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Studies on the development of a large scale Production system for 2-O-α -D-glucopyranosyl L-ascorbic acid, a stable vitamin C derivative

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2-*O*-α-D-Glucopyranosyl *L*-ascorbic acid (AA-2G), a stable derivative of L-ascorbic acid (AA), is produced by cyclodextrin glucanotransferase (CGTase, E.C.2.4.1.19) in a species of *Bacillus*. In contrast to AA, AA-2G was appeared to be very stable toward thermal and oxidative degradation in various aqueous solutions throughout wide range of pH and its non-reducibility. It was converted into AA and glucose by the action of mammalian α-glucosidase and showed AA-like biological activities in vivo and tissue culture. Therefore, enzymatic large-scale production of AA-2G will be very useful for industrial application. Our laboratory has succeeded to produce AA-2G from AA using CGTase with several kinds of sugar compound. However AA-2G production was limited to the small scale. In order to develop the method for mass production of AA-2G, *Paenibacillus* sp. JB-13 producing CGTase was cultured by 5 ℓ jar fermenter. Optimal conditions for CGTase production was 5% (v/v) of inoculum size of preculture, 250rpm of agitation speed, 4vvm of aeration rate, 24 hours of inoculum age of preculture. The synthesis of AA-2G uging CGTase was maximal when the condition was as follows : pH 5.0, temperature 37℃, 30% substrate solution containing 24% (w/v) of sodium

ascorbic acid and 6% (w/v) dextrin, 200rpm, addition of 40mM thiourea, 50mM acetic acid buffer, CGTase 1,200 unit/㎖ (for 48hrs) and with glucoamylase 15units/㎖ (for 24hrs). Under the above condition, 75.03mM of AA-2G, 10.57% of conversion yield based on AA was produced (reaction volume 3,000㎖). The production of AA-2G using ultrafiltrantion membrane reactor for the reuse of CGTase, a method that can reduce the production cost, was performed over 11 cycles at 37℃ in reaction volume 20㎖ for 22 days including reaction and filtration. In the 11 times' repetitive reaction, the yield of AA-2G synthesis was 74% of the initial yield. With the addition of 20% fresh CGTase in every reaction, the yield of AA-2G production was maintained more than 14%. AA-2G from reaction mixture was purified by Amberlite IRA-900 column chromatography and purified material was identified as AA-2G by HPLC.