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Human Argonaute, Dicer and TRBP protein expression in *Pichia pastoris*: An attempt to reconstitute human RNA interference

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Background: The RNA interference (RNAi) involves in target specific regulation of gene expression via degradation of mRNA during transcription. This phenomenon opened the gates for the development of therapeutics by silencing disease genes by administration of siRNA which leads to sequence specific degradation of the target mRNA. Designing of such siRNA requires very little effort as adequate online resources to generate siRNA are available. However, testing the therapeutics for RNAi in a laboratory is a major challenge as maintaining cell cultures have several limitations including need for specialized expertise, infrastructure, equipment and other expensive facilities. In such instances, *Pichia pastoris* can be used as a model organism to reconstitute RNAi pathway by introducing the RNA Induced Silencing Complex (RISC) genes; human Argonaute, Dicer and TRBP.
Objective: Expression of RISC genes in *Pichia pastoris* (*P. pastoris*).
Method: *P. pastoris* GS115 strain was transformed with RISC genes which are considered to be essential in the RNAi pathway. Since all the genes were cloned using gateway vectors under the control of GAL1 promoter, galactose induction was carried out to express the genes. Total RNA of the transgenic *P.pastoris* strain was isolated using hot phenol method. Reverse-Transcription PCR (RT-PCR) was carried out using gene specific primers. The cell lysate was also prepared and dilution series of the lysate were analyzed by dot blot assay.
Results: RT-PCR confirms the transcription of RISC genes when the yeast strain was induced in galactose. Production of RISC proteins in the transgenic *Pichia* was also successful according to the antibody-based detection in dot blot assay.
Conclusion: The genes involved in human RNAi pathway can be successfully expressed in *P. pastoris* which can be used in prospective RNAi analysis.

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