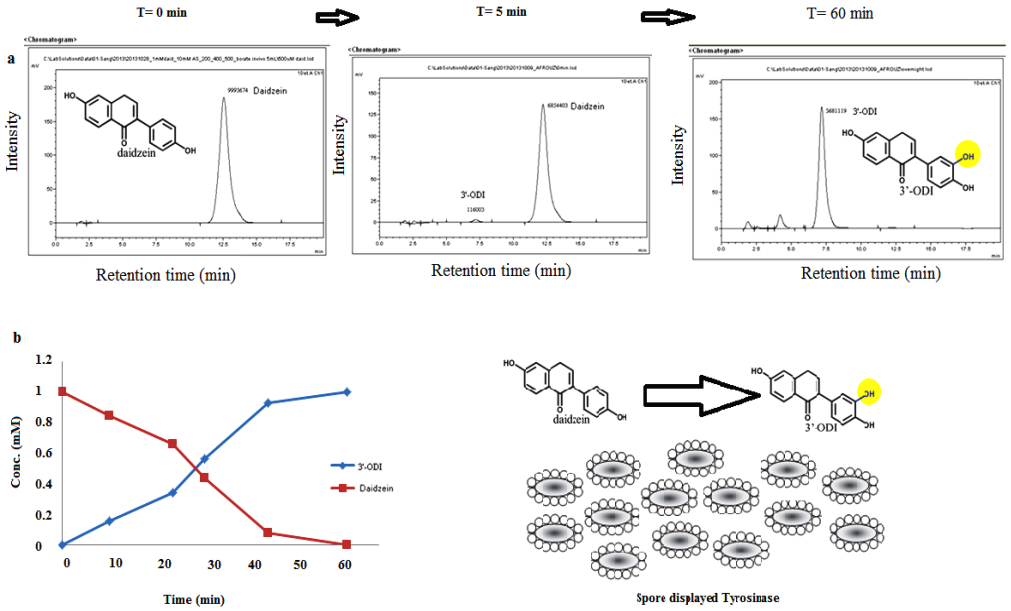

Fig. 1- HPLC result of the spore displayed tyrosinase reaction for producing 3′-ODI. (a) The peaks at retention time 12.5 min and 7.5 min are related to daidzein and 3′-ODI, respectively. (b) Time profile of daidzein consumption and 3′-ODI production.

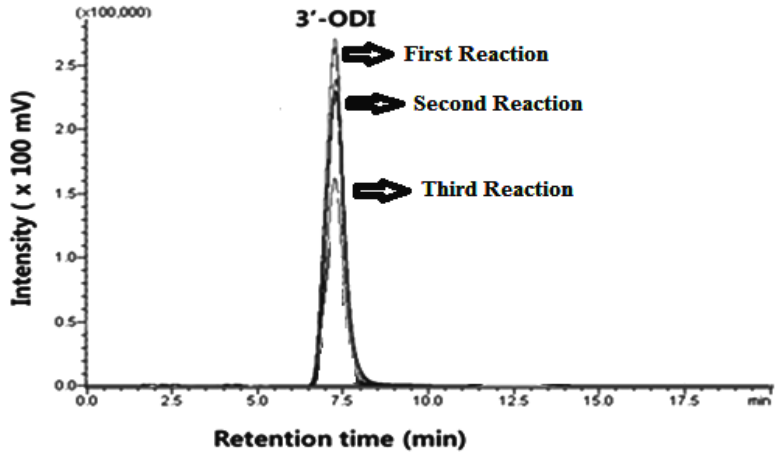

Fig. 2- HPLC result of the reusability of spore displayed tyrosinase after three times washing

Discussion and Conclusion

Daidzein, a natural bioactive compound in soybean was easily converted to 3′-ODI by spore displayed tyrosinase during 1 hour. 3′-ODI is not present naturally and because of the extra hydroxyl group, it is more active

than daidzein (4). Due to the spore resistance to harsh conditions such as low or high temperatures, acidic or alkali pH, the spore displayed enzymes also are very active in various industrial conditions (15). It shows the merit of the spore displayed tyrosinase than the pure tyrosinase. Recently, enzyme immobilization has been considered as an essential tool to improve stability and reusability of industrial enzymes (17). Our results also demonstrated 58% retained activity after three times usage. In our previous work, tyrosinase from *Bacillus megaterium* was displayed on the surface of *Bacillus subtilis* spores by CotE as an anchor protein. Our results revealed that the spore displayed tyrosinase can be active during 15 days maintenance in room temperature; however, the free enzyme was active for a few hours. The spore displayed tyrosinase was also active during 6 times washing steps (12). All the results showed that the spore displayed tyrosinase is a good candidate for being used in industry and bioconversion of natural useful compounds. Our previous research also showed 62% retained activity when spore displayed tyrosinase was used to produce dopachrome from L- Tyrosine after six times washing (12). In another research, Tsai and Meyer observed 83% retained activity of spore displayed esterase (18). The need to produce strong enzymes have been made the spore display technique as a suitable novel technique for immobilization.

In conclusion, the spore displayed tyrosinase was used as an efficient tool for bioconversion of daidzein to 3′-ODI. The genetically immobilized enzyme showed reusability potential for several times. It suggests that the spore displayed tyrosinase has an interested potential for industrial usage.

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References

(1) Vránová D. Quantification of soy isoflavones in meat products by HPLC. *Scripta Medica* (BRNO) 2005; 78 (4): 235–242.

(2) Suna MY., Yeb Y., Xiaob L., Rahmanc K., Xiad W., Zhang H. Daidzein: a review of pharmacological effects. *African Journal of Traditional, Complementary and Alternative Medicines* 2016; 13(3):117-132.

(3) Adjakly M., Ngollo M., Boiteux JP., Bignon YJ., Guy L.,Bernard-Gallon D. Genistein and daidzein: different molecular effects on prostate cancer. *Anticancer Research* 2013; 33: 39-44.

(4) Park JS., Park HY., Kim DH., Kim HK. Ortho-Dihydroxyisoflavone derivativesfrom aged Doenjang (Korean fermented soy paste) and its radical scavenging activity. *Bioorganic & Medicinal Chemistry Letters* 2008; 18(18):5006–5009.

(5) Lee NH., Kim EJ., Kim BG. Regioselective hydroxylation of trans-resveratrol via inhibition of tyrosinase from *Streptomyces avermitilis* MA4680. *ACS Chemecial Biology* 2012; 7(10):1687–1692.

(6) Lo YL., Wang W., Ho CT. 7,30,40-Trihydroxyisoflavone modulates multidrug resistance transporters and induces apoptosis via production of reactive oxygen species. *Toxicology* 2012; 302(2–3):221–232.

(7) Pandey BP., Roh C., Choi KY., LeeN., Kim EJ., Ko S., Kim T., Yun H., Kim BG. Regioselective hydroxylation of daidzein using P450 (CYP105D7) From *Streptomyces avermitilis* MA4680. *Biotechnology and Bioengineering* 2010; 105(4): 697-704.

(8) Using Tyrosinase as a Monophenol Monooxygenase: A Combined Strategy for Effective Inhibition of Melanin Formation. Lee SH., Baek K., Lee JE., Kim BG. *Biotechnology and Bioengineering* 2016; 113(4): 735-743.

(9) Freire RS., Durán N, Kubota LT., Electrochemical biosensor-based devices for continuous phenols monitoring in environmental matrices. *Journal of the Brazilian Chemical Society* 2002; 13(4): 456-462.

(10) Zaidi KU., Ali AS., Ali A., Naaz I. Microbial tyrosinases: promising enzymes for pharmaceutical, food bioprocessing, and

environmental industry. *Biochemistry Research International* 2014; 1-16.

(11) Surwase SN., Jadhav JP. Bioconversion of L-tyrosine to L-DOPA by a novel bacterium *Bacillus sp*. JPJ. *Amino Acids* 2010; 41 (2): 495–506.

(12) Hosseini-Abari A., Kim BG., Lee SH., Emtiazi G., Kim W., Kim JH.Surface display of bacterial tyrosinase on spores of *Bacillus subtilis* using CotE as an anchor protein. *Journal of Basic Microbioliology* 2016; 56, 1–7.

(13) Kim J., Schumann W. Display of proteins on *Bacillus subtilis* endospores. *Cellular and Molecular Life Sciences* 2009; 66: 3127–3136.

(14) Wernerus H., Stahl S. Biotechnological applications for surface-engineered bacteria. *Biotechnology and Applied Biochemistry* 2004; 40: 209–228.

(15) Nicholson WL., Munakata N., Horneck G., Melosh, HJ., Setlow P. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiology and Molecular Biology Reviews* 2000; 64: 548–572.

(16) Harwood C., Cutting S. Molecular biological methods for Bacillus. England: John Wiley & Sons Ltd, Chichester. (pp.16-30); 1990.

(17) Mattossovich R., Iacono R., Cangiano G., Cobucci-Ponzano B., Isticato R., Moracci M., Ricca E. Conversion of xylan by recyclable spores of *Bacillus subtilis* displaying thermophilic enzymes. *Microbial Cell Factories* 2017; 16:218.

(18) Tsai CT., Meyer AS. Enzymatic cellulose hydrolysis: enzyme reusability and visualization of β-glucosidase immobilized in calcium alginate. *Molecules* 2014; 19: 19390-19406.