Isolation, Identification and Optimal Cultrul Condition of Antioxidant Producing Bacterium Isolated from the Marine Sources

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Abstract

The isolated strain, SC2-1 was Gram-positive, catalase positive, facultatively anaerobic, oxidase negative, motile and small rods. The strain utilized sucrose, dextrose, fructose, mannitol and maltose as a sole carbon and energy source and sodium chloride required for the bacteria growth. The radical scavenging activity of the culture supernatants was determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) method. This bacterium was identified based on cellular fatty acids analysis and 16S rDNA sequencing then named *Exiguobacterium* sp. SC2-1. The optimum culture conditions for production of antioxidant were 25℃, pH 7.8 and NaCl concentration were 4%. The modified optimal medium compositions were maltose 2.5% (w/v), yeast extract 1.5% (w/v) and KH_2PO_4 0.05% (w/v). Free radical scavenging activity of under optimal culture conditions were 93%.

Introduction

Antioxidants are usually used as additives in the food industry to prevent lipid peroxidation. Although synthetic antioxidants have been widely applied in food processing, they have been reassessed for their possible toxic and carcinogenic components formed during their degradation[3]. Due to these health concerns, natural antioxidants have been extensively employed instead of synthetic ones in recent years. Oxidative stress has been implicated both in the physiological process of aging and in many pathological progression in the central nervous system (CNS) leading usually to some neurodegenerative disorders such as Parkinson's and Alzheimer's diseases[1]. Free radicals are known to take part in lipid peroxidation, which causes food deterioration, aging in organisms, and cancer

promotion. Microbial sources have been shown to be a potential means of producing natural antioxidants[2]. There are microorganisms living in environments of extreme temperature, pH, salinity, and hydropressure. There microorganisms have apparently acquired the ability to survive under such environmental conditions through long-term evolutionary processes, and they possess specific mechanisms for survival in such extreme environments. Antioxidants act as radical scavengers. Screening for natural antioxidants has been mainly done among secondary metabolites of terrestrial plants. Our attention has been focused on marine microorganisms, which are known to contain much polyunsaturated fatty acids. Marine microorganisms, the subject of a growing number of natural product researches, are now considered as efficient producers of biologically active and/or chemically novel compounds. Natural antioxidants are usually more expensive and inferior in effect, however and finding safer, more effective and low-cost natural antioxidants is highly desirable. In this paper we wish to describe the simple screening procedure for antioxidant producing microbes and isolation and identification of a few antioxidants from marine bacteria isolated from sea water and marine animal.

Materials and method

Screening method for antioxidant-producing strains

Sea water, marine algae and marine animal were homogenized in sterilized sea water (20 ml). Each suspension was diluted with sterilized from 10^{-1} to 10^{-4}. The suspensions from 10^{-4} to 10^{-6} (0.2 ml) were spread on agar plates made from the medium and cultured at 25℃ for a few day. A sterilized filter paper was placed on the agar plate so that colonies and their metabolites were replaced on the agar plate so that was further continued at 25℃ for a few days. Then the filter paper was taken out and sprayed with a DPPH solution (1×10-4 M in EtOH) after drying. Strains showing a white-on-purple spot were regarded as antioxidant-producing strains.

16S rDNA and fatty acid analysis

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).

Free radiacl(DPPH), Hydroxyl radicals and superoxide radical scavenging ability

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. The absorbance of the mixture was measured at 525 nm, and DPPH radical scavenging ability (%) was defined as follows : EDA(Electron donating ability) = $[1-(A_{525}(\text{sample})/A_{525}(\text{control}))] \times 100\%$.

The procedure was performed using terephthalic acid (THA) as a chemical dosimeter for hydroxyl radicals. SOD activity was assayed by measuring its ability to inhibit the pyrogallol autoxidation according to the method of Marklun().

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 2 days.

Result and discussion

An antioxidant-producing bacterium was isolated from sea water in Jeju island. The isolated strain, SC2-1 was Gram-positive, catalase positive, facultatively anaerobic, oxidase negative, motile and small rods. The strain utilized sucrose, dextrose, fructose, mannitol and maltose as a sole carbon and energy source and sodium chloride required for the bacteria growth. The radical scavenging activity of the culture supernatants was determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Phylogenetics analysis

based on 16S rRNA gene sequencing and chemotaxonomic data indicated that strain SC2-1 is a member of the genus *Exiguobacteriu*. Major fatty acids are iso$C_{11:0}$, $_{anteiso}C_{12:0}$, $_{iso}C_{13:0}$, $_{anteiso}C_{13:0}$, $_{iso}C_{14:0}$, $_{iso}C_{15:0}$, $_{iso}C_{16:0}$, $_{iso}C_{17:0}$, $_{anteiso}C_{17:0}$, and $_{iso}C_{18:0}$. The optimum culture conditions for production of antioxidant were 25℃, pH 7.8 and NaCl concentration were 4%. The modified optimal medium compositions were maltose 2.5% (w/v), yeast extract 1.5% (w/v) and KH_2PO_4 0.05% (w/v). The radical scavenging ability of *Exiguobacterium* sp. SC2-1 culture supernatant lower than butylated hydroxyanisole(92%), butylated hydroxytoluene(93%) and α-tocopherol(90%). Hydroxyl radical activity of the supernatant of *Exiguobacterium* sp. SC2-1 was estimated to be 73%. The SOD activity of the culture supernatant was estimated about 35%. Free radical scavenging activity of under optimal culture conditions were 92%.

Reference

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