

MS1-P1 Cultivation at 6-10 is Effective to Overcome the Insolubility of Recombinant Proteins. Young Jun An, Min-Kyu Kim, SangMin Lee, Chang-Sook Jeong and Sun-Shin Cha. *Marine Biotechnology Research Center, Korea Ocean Research & Development Institute, Ansan 426-744, Republic of Korea*
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Protein expression in *Escherichia coli* at 15–25 °C is widely used to increase the solubility of recombinant proteins. However, many recombinant proteins are insolubly expressed even at those low temperatures. Here, we show that recombinant proteins can be expressed as soluble forms by simply lowering temperature to 6–10 °C without cold adapted chaperon systems. By using *E. coli* Rosetta-gami2 (DE3), we obtained 1.8 and 0.9 mg of *Cryptopygus antarcticus* mannanase (CaMan) and cellulase (CaCel) from 1 l culture grown at 6 and 10 °C, respectively. Cultivation at 10 °C also led to successful expression of EM3L7 (a lipase isolated from a metagenomic library) in a soluble form in *E. coli* BL21 (DE3). Consequently, *E. coli* cultivation at 6–10 °C is an effective strategy for overcoming a major hurdle of the inclusion body formation. Mannanase (CaMan) and cellulase (CaCel) from *Cryptopygus antarcticus* was purified and crystallized at 295 K. A 2.6 Å resolution data set of mannanase crystal has been collected using synchrotron radiation. The crystals belong to space group $P2_12_12_1$ with unit-cell parameters $a = 74.02$, $b = 82.45$, $c = 164.32$ Å. A 2.6 Å resolution data set of cellulase crystal has been collected using synchrotron radiation. The crystals belong to space group $P3_12_1$ with unit-cell parameters $a = 81.71$, $b = 81.71$, $c = 89.35$ Å, $\alpha = 90$, $\beta = 90$, $\gamma = 120$.

[1] Song JM, An YJ, Kang MH, Lee YH, Cha SS. (2012). Protein Expr Purif. 82(2):297-301

Keywords: Low temperature, Crystal Structure, Mannanase, Cellulase

MS1-P2 Towards the Co-crystallization of Dihydroorotate dehydrogenase in *Plasmodium falciparum* and *Plasmodium vivax* with novel inhibitors Rhawnie Caing-Carlsson^a, Leah Kago^a, Colin Fishwick^b, Peter Johnson^b and Rosmarie Friemann^a ^aUniversity of Gothenburg, Sweden, ^bSchool of Chemistry, University of Leeds, England
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As the *Plasmodium* species continue to acquire resistance to the mainstay anti-malarial agents, the pressure in discovering new drugs for novel anti-malarial chemotherapies is raised. Malaria remains to be a major global burden causing high rates of morbidity and mortality. *Plasmodium falciparum* Dihydroorotate dehydrogenase (DHODH), a flavin enzyme that catalyzes the fourth and rate limiting step in *de novo* biosynthetic pathway, is identified as a “druggable” target. Sharing a high homology with *Plasmodium vivax* DHODH, an attempt to crystallize and solve the three dimensional structure bound to novel inhibitors was made. Using ligation-independent cloning, the N-terminally truncated cDNA was cloned to *Escherichia coli* expression vector. Subsequent loop deletion was carried out. Following large scale protein expression *Plasmodium falciparum* and *Plasmodium vivax* DHODH were subjected to Immobilized metal ion affinity chromatography (IMAC) and size exclusion. Viable crystals yielded after crystallization through both hanging and sitting drop vapor diffusion methods. The *Plasmodium falciparum* DHODH crystals diffracted to 6.5 Å.

[1] Heikkila, T., Thrumalairajan S., Davies, M., Johnson, A.P., McConkey, G., and Fishwick, C.W.G., (2005) *Bioorg. Med. Chem. Lett.*

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