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Inositol and its deviates were important in signal transduction, stress response, cell wall biogenesis and other pathways in a broad spectrum of species including animals, plants, fungi and bacteria. Now the cost of inositol production is very high. To efficient produce the inositol from abundantly available sugars, in current study, for the first time, inositol was synthesized in one-pot reaction using four hyperthermostable enzymes system from the maltoheptaose and cheap material dextrin. The four enzymes are all involved in the inositol pathway in bacteria including α-glucan phosphorylase (TM1168), inositol monophosphatase (TM1415), Inositol 1-phosphate synthase (TM1419) from hyperthermophilic bacterium *Thermotoga maritima*, phsophoglucomutase/phosphomanomutase (PGM/PMM TK1108) from *Thermococcus kodakaraensis*, all these 4 enzymes were over expressed in *Escherichia coli*. And the recombinant protein was purified to homogeneity by Ni-NTA column.

To establish the reaction system and get the optimal condition of inositol synthesis, heat stable inositol 1-phosphate synthase (IPS, TM1419) was subjected to be characterized. In a series of assays, recombinant IPS exhibited maximal activity of at pH 8, 90℃. It was quite stable after exposure to 60℃ for 1 day. Under the optimal conditions, The K_m and k_{cat} values of TM1419 were 1.76 mM and 0.042 s^{-1}, respectively. The catalytic efficiency, k_{cat}/K_m, was 0.024 $s^{-1}mM^{-1}$, similar to that of other IPS which was among 0.12 to 0.233 $s^{-1}mM^{-1}$. Mg^{2+} and Mn^{2+} enhanced the activity of TM1419 significantly, indicating it belongs to class II aldolase enzymes. The reaction system was also optimized by response surface methodology with 78mM maltoheptaose. The TK1108,TM1415 and TM1419 were turned out to be the 3main factors in this system by one order selection- the first order fractional factorial design 2^{6-2}. Central composite design (CCD) with 3 independent variables, TK1108 (x_1) and TM1419 (x_2), TM1415 (x_3) was applied to obtain the maximal inositol production. After CCD optimization, the predicted maximum inositol yield was 167 mmol/L with corresponding TK1108 0.373 mg/ml and TM1419 0.38 mg/ml. TM1415 0.175 mg/ml. The optimal one pot enzymatic reaction for synthesizing inositol predicted by the model (per milliliter) was: 50 mM PBS (pH 8.0) TK1108 0.373 mg/ml, TM1419 0.81 mg/ml, TM1415 0.172 mg/ml, TM1168 0.84 mg/ml, NAD^+ 0.1 mM, G 1,6P 0.5 μg/ml, Mg^{2+} 20 mM. The 155 mM inositol was obtained in the optimal condition, at the same level as predicted production. Also 115.2 mM inositol was obtained under this optimal system from 165 mg/ml cheap material Dextrin within in 8h.