Optimal Conditions for the Production of Antioxidant by *Nocardiopsis* sp. S-1

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Abstract

This study investigated the production of antioxidant from Actinomyces culture supernatant. For the research of the natural marine antioxidant, several bacteria were isolated from the coast of Je-ju in Korea. An actinomycetes strains, S-1 was identified to a genus level 16S ribosomal DNA sequence and fatty acid analysis. From these results and other characteristics described in the Bergey's Manual, this strain was identificated as a *Nocardiopsis dassonvillei*. Strain S-1 showed high activity of 1,1-diphenyl-2-picrylhydrazyl radical scavenging. The hydroxyl radical scavenging ability of *Nocardiopsis* sp. S-1 supernatant was 53%. Nutritional and cultral conditions for the production of antioxidant by this organism under shake-flask conditions have optimized. Similary initial medium pH 7.6, incubation temperature of 25℃, sodium chloride concentration 2.5% and incubation time of 8 day were found to be optimal.

Introduction

Radicals are known to take part in lipid peroxidation, which causes food deterioration, aging in organisms, and cancer promotion[1,2]. Antioxidants act as radical scavenger.

During the biological oxidation process for the production of energy form fuels, the generated oxidative stress can damage some biological molecules. It has been well established that a wide variety of oxygen-centered free radicals and other reactive oxygen species (ROS) are continuously produced in the human body and food system[3,4]. Including aging, it is well known that the oxidative damage plays an important pathological role in cancer, emphysema,

cirrhosis, atherosclerosis, and arthritis. Microbial sources have been shown to be a potential means of producing natural antioxidants. Both eukaryotic and aerobic prokaryotic organisms have developed an overall antioxidative defense system to mitigate the damaging effects of ROS. The important components of the cellular defense system are reduced glutathione (GSH) and antioxidative enzymes like superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalse (CAT). The genus *Nocardiopsis* was created by Meyer(1) to harbour *Actinomadura dassonvillei* on the basis of morphological and chemotaxonomic properties.

The genus *Nocardiopsis* includes aerobic-forming actinomycetes that produce a branched, vegetative mycelium and aerial hyphae. *Nocardiopsis dassonvillei*, isolated from the sea water. In this paper, the characterization of *Nocardiopsis* sp. S-1 and optimization of medium and cultural conditions for maximum production of antioxidant are reported.

Material and method

Isolation and maintenance

Antioxidant producing bacteria were collected along Jeju Island coast of Korea during a period from August 2003 to October 2003. The strain S-1 has been derived from the sediments sand of Hamdeok beach. It was isolated on MA (Marine Agar. Difco. Co.) while incubating at 25℃. Plates containing the culture were stored at 4℃. For long storage, it was grown in MB (Marine Broth. Difco. Co.) for 7 days. To it glycerol was added to a final concentration of 25% (v/v) and stored at -20℃.

Identification (Physiological, biochemical, cultural characteristics, 16S rDNA and fatty acid)

Media used were those recommended by Shirling and Gottlieb (1966) in the international Streptomyces Project (ISP) and by Waksman (1961). Mycelium was observed after incubation at 25℃ for 2 weeks. Colors were determined according to Prauser (1964). Carbohydrate utilization was determined by growth on carbon utilization medium (ISP 9) (Pridham and Gottlieb, 1948) supplemented with 1% carbon sources at 25℃. Temperature range for growth was determined on

inorganic salts starch agar medium (ISP 4) using a temperature gradient incubator. Reduction of nitrate and production of melanoid pigment were determined by the method of ISP (Shirling and Gottlieb, 1996). All cultural characteristics were recorded after 2 weeks.The resultant sequence of strain SC2-1 was manually aligned with representatives of the genus *Exiguobacterium* and related taxa using known 16S rDNA secondary structure information. Phylogenetic trees were inferred by using the neighbour-joining method. The resultant unrooted tree topology was evaluated in bootstrap analyses of the neighbour-joining method based on 1000 resamplings. Fatty acid methyl esters (FAME) mixtures were analysed by capillary GC using a Hewlett Packard model 5898A GC run by Microbial Identification software (Microbial ID).

Measurement of growth and radical, hydroxylradical scavenging activity

Growth of the isolated was measured ad dry weight of the mycelium. Antioxidant activity was performed by DPPH method. DPPH solutrion was prepared at the concentration of 150 uM in methanol. During the assay, the supernatant of 1 ml was mixed 3.0 ml DPPH solution. The mixture was incubated in the room temperature for 30 min. After standing for 30 min, absorbance at 525 nm, and the percentage of inhibition was defined by the absorbance at 525 nm in the absence of supernatant to that measured with sample. The control sample contained a MB medium instead of sample. The absorbance of the mixture was measured at 525 nm, and DPPH radical scavenging ability (%) was defined as follows : EDA(Electron donating ability) = $[1-(A_{525}(\text{sample})/A_{525}(\text{control}))] \times 100\%$.

Optimization of nutritional and cultural conditions

To determine the optimal nutritional and cultural conditions for growth and antioxidant prodution, MB medium was used as the base. It was supplemented with different carbon and nitrogen sources to study their effect on growth and antioxidant production. The medium (50 ml in 250 ml Erlenmeyer flask) was inoculated with 2% (v/v) bacteria suspension and incubated with at 25℃ on a rotary shaker (120 rpm) for 8 days.

Result and disccusion

Recently many reseachers are interested in finding any natural antioxidants having safety and effectiveness, which can be substituted for current commercial synthetic antioxidants, BHA and BHT. Actinomyces have become good candidates for the source of natural antioxidants due to a number of studies recently revealed. Natural antioxidative strain of *Nocardiopsis* sp. S-1 were from the coast of Je-ju in Korea. An actinomycetes strains, S-1 was identified to a genus level 16S ribosomal DNA sequence, fatty acid analysis and International Streptomyces Project (ISP). From these results and other characteristics described in the Bergey's Manual, this strain was identificated as a *Nocardiopsis dassonvillei*. Strain S-1 showed high activity of 1,1-diphenyl-2-picrylhydrazyl radical scavenging. The hydroxyl radical scavenging ability of *Nocardiopsis* sp. S-1 supernatant was 53%. Nutritional and cultral conditions for the production of antioxidant by this organism under shake-flask conditions have optimized. Similary initial medium pH 7.6, incubation temperature of 25℃, sodium chloride concentration 2.5% and incubation time of 8 day were found to be optimal. The results of this study demonstrate the antioxidative potential of marine bacteria, and suggest these strains are useful for functional food industrial and probiotics.

Reference

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