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Original Research Paper

Evaluation of environmental parameters for production of 7, 10-dihydroxy-8(*E*)-octadecenoic acid from olive oil by *Pseudomonas aeruginosa* PR3

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ABSTRACT

Microbial conversions of the free unsaturated fatty acids often generate novel hydroxy fatty acids (HFA), which are known to have special properties such as higher viscosity and reactivity. Among microbial strains known to produce HFAs, *Pseudomonas aeruginosa* PR3 has been well studied to produce 7,10-dihydroxy-8(*E*)-octadecenoic acid (DOD) from the free oleic acid. Previously we reported that the strain PR3 could utilize olive oil instead of oleic acid as a substrate for production of DOD (Suh et al., 2011. Applied Microbiology and Biotechnology, 89, 1721–1727). In this study, we evaluated the environmental parameters for DOD production from olive oil by PR3. DOD production was closely related to the species and concentration of carbon source. Glucose and galactose showed optimal DOD production at 0.2% and 0.4%, respectively. Addition of 1.5% oil substrate to the culture 36 h after initiation of cultivation under 200 rpm represented a maximal 318 mg DOD production in 50 ml culture, corresponding to 85% conversion yield over the oleic acid content in olive oil.

1. Introduction

Microbial transformation of the natural compounds is one of the efficient ways to add new value to them. Oxygenation of the free unsaturated fatty acids by microbial conversion is a good example of those modifications. Microbial oxygenation of the unsaturated fatty acid can produce oxylipin including hydroxyl lipid. Hydroxy fatty acids (HFA) are known to have special properties such as higher viscosity and reactivity compared with other normal fatty acids (Bagby and Calson, 1989) rendering them to gain high potential in a wide range of industrial applications including resins, waxes, nylons, plastics, lubricants, cosmetics, and additives in coatings and paintings. In addition, some of the HFAs are known to have antimicrobial activities (Bajpai et al., 2004; Hou and Forman, 2000; Kato et al., 1984; Shin et al., 2004).

Among microbial strains tested for hydroxylation of the free fatty acids, *Pseudomonas aeruginosa* PR3 has been well studied to produce mono-, di-, and trihydroxy fatty acids from various unsaturated fatty acids. Specifically the strain PR3 converted oleic acid into a 7,10-dihydroxy-8(E)-octadecenoic acid (DOD) with 60% production yield (Hou et al., 1991) and its production yield was improved over 80% via modification of culture conditions (Kuo et al., 1998). 10-hydroxy-8(*E*)-octadecenoic acid was identified as an intermediate in this bioconversion Hou and Bagby, 1992; Kim et al., 2000. Recently triolein, rather than free oleic acid, was efficiently used as a substrate by *P. aeruginosa* PR3 to produce DOD (Chang et al., 2007). This result suggested that the vegetable oils containing oleic acid could be used as a substrate for DOD production by *P. aeruginosa* PR3. Based on these observations, we studied and reported that olive oil containing high content of oleic acid was efficiently used as a substrate for DOD production by *P. aeruginosa* PR3 (Suh et al., 2011). In this study we focused on the evaluation of the environmental parameters for DOD production from olive oil by *P. aeruginosa* PR3.

2. Materials and methods

2.1. Chemicals

Olive oil was purchased from a local market and heptadecanoic acid (C17:0) was purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA). Mixture of trimethylsilylimidazole (TMSI) and pyridine (1:4, v/v) was purchased from Supelco Inc. (Bellefonte, PA, USA). All other chemicals were reagent grade and were used without further purification. Thin-layer pre-coated Kieselgel 60F_{254} plates

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were obtained from EM Science (Cherry Hill, NJ, USA). Other chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA), unless mentioned otherwise.

2.2. Microorganism and bioconversion

P. aeruginosa NRRL strain B-18,602 (PR3) was kindly provided by Dr. Hou of NCAUR (National Center for Agricultural Utilization Research, Peoria, IL, USA). The strain was aerobically grown at 28 °C with shaking at 200 rpm in a 125 ml Erlenmeyer flask containing 50 ml of standard medium. The standard medium used hereafter contained (per liter) 4 g dextrose, 2 g K_2HPO_4, 2 g $(NH_4)_2HPO_4$, 1 g NH_4NO_3, 1 g yeast extract, 0.056 g $FeSO_4 \cdot 7H_2O$, 0.1 g $MgSO_4$, and 0.01 g $MnSO_4 \cdot 7H_2O$. The medium was adjusted to pH 7.0 with diluted phosphoric acid. For bioconversion, as a standard condition, olive oil (0.5 g) was added to the 24-h-old pre-culture, followed by additional incubation for 72 h at 28 °C. At the end of cultivation, the culture was acidified to around pH 2.0 with 6 N HCl, followed by two immediate extractions with equal volume of ethyl acetate and diethyl ether. The solvent was evaporated from the combined extract with a rotary evaporator. For optimization study, individual nutritional component was replaced as needed from the standard medium and conversion was carried out under standard condition, unless mentioned otherwise. Cell growth was determined spectrophotometrically by measuring absorbance of the cell culture at 610 nm. In brief, cells were harvested from one ml of the culture by centrifugation at 3000 rpm for 5 min followed by washing once with 10% saline solution prior to measuring the absorbance. High density culture was properly diluted with saline solution before measuring absorbance and the final cell density was determined by multiplication of the absorbance value by dilution factor.

2.3. Analysis of products

The extracted reaction products were analyzed by thin-layer chromatography (TLC) and gas chromatography (GC). The TLC was developed with a solvent system consisting of toluene:dioxane: acetic acid (79:14:7, v/v/v). Spots were visualized by spraying the plate with 50% sulfuric acid and heating it in a 100 °C oven for 10 min. For GC analysis, the samples were first methylated with diazomethane for 5 min at room temperature, followed by derivatization with a mixture of TMSI and pyridine (1:4, v/v) for at least 20 min at room temperature. The TMS-derivatized sample was analyzed with Younglin AMCE 6100 GC (Younglin, Seoul, Korea) equipped with a flame-ionization detector and a capillary column (SPB-1™, 15 m × 0.32 mm i.d., 0.25 μm thickness, Supelco Inc., Bellefonte, PA, USA). GC was run with a temperature gradient of 20 °C/min from 70 °C to 200 °C, 1 min with a hold at 200 °C, and then 0.7 °C/min to 240 °C followed by a 15 min hold at 240 °C (nitrogen gas flow rate = 0.67 ml/min). Injector and detector temperatures were held at 250 °C and 270 °C, respectively. Heptadecanoic acid (C17:0), as an internal standard for quantification, was added to the sample before derivatization.

Chemical structure of the target compound was confirmed by GC/mass spectrometry (GC/MS) analysis. Electron-impact (EI) mass spectra were obtained with a Hewlett Packard (Avondale, PA, USA) 5890 GC coupled to a Hewlett Packard 5972 Series Mass Selective Detector. The column outlet was connected directly to the ion source. Separation was carried out in a methylsilicone column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with a temperature gradient of 20 °C/min from 70 °C to 170 °C, 1 min hold at 170 °C and 5 °C/min up to 250 °C followed by a 15 min hold at 250 °C (helium gas flow rate = 0.67 ml/min).

3. Results

3.1. Effect of carbon source concentration on DOD production

Carbon source seems to be closely related to DOD production by *P. aeruginosa* PR3 because DOD was further consumed by the strain PR3 when most of the carbon sources in the medium were exhausted (Chang et al., 2007). In this point of view, it was quite necessary to investigate the effect of concentration of optimal carbon sources on DOD production by *P. aeruginosa* PR3. Therefore, we determined the effect of concentration of glucose and galactose on DOD production since these carbon sources were found to be most efficient for DOD production from olive oil by *P. aeruginosa* PR3 (Suh et al., 2011). DOD production was highly dependent upon the concentration of each carbon source although the optimal concentrations were different. DOD productions were maximized at 0.1 g of glucose in 50 ml culture (0.2%) (Fig. 1) and at 0.2 g of galactose in 50 ml culture (0.4%) (Fig. 2) followed by a significant decrease thereafter while cell growths were maintained high. These results demonstrated that DOD production was reversely related with the concentration of the carbon sources beyond certain level and surplus amount of carbon source could cause negative effect on DOD production by *P. aeruginosa* PR3 although the optimal concentration of carbon source could be affected by other parameters.

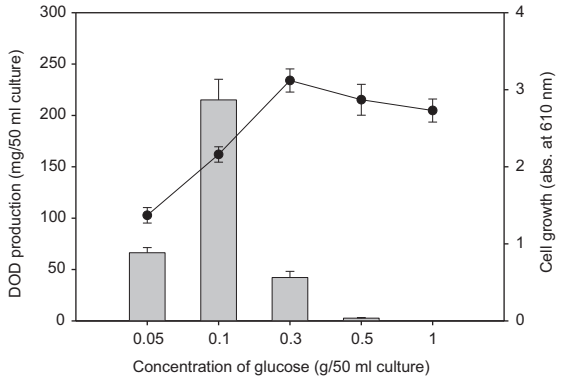

Fig. 1. Effect of glucose concentration on DOD production from olive oil by *P. aeruginosa* PR3. Different amount of glucose were added as single carbon source to the standard medium. Bar and line graphs represented DOD production and cell growth, respectively. Other reaction conditions were same as standard condition explained in materials and methods.

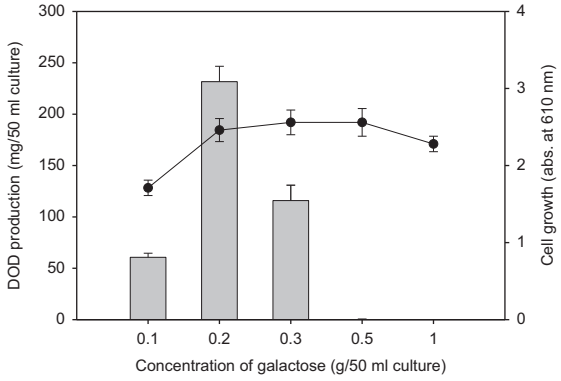

Fig. 2. Effect of galactose concentration on DOD production from olive oil by *P. aeruginosa* PR3. Different amount of galactose were added as single carbon source to the standard medium. Bar and line graphs represented DOD production and cell growth, respectively. Other reaction conditions were same as standard condition explained in materials and methods.