Production of γ - poly-glutamic acid by microorganism isolated from soy sauce

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ABSTRACT

Poly- γ -glutamate (PGA) is water soluble, biodegradable, edible and non-toxic toward humans and the environment. It has potential application in the field of food, cosmetics, medicine, and environmental. Therefore, the development of this material is both environmentally and economically valuable. A bacterium, strain C1, capable of PGA production was isolated from the soy sauce. It was Gram-positive, catalytic, aerobic, and formed spores in the presence of Mn2+. The 16S rDNA (ribosomal DNA sequence) and Microbial Identification System of the isolated strain showed similarity of 97% to that of Bacillus subtilis C1. The study of nutrient requirement for Bacillus subtilis C1 showed that the C1 strain is glutamate-independent bacteria that does not require L-glutamic acid for γ -PGA production, contrary to many γ -PGA producing bacteria reported previously. In contrast, the citric acid and glycerin were essential for biopolymer production. Accordingly, Medium T2 composed of citric acid (22g/L), glycerin (170g/L), NH4C1 (7.0g/L), MgSO4 · 7H2O (0.5g/L), K2HPO4 (0.5g/L), and CaCl2 · 2H2O (0.15g/L) was chosen for the biopolymer synthesis by B. subtilis C 1. After incubation for 6 days in Medium T2 at 37℃, 150rpm, pH=6.5, the biopolymer reached a maximum of 21.44g/L. The number-average molecular weight (Mw) of the product was determined by gel permeation chromatography and found to be over 1×l07. The 6N HCl hydrolysate of the purified viscous material was composed solely of glutamic acid. The phenol-sulfuric acid method showed that the polysaccharide contained less than 1% (w/w) of total sugar. However, the H1-NMR spectra indicated otherwise. The chemical shifts in ppm, 1.8-2.1 (m, β , 2H), 2.3-2.4 (b, γ , 2H), 4.1-4.2 (b, α , 1H), and 7.8 (N-H) corresponds to the peak position of authentic γ -PGA previously synthesized. In addition, the chemical shifts in ppm, 3.50-3.54 (dd, 2H), 3.60-3.63 (dd, 2H), 3.72-3.77 (m, 1H) corresponds to the peak position of glycerol. The glycerin could be removed after the product was hydrolyzed in 0.5% HCI solution (150℃, 30 min), suggesting that the product was a glycerin conjugated γ -PGA derivative. The glycerin-conjugated γ -PGA derivative is unusual in that no such material has ever been reported in the literature. We have also shown that the molecular weight of the biopolymer decreased with the increased salt concentration in that biopolymers of comparatively low molecular weight were preferentially produced under high saline conditions. The molecular weight were decreased by a factor of approximately 10 from 7.94 to 0.73 dalton for cultures at 4 day cultivation time which contained 0.05% and 5% NaCl, respectively. In addition, the stereo-chemical compositions determined by HPLC with a CROWNPAK CR(+) column showed that the D-glutmate content was indifferent to the Mn2+ concentration under study, a phenomenon contrary to that of many γ -PGA producers.

Keywords : poly- γ -glutamate ; soy sauce

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