Galactosidase Gene Expression in Yeast Cells Enhanced by Nanoparticles

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ABSTRACT

Nanoparticels Au and Ag, and their nanocomposites, silica@Au and silica@Ag were prepared and used to enhance gene expression. The rice α-galactosidase gene was cloned into the yeast chromosome, where the cloned cells were pre-cultured and induced into a medium containing each of the testing nanomaterials. The enzyme activity was determined by a synthetic substrate, p-nitrophenyl-α-D-galctopyranoside, and the yellow product yield was recorded in a spectrophotometer at 400 nm. When Au and Ag nanoparticles were added to the culture, a 3-5 fold enhancement in α-galactosidase was observed for intracellular activity as well as the secreted activity into the medium. The secreted protein was analyzed to have a pure form and displayed as a single protein band in the SDS-gel electrophoresis.

Keywords: **nanomaterials, gene expression, galactosidase, yeast cells, secreted proteins**

1. INTRODUCTION

Metal oxide nanoparticles and nanocomposites, i.e., TiO_2 and TiO_2@Au have been employed in antimicrobial coatings.[1] Silver materials have been medically proven to kill many disease-causing organisms in human body, such as *Streptococcus sanguis and Escherichia coli*.[2] The recent advancement in nanobiotechnology research is expected to have a major impact leading to new drug delivery and gene therapy systems, and diagnostic, detection, and separation tools for biomedical applications. The use of gold nanoparticle-oligonucleotide complexes as the intracellular gene regulation agents for controlling protein expression in cells has been reported.[3] Moreover, the application of organically modified silica nanoparticles as a non-viral vector for efficient *in vivo* gene delivery has been communicated.[4]

The rice α-galactosidase gene was isolated from the stem portion of taro.[5] In this paper, we report the effect of nanomaterials (Au, Ag, silica@Au and silica@Ag) on gene expression while rice α-galactosidase gene is cloned into the yeast cells.

2. EXPERIMENTAL

2.1 Materials

Rice α-galactosidase gene was cloned from cDNA library. The yeast strain, *Pichia pastoris* SMD1168 and pPIC-9k plasmid were obtained from Invitrogen (Carlsbad, CA). Silica colloidal particles were processed following the method of Stöber et al.[6] The aqueous colloidal solution of Au, and Ag, individually was prepared following a chemical reduction method employed by Turkevich et al.[7] The core-shell nanomaterials, silica@Au and silica@Ag were prepared by the procedure described previously.[8] The size, shape, and distribution of the synthesized nanomaterials were characterized by a field emission SEM (LEO Gemini 1530) and a UV-Vis spectrophotometer

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(Hitachi U-2000).

2.2 Gene cloning and Gene expression in yeast cells

Rice α-galactosidase gene was cloned in pPIC-9k plasmid (Invitrogen, Carlsbad, CA), and transformed into SMD 1168 yeast strain chromosomal DNA by electroporation according to the Invitrogen protocol described in EasySelect (www.Invitrogen.com) and given by Higgins et al.[9] The gene expression is done also by following the Invitrogen EasySelect procedure. The cell mass was measured spectrophotometrically by recording its optical density at 600 nm (OD600).

2.3 Determination of enzyme activity and Enzyme assay

After each period of induction in the gene expression (24 hr, 48 hr, and up to 8 days), the enzyme activity was determined by a synthetic *p*NP-substrate, p-nitrophenyl-α-D-galactopyranoside. The released *p*-nitrophenol, *p*NP, representing the secreted activity was read in a spectrophotometer at 400 nm.[5] One unit of enzyme activity is defined as the amount of enzyme that can produce 1 μmol of *p*NP/min at 37 ^{o}C. The secreted proteins were analyzed by SDS-Polyacrylamide Gel Electrophoresis (PAGE).[10]

3. RESULTS AND DISCUSSION

3.1 Size and Size distribution of nanomaterials

The particle size and size distribution of nanomaterials synthesized and used for gene expression enhancement are shown in Figure 1. In image 1b, the SiO_2 particles processed by sol-gel method show a relatively uniform particle size distribution of ~ 100 nm in diameter. In image 1a, the size of Au nanoparticles is not very uniform but they are all in the ranges between 20 nm and 50 nm. The size of core-shell nanocomposite of SiO_2 and Au, i.e., silica@Au is quite similar to that of silica-@Ag (not shown), and is observed to be ~ 150 nm as shown in image 1c with a fairly uniform distribution. The sizes of Ag particles (not shown) are also not very uniform ranging from 20 nm to 50 nm, and they tend to form some larger agglomerates. These agglomerates seem to disappear when Ag nanoparticles form core-shell structure with SiO_2 particles. It is important to mention that the size and size distribution of nanomaterials are critically important for gene delivery and gene expression in cells.

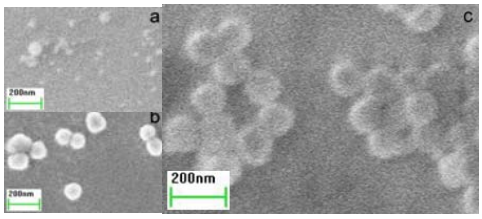

Fig. 1. SEM images of the synthesized nanomaterials

3.2 α-galactosidase activity in cells assisted by nanomaterials

The size and surface area are crucial factors in delivery and expression of genes. Xu et al. have shown[11] that Ag nanoparticles with sizes ranging up to 80 nm can effectively accumulated in living microbial cells. They have demonstrated that these Ag nanoparticles with size of 80 nm or smaller can transport through the inner or outer membrane of the *P. aeruginosa cells.*

In this study, the gene expression experiments in yeast (SMD1168/α-Gal) are carried out by adding nanomaterials (30% of the total volume of the medium) to 2 mL of the cell cultures for every 24 hrs induction. Figure 2 shows the enhanced gene expression, α-galactosidase activity, in yeast cells by adding Au (20 nm - 50 nm) and silica@Au

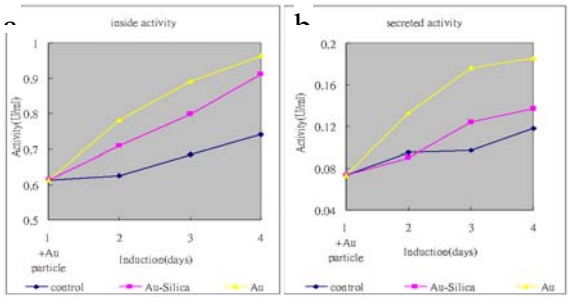

Fig. 2. Gene expression enhanced by Au nanomaterials

(~ 150 nm) nanomaterials. The intracellular and secreted enzyme activities resulting from day 1 to day 4 inductions are plotted in Figure 2a and 2b, respectively. The α-galactosidase activity is plotted in yellow line (- ▲ -), pink line (- ▪ -), and blue line (- ♦ -), separately for the addition of Au, silica@Au and the control samples. It shows that both Au and silica@Au nanomaterials are not toxic to yeast cells, rather they act to increase the transfection activity. The enhancement of gene expression is higher for Au nanoparticle than silica@Au nanocomposite. From Figure 2, the intracellular α-galactosidase activity is shown to be about five-seven times higher than the secreted (extracellular) activity in the medium.

Figure 3 shows the intracellualr (a) and secreated (b) α-galactosidase activity assisted by adding different amount of silica@Ag, 450 μL (yellow line, - ▲ -), 225 μL (pink line, - ▪ -), and control (blue line, - ♦ -). The activity is labeled in unit/mL, where one unit of enzyme activity

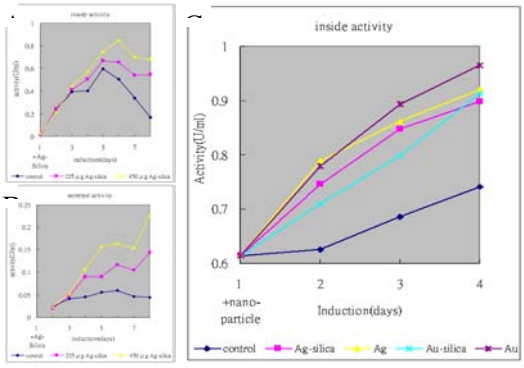

Fig. 3. Gene expression enhanced by different dose (A) and (B), and different type (C) of nanomaterials.

was defined as the amount of enzyme that can release 1 μmol of *p*NP/min at 37 °C. For intracellular activity, the activity at day 5 induction was measured to be 0.595, 0.66, and 0.74 unit/mL for the control sample, 225 μL added silica@Ag sample, and 450 μL added silica@Ag sample, respectively. The results indicate that cells incubated with silica@Ag enhance the intracellular activity; with a double dose of silica@Ag gives a double enzyme activity. At the day 8 induction, the intracellular activity was changed to 0.18, 0.55, and 0.68 unit/mL for the control sample, 225 μL added silica@Ag sample, and 450 μL added silica@Ag sample, respectively. This corresponds to a 206% and 278% enhancement in activity for the 225 μL and 450 μL added silica@Ag samples, respectively to the control sample. It is noted that the amount of enzyme activity in unit/mL is about four times smaller in the secreted medium (Figure 3b) as compared to that inside the yeast cells (Figure 3a). However, the percent enhancement of extracellular activity assisted by silica@Ag is much larger than that shown in the intracellular activity. At the day 8 induction, a 233% and 423% enhancement in extracellular activity was observed for the 225 μL and 450 μL added silica@Ag samples, respectively to the control sample. Again, a double enzyme activity has been expressed when a double dose of silica@Ag was given to the yeast cells.

Figure 3c compares the intracellular enzyme activity assisted by the same amount of nanomaterials, Au (purple line, - ∗ -), Ag (yellow line, - ▲ -), silica@Au (light blue, - x -), silica@Ag (pink line, - ▪ -), and control (blue line, - ♦ -). The Au and Ag nanoparticles (with particle size of ~ 50 nm or smaller) give a higher enhancement in enzyme activity than the corresponding core-shell silica@Au and silica@Ag nanocomposites (with a particle size of ~ 150 nm) that presumably are due to the different in their particle sizes. The results indicate that the highest enhancement in intracellular enzyme activity goes to Au nanoparticles assisted gene expression, following by Ag nanoparticles, silica@Au nanocomposites, and then silica@Ag nanocomposites. When Au and Ag nanoparticles were added to and incubated in the yeast culture, a 3-5 fold enhancement in α-galactosidase activity was observed for the intracellular activity as well as in the sectreted medium as compared to that of the control group. The secreted protein in the medium was shown to have a pure form and displayed as a single protein band at 40 kDa[12] in the SDS-gel electrophoresis.

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4. CONCLUSION

We have cloned rice α-galactosidase gene into yeast chromosome cells. Using different nanomaterials, the gene expression of α-galactosidase activity as the parameter for monitoring the effectiveness of particle's catalytic activity has been investigated. Au and Ag nanoparticles have shown to enhance gene expression in yeast cells with the cloned rice α-galactosidase gene. Under our experimental conditions, Ag, Au, silica@Ag, and silica@Au nanomaterials are not toxic, and do not show to kill the cells. On the contrary, they act to enhance gene expression inside the yeast cells and also secrete into the cultural medium. It is suggested that SMD 1168–α-Gal yeast cells may be a choice of model system for studying other non-viral transfer of gene or drug into cells assisted by nanomaterials.

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