

High-Level Expression of Recombinant Protein from Rice Cell Suspension Culture with Minimal Protease Production and Activation Using RNA interference

Nan-Sun KIM, Ok-Hyun KIM, Yun-Ji SHIN¹, Tae-Ho KWON¹ and Moon-Sik YANG*

Division of Biological Sciences, Chonbuk National University, Jeonju 561-756, Korea. ¹Jeonju Bio-materials Institute, Jeonju 561-360, Korea.

TEL: +82-63-270-3569, Fax: +82-63-270-4334

Abstract

Recombinant protein production in plant cell suspension culture is limited by secreted proteases with high level. Two-dimensional (2-D) electrophoresis and spot-identification methodology were used to identify secreted proteases during rice cell suspension culture. The internal amino acid sequence of five spots correspond to cysteine endopeptidase, encoded by Rep1. This result indicates that cysteine endopeptidase is major secreted protease during rice cell suspension culture. RNA interference (RNAi) was utilized to examine the effects of suppressed expression of cysteine endopeptidase in rice cell suspension culture. Analysis of secreted protease activity in the supernatant of transgenic rice cell suspension culture showed lower activity than that of wild type. Incubated rhGM-CSF in transgenic rice cell culture soup was stable constantly for 3 hours, but rhGM-CSF in wild type soup was unstable and rapidly degraded after 15 minutes by ELISA. This study demonstrated that the expression of cysteine endopeptidase, major secreted protease in rice cell suspension culture was suppressed by siRNA, and degradation of rhGM-CSF was protected from protease attacks.

Reference

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