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Novel Construction of Secretion Factor for Human Protein in Yeast Expression System

Eun-Ju Do[1], Se-Lyun Yoon[1], Ji-Sun Lee[1] and Sun-Hee Leem*

[1]*Laboratory of Functional Genomics, Department of Biological Science, Dong-A University, Busan, 604-714, Korea*

To make a construction of yeast strain for the secretory production of most of human pharmaceutical proteins, we analyzed the secretion factors to elevate the proper protein folding. Prokaryotic expression system can grow faster than eukaryote as well as can produce several human therapeutic proteins but they cannot make active form. The yeast expression pathway such as post-translational modifications is a similar to in higher eukaryote of protein and yeast is a eukaryotic microorganism. This study is found optimal conditions will be designed using a combination of useful factors for the secretion from yeast for improvement of secretion capacity. Transcriptome of strains with control cells (empty vector) and over-expressed cells for human protein G-CSF (Granulocyte-colony stimulating factor) was analyzed by using DNA chip. The selected 30 candidates up-expressed) were tested for protein secretion using a secretory selectable marker (lipase) on a lipid plate and RT-PCR for validating genes. Selected secretion factors are functionally characterized of their impacts on the secretion of several human proteins and might be used to study the functional geneomics of yeast *S. cerevisiae*.

Key words: Secretion pathway, post-translation modification, DNA chip, RT-PCR, G-CSF

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Isolation, Purification of Thrombin Inhibitior from *Pinus densiflora*

Min-Jeong Seo[1], Tae-Yeon Kim[2], Hye-Rin Lee[2], Hye-Hyeon Lee[2], Hak-Seob Lim[1], Kang Byoung Won[5], Woo-Hong Joo[3], Hye Jung Choi[4] and Yong-Kee Jeong[1,2]*

[1]*Bioinstitute, MILLENNIUM PROMISE CO.,LTD., Gijang-gun, Busan 619-961, Korea,*
[2]*Department of Biotechnology, Dong-A University, Busan 604-714, Korea,*
[3]*Department of Biology, Changwon National University, Changwon City, 641-773 Korea*
[4]*Interdisciplinary Program in Biotechnology, Changwon National University, Changwon City, 641-773 Korea*
[5]*BK21 Center for Silver Bioindustrialization, Dong-A, University, Busan, Korea*

The importance of thrombosis in cardiovascular disorders pushed the researcher to research for better antithrombolytic agents. In this study, the thrombin inhibition activity of dried needle extract of *P. densiflora* was investigated for developing blood circulation agent. The *P. densiflora* was extracted with absolute EtOH at 80℃, partitioned into BuOH, MeOH, n-hexane and H_2O to isolate the thrombin inhibitory assay. The MeOH fraction showed th most higher acticity than after fractions. The aqueous MeOH fraction was subjected to RP-MPLC, and then elute to afford 14 fraction. Fraction 4 was further separated by RP-HPLC, isolated one compound. The thrombin inhibition activity was increased concentration dependently. The compound inhibited fibrin polymerization by the interaction of thrombin and fibrinogen.

Key words: *Pinus densiflora,* thrombin, thrombosis