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UV Mutagenesis Enhanced Biotransformation Efficiency of Rutin to Isoquercitrin by *Bacillus litoralis*

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Abstract: *In order to obtain high biotransformation efficiency of rutin to isoquercitrin (quercetin-3-O-glucoside), Bacillus litoralis C44 was treated by UV mutagenesis to screen the thermo- and alkali-tolerant mutants, for these conditions allow for a very high substrate concentration. The optimal mutagen dose for strain C44 was irradiation for 50s with a 15W UV lamp from 30 cm away. The mutants were preliminary screened by quantitative TLC, and 16 mutant strains were faced to second-screening by HPLC. As a result, a genetic stable mutant strain UV-2-45 was obtained, which got a biotransformation rate of 3.9 times more than the original strain Bacillus litoralis C44, and its mole yield reached as high as 91% from 3 g/L of rutin in glycine-sodium hydroxide buffer (pH 9.0) at 45°C for 2 days.*

Keywords: *Isoquercitrin, UV Mutagenesis, Bacillus litoralis*

I. Introduction

The flavonol glycoside isoquercitrin (quercetin-3-O-β-d-glucopyranoside) is commonly found in medicinal herbs, fruits, vegetables and plant-derived foods and beverages [1]. Isoquercitrin can be obtained by enzymatic hydrolysis of rutin with α-l-rhamnosidase. It possesses a wide range of biological activities, such as anti-oxidant [2], anti-diabetic [3], anti-influenza [4], and diuretic activities [5]. Enzymatically modified (α-glucosylated) isoquercitrin (EMIQ) has higher bioavailability of anti-tumor and anti-allergy [6-10]. To produce isoquercitrin at a reasonable price, the transformation of rutin to isoquercitrin has been proved to be a feasible procedure because rutin is much more widely distributed in numerous plants than isoquercitrin [11-13]. Rutin has a very low solubility in neutral aqueous, which limited the use of neutrophilic microorganisms and there enzymes. However, as it known to all, rutin has higher solubility at high temperatures and in alkaline conditions.

Mutation breeding of microorganisms to improve the product yield or activity using a variety of means has been a hot research field all over the world. UV mutagenesis techniques are conventional methods of breeding superior strains of mutagenesis and screening. Because of its simple equipment, high mutagenic efficiency, safe and easy operation, and is widely used.

In the previous study, we reported that *Bacillus litoralis* C44 could degrade rutin to produce isoquercetin by producing α-L- rhamnosidase[14]. In this article, to increase the transformation efficiency, UV mutagenesis method was used to treat strain C44 and the thermo- and alkali-tolerant mutants with high α-L-rhamnosidase were screened using quantitative TLC and HPLC methods.

II. Materials And Methods

2.1 Materials

2.1.1 Bacteria strain

The initial strain *Bacillus litoralis* C44 was screened and preserved by our laboratory, which can hydrolyze rutin to form isoquercitrin [14].

2.2 Methods

2.2.1 The preparation of cell suspension

The strain was cultured in the 30 mL beef extract-peptone liquid medium(7.0) that contains (per liter distilled water): 3g beef extract, 10g peptone, 5g NaCl, the pH was monitored and adjusted to 7.0. After incubated at 200r/min 28℃ for 12 h, 10% inoculum size was transferred to fresh liquid medium and cultured for 6 h to reach the logarithmic growth phase. Subsequently, the cells were harvested by centrifugation at 10000 r/min for 5min at 4°C, washed twice with physiological saline and make the cell concentration about 1×10^8CFU/mL[15].

2.2.2 UV mutagenesis

To obtain the optimum dose of UV irradiation for *Bacillus litoralis* C44, the cell suspensions were exposed to UV rays at a distance of 30 cm from the UV lamp (λ = 254nm) for 0s, 10s, 20s, 30s, 40s, 50s, and

60s. And the lethality rates of various doses were determined by calculating the survived CFU/mL cell suspension vs. the untreated control with dilution spread plate method [15].

2.2.3 Screening of thermo- and alkali-tolerant mutants

Thermo- and alkali-tolerant mutants were obtained by spreading the optimal dose of UV treated cell suspension on alkaline beef extract-peptone agar medium plates (pH 9.0), and culturing at 45°C for 48 hours.

2.2.4 Screening of thermo- and alkali-tolerant efficient mutants with high isoquercitrin yield

2.2.4.1 Quantitative TLC determination

Quantitative thin layer chromatography (Q-TLC) method was used to screen the high isoquercitrin producing mutant strains preliminary. The mutant strains were inoculated in 30 mL of beef extract-peptone medium (pH 9.0) in 250 mL flask, cultured at 45°C in a thermostat shaker by shaking at 220r/min for 24h. The liquid culture was centrifuged at 10000r/min for 5min at room temperature to separate the cell. The cell pellet was washed with 10 mL of 0.05mol/L glycine-sodium hydroxide buffer (pH 9.0) and centrifuged again to harvest the biomass. Then the biomass was resuspended with 1 mL of rutin containing glycine-sodium hydroxide buffer (pH 9.0, 3g/L of rutin) per 40mg and transformed for 48h by shaking at 220r/min and 45°C. At last, TLC analysis was performed to check the activity. The TLC analysis was carried out by loaded the 2 μL of converted products on polyamide film and developed with 75% microemulsion consisted of SDS/n-butyl alcohol/n-heptane/water, 0.27: 0.63: 0.10: 36 (m/m). The developed polyamide film was stained by spraying 1% AlCl3-ethanol solution and dried under room temperature. Then, the TLC polyamide film were scanned and analyzed by UV-scan-it gel scanning software. Data processing and quantitative analysis were used Quantity One software [16-18].

2.2.4.2 HPLC determination

High performance liquid chromatography (HPLC) was used to screen the efficient thermo- and alkali-tolerant mutants further. The HPLC sample was prepared as following: the transformed solution was centrifuged to get rid of the cell, and then the supernatant was filtrated by 0.22μm membrane and ready for HPLC [17]. The chromatography conditions were as following: separated by pre-packed Hypersil ODS (C18) column (250×4.6mm, Elite, China) column at 24°C±1°C and detected by UV detector at 360nm, the mobile phase composed of acetonitrile: 80% methanol: water: phosphoric acid (100:10:340:0.3, v/v) was used as the eluent at a flow rate of 1.0 mL/min, sample volume was 10μL [19].

III. Results And Discussion

3.1 Optimum UV mutagenesis does

The time of ultraviolet irradiation has a great impact on mortality of strain C44 (Fig. 1). When the irradiation time was 60s, the lethality rate was 98.1%. As many scientists think the mortality rate of 80-90% is conducive to produce positive mutation, we select the irradiation time of 50s in the further mutagenesis.

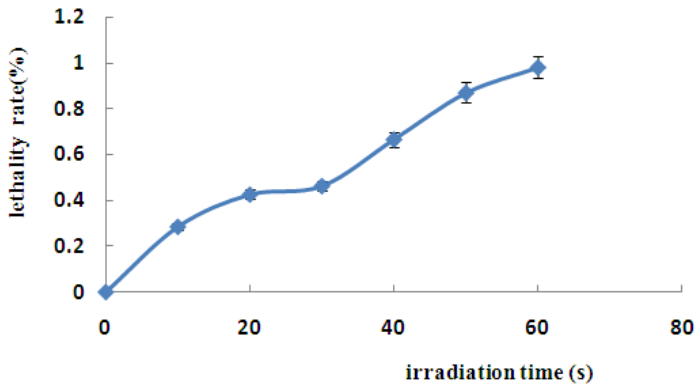

Fig.1 UV mutagenesis lethality rate curve of *Bacillus litoralis* C44

3.2 Screening of thermo- and alkali-tolerant mutants

The cell suspension of *Bacillus litoralis* C44 was irradiated 50s and spread to the alkaline beef extract-peptone agar medium plates (pH 9.0), inverted cultured in the incubator at 45°C for 48 h. Finally, 98 vigorous mutant strains were obtained.

3.3 Quantitative TLC determination

The ability of transforming rutin to isoquercitrin of 98 thermo- and alkali-tolerant mutant strains were assayed with quantitative thin layer chromatography (Q-TLC) method. At last, there are 32 strains with α-L-rhamnosidase activity. TLC screening result of some mutant strains was shown in Fig. 2, and the quantitative analysis results of some mutants were shown in Fig. 3 compared with the original strain *Bacillus litoralis* C44.

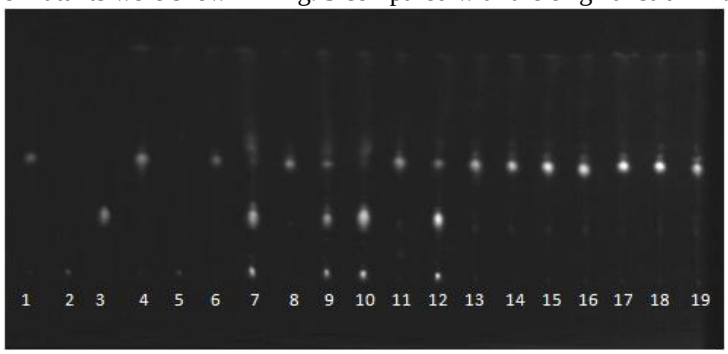

Fig. 2 TLC chromatogram of some mutants

1 Rutin standard, 2 Quercitrin standard, 3 Isoquercitrin standard, 4 Negative control, 5-11 and 13-9 transformation products of mutants, 12 transformation products of original strain C44.

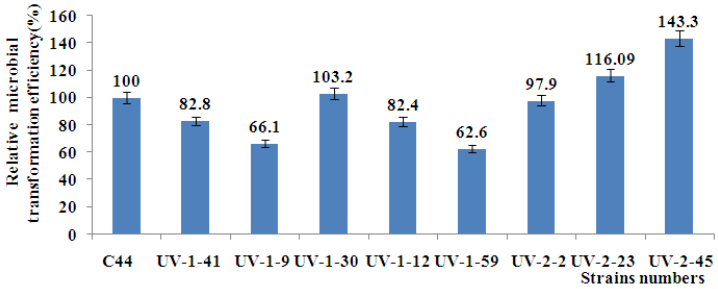

Fig. 3 Quantitative analysis results of some mutants

Cells of strain C44 was suspended with 1mL of rutin containing phosphate buffer (pH 7.0, 3g/L of rutin) per 40mg and reacted for 72h by shaking at 220r/min and 28°C, the transformation efficiency was set as 100%. Cells of the mutants (UV-1-41 UV-1-9 UV-1-30 UV-1-12 UV-1-59 UV-2-2 UV-2-23 UV-2-45) was suspended with 1 mL of rutin containing glycine-sodium hydroxide buffer (pH 9.0, 3g/L of rutin) per 40mg and reacted for 48h by shaking at 220r/min and 45°C.

3.4 HPLC determination

16 mutants that showed higher transform efficiency than C44 were further determined by HPLC. And mutant strain UV-2-45 strain reached a biotransformation rate of 4.9 times of the original strain *Bacillus litoralis* C44, and its mole yield reached as high as 91% from 3 g/L of rutin in glycine-sodium hydroxide buffer (pH 9.0) at 45°C for 48h (Fig. 4).

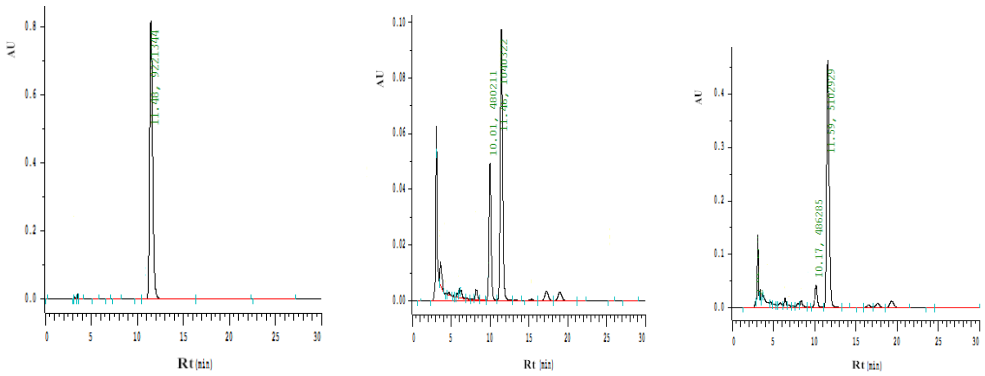

Fig. 4 HPLC determination result of mutant strain UV-2-45 comparing with strain C44

(A) HPLC chromatogram of isoquercitrin standard (B) HPLC chromatogram of transformation products of *Bacillus litoralis* C44 (C) HPLC chromatogram of transformation products of mutant UV-2-45

3.5 Genetic Stability test results

To check the genetic stability of the strainUV-2-45, we continuous passage cultured it for 10 generations within one month and determined the biotransformation efficiency of rutin to isoquercitrin by mutant strain UV-2-45. Its mole yield reached as high as 91.2±0.46% from 3 g/L of rutin in glycine-sodium hydroxide buffer (pH 9.0) at 45°C for 48h.The results clearly showed that this strain is genetically stable in isoquercitrin production.

IV. Conclusion

In this project, the initial low- biotransformation efficiency of rutin to isoquercitrin strain *Bacillus litoralis* C44 was subjected to UV mutagenesis. A genetic stable mutant strain UV-2-45 was obtained, which biotransformation time was reduced by 0.67-fold, while the yield of isoquercitrin was increased by 4.9-fold which reached as high as 91% at the end. The results suggest that the thermo- and alkali-tolerant strain have great potential to enhance the biotransformation of rutin for isoquercitrin production.

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