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Short Communication

Optimal Production Conditions of *Streptomyces griseus* Trypsin (SGT) in *Streptomyces lividans*

Bon-Joon Koo[†], Joung Mee Kim, Si-Myong Byun[‡] and Soon-Kwang Hong*

Department of Biological Science, Myongji University, San 38-2, Nam-dong, Yongin-city, Kyunggi-do, Korea

[†]R&D Center, TS Corporation, 6-14 1Ka, Buksung-dong, Chung-ku, Inchon 400-201, Korea

[‡]Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), and Research Center for New Bio-Materials in Agriculture (RCNBMA), 373-1, Kusong-dong, Yusong-Gu, Taejon 305-701, Korea

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The *sprT* gene encoding *Streptomyces griseus* trypsin (SGT) was introduced into *Streptomyces lividans* TK24 and *Streptomyces lividans* 1326 to study which strain would be better to overexpress the extracellular proteinase. Various media with different compositions were also used to maximize the productivity of SGT in heterologous hosts. The SGT productivity was best when the transformants of *S. lividans* TK24 and 1326 were cultivated in R2YE medium, and their relative trypsin activity of the culture broth measured with an artificial chromogenic substrate, N-α-benzoyl-DL-arginine-ρ-nitroanilide, were 382 units/ml and 221 units/ml, respectively. They produced high levels of SGT in GYE medium but relatively lower than those in R2YE medium, and negligible amount of SGT was produced in Ferm, RASF, LIVID, and NDSK media. Considering non-SGT associated activity in Pronase powder, it was estimated that the transformant of *S. lividans* TK24 can produce SGT in R2YE 3.5 times more than the amount by *S. griseus* 10137 from which the *sprT* gene had been originated. The growth of *S. lividans* reached the maximum level of cell mass at 5 d of culture, but SGT production started in the stationary phase of cell growth and kept increasing until the ninth day of culture in R2YE medium, but in GYE media the productivity reached at the maximum level at 7 d of cultivation.

Keywords: Optimization, *Streptomyces griseus* trypsin, *Streptomyces lividans*.

* To whom correspondence should be addressed.
Tel: 0335-330-6198; Fax: 0335-335-8249
E-mail: skhong@bioserver.myongji.ac.kr

Introduction

Proteinases of commercial importance are produced from microbial, animal, and plant sources. The proteinases constitute a very large and complex group of enzymes which differ in properties such as substrate specificity, catalytic mechanism, and stability profiles depending on pH, temperature, and ionic strength. Commercial proteinases have application in a range of processes which take advantage of the unique physical and catalytic properties of individual proteolytic enzyme types.

Streptomyces griseus produces a mixture of proteinases which has been sold under the commercial name of Pronase with a variety of industrial use. Pronase has also been widely used for the amino acid sequencing in a mixed state with different proteinases, which gave a limit to broaden its use (Trop *et al.*, 1970). Therefore, there have been many efforts to isolate each component of Pronase, but the low content of each proteinase makes it difficult (Awad *et al.*, 1976; Tsuyuki *et al.*, 1991). For an efficient purification of the Pronase component, we had cloned genes which encode proteinases and studied their overexpression system.

Streptomyces griseus trypsin (SGT) is a bacterial serine proteinase with an active serine sequence, Asp-Ser-Gly, and has more similarity to mammalian trypsin than to *S. griseus* proteinase A and B, two other chymotrypsin-like serine proteinases from the same bacterium (Olfason *et al.*, 1975; Henderson *et al.*, 1987). The general similarity of the substrate binding regions of SGT and bovine trypsin (BT) is consistent with the similarity of their interactions with substrates and inhibitors. SGTs act in a specific manner for the hydrolysis of lysine and arginine residues in the β-chain of oxidized insulin, in the same manner as BT, as well as the synthetic substrate N-α-benzoyl-L-arginine ethyl ester, and is inhibited by $iPr_2P\text{-}F^1$ and by naturally occurring trypsin inhibitors (Trop *et al.*, 1968). The *sprT* gene (Genbank

accession No. M64471) encoding *S. griseus* trypsin has been isolated from a *S. griseus* genomic library (Kim *et al.*, 1991). We constructed an overexpression system of the *sprT* gene using *S. lividans* TK24 as a heterologous host and purified the SGT from the transformant and Pronase, and compared their enzymatic properties to each other (Koo *et al.*, 1998). The SGT with a molecular weight of 23 kDa calculated from its deduced amino acid sequence was confirmed to be the protein band with a molecular weight of 28 kDa in SDS-polyacrylamide gel electrophoresis. Another overexpressed protein with a molecular weight of 42 kDa was thought to be a premature form of the SGT protein. Obviously, overexpression in heterologous host makes it easier to isolate SGT from the cultural filtrate (Koo *et al.*, 1998).

In this report, we studied for the optimal conditions to maximize overexpression efficiency of the *sprT* gene by changing the host in various media. The productivity of SGT by the transformant in different conditions was compared with those by *S. griseus* 10137.

Materials and Methods

Preparation of *S. lividans* transformant harboring the *sprT* gene Two strains of *S. lividans* TK24 (str-6) and *S. lividans* 1326 (SLP2 and SLP3) were used as hosts for *sprT* overexpression because they have low levels of milk protein-hydrolyzing proteolytic activity (Lichenstein *et al.*, 1988; Bender *et al.*, 1990). *S. lividans* 1326 and TK24 were obtained from the John Innes Institute, United Kingdom (Hopwood *et al.*, 1985). *S. griseus* 10137, from which the *sprT* gene had been cloned, was obtained from the American Type Culture Collection. *Streptomyces* strains were maintained on R2YE agar, and *S. lividans* TK24 and 1326 grown in R2YE liquid broth at 28°C were used for the preparation of protoplasts and the isolation of plasmid DNAs (Hoopwood *et al.*, 1985).

Protoplasts of *S. lividans* 1326 and TK24 were prepared as described by Sohng *et al.* (1996). Cells were grown in 100 ml of R2YE containing 0.5% glycine in a 500 ml baffled flask and incubated for 18 to 24 h. The obtained cells were treated with 20 ml of P buffer containing 80 mg of lysozyme, and the resulting protoplasts were suspended in P buffer at a concentration of ~10^9/ml and frozen at −70°C. P buffer had a pH of 7.2 and contained 103 g/l sucrose, 0.25 g/l K_2SO_4, 2.02 g/l $MgCl_2.6H_2O$, 0.05 g/l KH_2PO_4, 3.68 g/l $CaCl_2.12H_2O$, 5.73 g/l TES, 0.2%(V/V) of trace element (Okanishi *et al.*, 1974). *Streptomyces* protoplasts were transformed as described by Sohng *et al.* (1996) by using 100 μl of protoplasts (~10^8), 1 μg of plasmid DNA in 20 μl of TE buffer, and 500 μl of 25% PEG1000 in P buffer. Samples (100 μl) were plated in 2.5 ml of 0.6% soft R2YE agar on R2YE regeneration plates. After incubation at 28°C for 18 to 24 h, the plates were overlaid with 2.5 ml of 0.6% soft R2YE agar containing 25 μg/ml of thiostrepton. Transformants were visible after incubation for an additional 3 to 5 d at 28°C.

The plasmid pWHM-T, which is an *E. coli-Streptomyces* shuttle vector containing the entire *sprT* gene, was constructed as previously described (Koo *et al.*, 1998) and their detailed map is shown in Fig. 1.

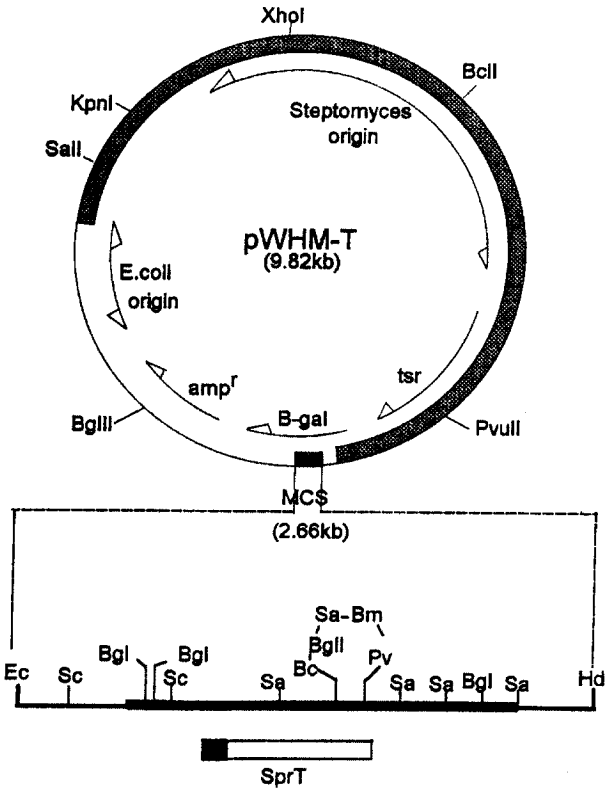

Fig. 1. Restriction map of expression vector pWHM-T containing an insert of the entire *sprT* gene at *Eco*RI and *Hin*dIII sites. The thick line indicates the *sprT* coding region. The organization of the structural gene, with pre-pro peptide (■) and mature proteinase (223 amino acids, □), is shown below the map. Abbreviations: Bm, *Bam*HI; Bgl, *Bgl*I; BglI, *Bgl*II; Ec, *Eco*RI; Hd, *Hin*dIII; Kp, *Kpn*I; Pv, *Pvu*II; Sa, *Sal*I; Sc, *Sac*I; Sm, *Sma*I.

Cultivation of cells and quantitation of SGT The transformant of *S. lividans* TK24 and 1326 harboring the pWHM-T recombinant plasmid were grown in 100 ml of R2YE media in 500-ml baffled flasks at 28°C with vigorous shaking at 250 rpm. After 2 d cultivation, 10 ml of cultural broth was used for inoculation into 100 ml of various liquid media listed in Table 1 in 500 ml baffled flasks at the same condition. Five ml of culture broth were sampled everyday and centrifuged at 5000 rpm for 10 min. The supernatant was used for the measurement of protein concentration and proteinase activity, and the cell pellet was used for the quantitation of protein after cell disruption by sonication.

Trypsin activity was measured spectrophotometrically by the release of *p*-nitroaniline due to the enzymatic hydrolysis of an artificial chromogenic substrate, N-α-benzoyl-DL-arginine-*p*-nitroanilide. The assay reaction mixture, which was composed of 890 μl of reaction buffer [50 mM Tris·HCl (pH 8.0), 20 mM $CaCl_2$] and 10 μl of 50 mM N-α-benzoyl-DL-arginine-*p*-nitroanilide was prewarmed for 5 min at 37°C, rapidly mixed with 100 μl of enzyme solution, and incubated for 15 min. The reaction was stopped by adding 400 μl of 30% acetic acid in dioxane and the absorbance at 405 nm was recorded. One unit of trypsin was defined as the amount of enzyme corresponding to 0.1 increase in absorbance at the above conditions. Protein concentrations were measured by the method of Bradford (1976).

Table 1. Proteinase production media used in this study.

Medium	Ingredient	Amount (g/l)
NDSK (in this study)	$NaNO_3$	4.28
	$K_2HPO_4 \cdot 3H_2O$	0.23
	HEPES	4.77
	$MgSO_4 \cdot 7H_2O$	0.12
	trace element	2 ml
	skim milk	10
	maltose	45.04
Livid (Jayant *et al.*, 1993)	MOPS	1.49
	Nutrient broth	1.0
	$CaCl_2 \cdot 2H_2O$	0.735
	KH_2PO_4	0.5
	K_2HPO_4	0.5
	fructose	0.25
	skim milk	20
	pH 7.2	
Ferm (Kim *et al.*, 1991)	glucose	20
	skim milk	10
	KH_2PO_4	1.0
	K_2HPO_4	3.4
	$MgSO_4 \cdot 7H_2O$	0.3
	$FeSO_4 \cdot 7H_2O$	0.01
	$ZnCl_2$	0.1
	$CuSO_4 \cdot 7H_2O$	0.01
	$MnCl_2 \cdot 4H_2O$	0.003
	$CaCl_2$	0.01
	NaCl	0.03
	pH 7.0	
RSAF (Daza *et al.*, 1990)	K_2SO_4	0.25
	$MgCl_2 \cdot 6H_2O$	10.12
	casamono acid	0.1
	yeast extract	5.0
	TES	5.73
	$CaCl_2$	2.9
	proline	3.0
	trace element	200 μl
	pH 7.0	
GYE (Gregory *et al.*, 1989)	glucose	12.5
	yeast extract	5.0
	$CaCO_3$	8.0
	NaCl	5.3
	pH 7.2	
R2YE (Hopwood *et al.*, 1985)	sucrose	103
	K_2SO_4	0.25
	$MgCl_2 \cdot 6H_2O$	10.12
	glucose	10
	casamino acid	0.1
	yeast extract	5
	K_2HPO_4 (0.5%)	10 ml
	$CaCl_2 \cdot 2H_2O$ (3.68%)	80 ml
	L-proline (20%)	15 ml
	TES (5.73%, pH 7.2)	100 ml
	trace element	2 ml

Results and Discussion

Production of SGT in various media Two *S. lividans* strains harboring pWHM-T and *S. griseus* 10137 were cultured in various liquid media to study the best compositions of media for the SGT overproduction. In *S. griseus* 10137, the enzymatic activity of the culture broth was 268 units/ml and 232 units/ml in R2YE and NDSK media, respectively (Fig. 2A). In Ferm, RASF, Livid, and GYE media, the level of SGT activity was relatively low (Fig. 2A). The SGT activity produced by *S. lividans* 1326 transformant with pWHM-T reached to 221 units/ml and 206 units/ml of culture broth in R2YE and GYE media, respectively (Fig. 2B). On the other hand, the *S. lividans* TK24 harboring pWHM-T produced SGT at 382 units/ml and 283 units/ml of culture broth (Fig. 2C). Both strains of *S. lividans* produced very low levels of SGT in Ferm, RASF, Livid, and NDSK media. All three strains showed highest level of SGT activity in R2YE and the *S. lividans* TK24 transformant produced 1.4 times more than the amount produced by *S. griseus* 10137. In the previous paper, we reported that more than 60% of the total trypsin activity in Pronase powder should be non-SGT associated activity (Koo *et al.*, 1998). Therefore, the real content of SGT in the culture broth of *S. griseus* 10137 will be lower than the amount estimated from the BAPNA assay. From this fact, it was expected that the *S. lividans* TK24 transformant may produce 3.5 times more SGT than the amount produced by *S. griseus* 10137 in R2YE media.

Comparison of SGT productivity and cellular growth Because the SGT productivity of tested strains were much better in R2YE and GYE media than those in other media, the productivity of SGT in R2YE and GYE by different strains were summarized in Fig. 3. *S. lividans* 1326 and *S. lividans* TK24 harboring pWHM3 vector, which were used as controls, showed negligible amounts of SGT activity, i.e, less than 10 units/ml in both media, which supports the idea that those strains are useful candidates for the host to overexpress foreign proteinases (Tomich, 1988). In R2YE medium, *S. lividans* TK24 showed 1.4 times higher titer of SGT than that of *S. griseus* 10137, but *S. lividans* 1326 showed only $\frac{4}{5}$ of the SGT activity produced by *S. griseus* 10137 (Fig. 3A). In GYE medium, *S. lividans* TK24 and 1326 transformant with pWHM-T produced 4.2 times and 3.0 times more SGT, respectively, than that by *S. griseus* 10137 (Fig. 3B). If we consider non-SGT associated activity by *S. griseus* 10137, both strains of *S. lividans* could overproduce the SGT more than that produced by *S. griseus* 10137 in both media.

Because of many insoluble ingredients in fermentation media, it was difficult to measure the cell growth and the concentration of extracellular proteins exactly, therefore, the SGT activity produced could not be compared in specific activity except in R2YE medium. In R2YE medium, the SGT activity kept increasing during 9 d of cultivation

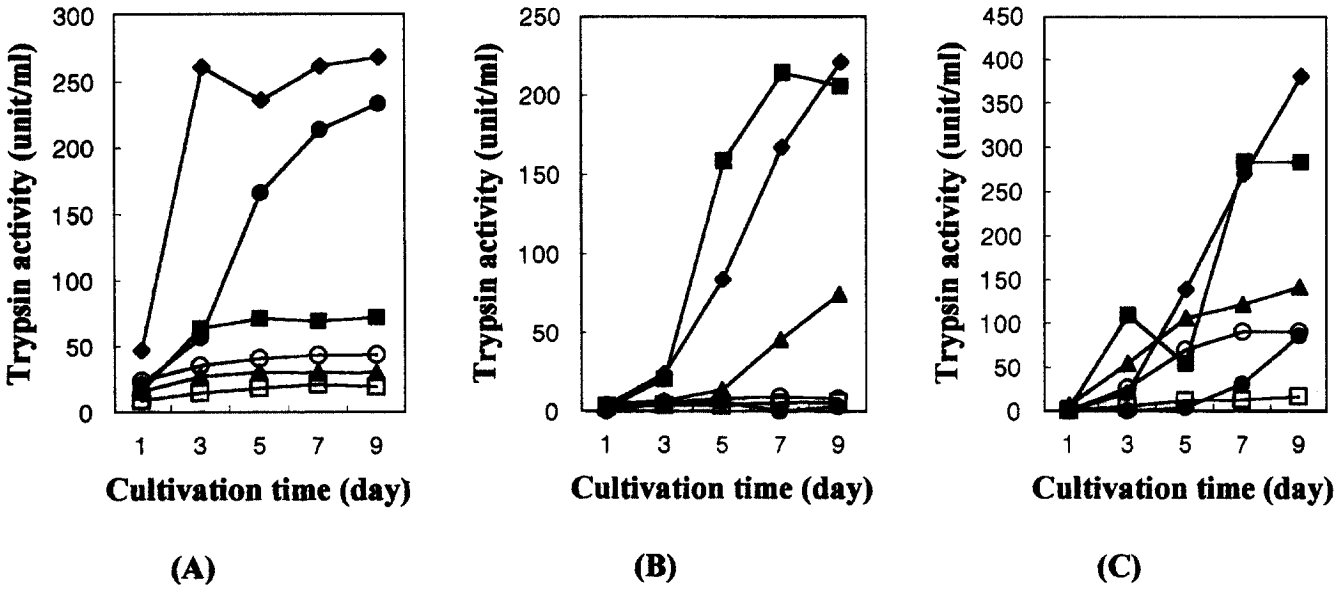

Fig. 2. Comparison of SGT activity produced by *S. griseus* 10137 (A) and the transformant of *S. lividans* 1326 (B) and TK24 (C) cultivated in a variety of media. The amount of enzyme produced was expressed in units/ml of culture filtrate depending on cultivation time because specific activity could not be calculated owing to insoluble ingredients in the production media. ◆−◆, R2YE medium; ■−■, GYE medium; ▲−▲, Ferm medium; ○−○, RSAF medium, □−□, Livid medium; ●−●, NDSK medium.

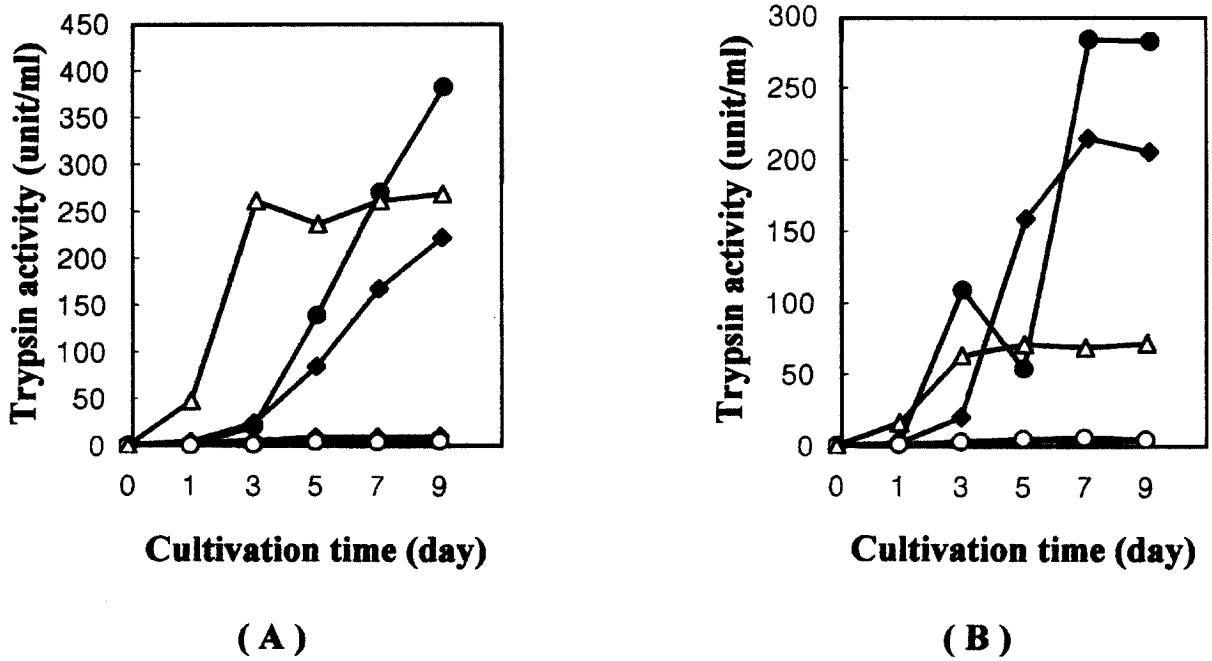

Fig. 3. Comparison of SGT activity produced in R2YE (A) and GYE (B) media by *S. griseus* and *S. lividans* recombinant strains. The amount of enzyme produced was expressed in units/ml of culture filtrate depending on cultivation time because of insoluble ingredients in the production media. □−□, *S. lividans* 1326 with pWHM3 as a control; ■−■, *S. lividans* 1326 with pWHM-T; ○−○, *S. lividans* TK24 with pWHM3, ●−●, *S. lividans* TK24 with pWHM-T; △−△, *S. griseus* 10137.

(Fig. 3A), but in GYE medium the productivity reached at the maximum level at 7 d of culture (Fig. 3B). The relationship between the cell growth and SGT production was studied in R2YE medium. The growth of *S. lividans* reached the maximum cell mass at 5 d of culture, but SGT production started in the stationary phase of cell growth and kept increasing up to the 9th day of cultivation (Fig. 4).

In the previous report, we showed that subcloning of *sprT* into pWHM3 resulted in the overexpression of SGT in *S. lividans* TK24, which made it convenient to purify

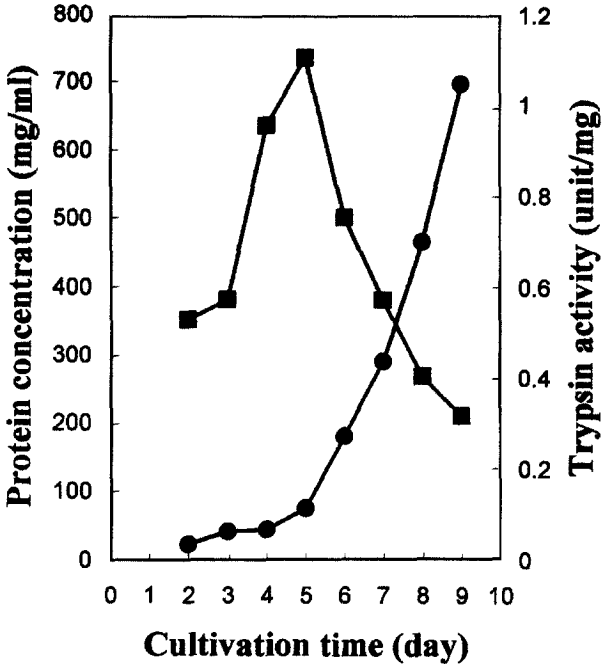

Fig. 4. Relationship between the cell growth and SGT productivity in *S. lividans* 1326 transformant cultivated in R2YE medium. The SGT activity of the cultural filtrate (●–●) was expressed in unit/mg of cellular protein and the concentration of cellular protein (■–■) was measured by the Bradford's method after cell disruption by sonication.

SGT. We tested two strains of *S. lividans* to choose a better strain for use as a host for SGT overexpression and found *S. lividans* TK24 to be more useful to our purpose in various media compositions. The highest productivity (382 units/ml) was obtained when the *S. lividans* TK24 transformant was cultivated in R2YE medium. We had used GYE (C5/L) medium for purification of SGT from the transformant (Koo *et al.*, 1998), but if we use R2YE medium as a production medium, the purification step should be much simpler because they produce 1.34 times more SGT than that in GYE (Fig. 2C). In general the production of protease can be regulated by many factors, especially medium component. Our results showed the use of yeast extract with appropriate carbon source such as sucrose and glucose could significantly improve the productivity. In addition, the presence of free amino acids or trace element and buffering capacity of the medium seem to be important factors to stabilize the productivity. At this time, we do not understand what differences of the host strain can affect production yield. However, selecting a host strain and optimizing culturing conditions of the selected strain are very important factors for the overexpression of the foreign genes, and our data cleary showed that great improvement in the productivity could be obtained by choosing the appropriate host and medium.

Proteinases are known to have many important intracellular functions such as cell differentiation, cell-to-cell recognition, cell growth, virulence, etc. (Park *et al.*, 1997). *Streptomyces* can produce many extracellular proteinases which can be divided as primary and secondary proteinases depending on production time. In general, secondary proteinases were produced after active cell growth had ceased because of nutritional depletion. These kinds of secondary proteinases are thought to have some functional relationship with secondary metabolism or cell morphogenesis which are also occurring after cell growth has stopped (Kim *et al.*, 1996; Moncheva *et al.*, 1997). The profile of SGT production depending on cultivation time implies that it belongs to secondary proteinases. *S. griseus* ATCC 10137 in R2YE medium and *S. lividans* TK24 transformant in GYE medium show typical production patterns of SGT with two peaks that represents primary and secondary proteases (Figs. 2A and 2C). However, most of the SGT activity was detected at the later stage of cell growth, which suggests that SGT is produced mainly as a secondary protease. Recently, we studied the *in vivo* effects of protease inhibitors (Choi *et al.*, 1997) on the morphological differentiation of *Streptomyces*, and found some inhibitors which severely inhibited the formation of aerial mycellia (unpublished data). Therefore, elucidating the biological function of SGT as a secondary proteinase will give us much valuable information to understanding the complex and elaborate system of living organisms.

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References

Awad, W. M. Jr., Brew, K., Powell, J. T., Russin, D. J., Seber, J. F., Siegel, S. and Vosbeck, K. D. (1976) The proteolytic enzymes of the K-1 strain of *Streptomyces griseus* obtained from a commercial preparation (Pronase). XI. Evolutionary Implications; in *Proteolysis and Physiological Regulation*, Ribbons, D. W. and Brew, K. (eds.), New York, pp. 77–91.

Bender, E., Vogel, R., Koller, K. P. and Engels, J. (1990) Synthesis and secretion of hirudin by *Streptomyces lividans*. *Appl. Microbial. Biotechnol.* **34**, 203–207.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.

Choi, J.-S., Shin, J.-S., Choi, H.-S. and Park, J.-S. (1997) Characteristics of the inhibitory action of protease inhibitors on the glucose-6-phosphate transporter. *J. Biochem. Mol. Biol.* (formerly *Korean Biochem. J.*) **30**, 157–161.

Daza, A., Gil, J. A., Vigal, T. and Martin, J. F. (1990) Cloning and characterization of a gene of *Streptomyces griseus* that increases production of extracellular enzymes in several species of *Streptomyces*. *Mol. Gen. Genet.* **222**, 384–392.

Gregory, D. G., Ordaz, D. E. and Strohl, W. R. (1989) Over-expression of extracellular protease activity by *Streptomyces*

C5-A13 in fed-batch fermentation. *Appl. Microbiol. Biotechnol.* **31**, 119–124.

Henderson, G., Krygsman, P., Liu, C. J., Davey, C. C. and Malek, L. T. (1987) Characterization and structure of genes for proteases A and B from *Streptomyces griseus*. *J. Bacteriol.* **169**, 3778–3784.

Hopwood, D. A., Bibb, M. J., Chater, K. F., Kieser, T., Bruton, C. J., Kieser, H. M., Lydiate, D. J., Smith, C. P. and Ward, J. M. (1985) *Genetic Manipulation of Streptomyces: A Laboratory Manual*, The John Innes Foundation, Norwich, England.

Jayant, S. A. and Strohl, W. R. (1993) Purification and properties of an extracellular aminopeptidase from *Streptomyces lividans* 1326. *J. Gen. Microbiol.* **139**, 417–424.

Kim, I. S., Kim, H. T., Lee, H. S. and Lee, K. J. (1991) Protease inhibitor production using *Streptomyces* sp. SMF13. *J. Microbiol. Biotechnol.* **1**, 288–292.

Kim, I. S. and Lee, K. J. (1996) Trypsin-like protease of *Streptomyces exfoliatus* SMF13, a potential agent in mycelial differentiation. *Microbiology* **142**, 1797–1806.

Kim, J. C., Cha, S. H., Jeong, S. T., Oh, S. K. and Byun, S. M. (1991) Molecular cloning and nucleotide sequence of *Streptomyces griseus* trypsin gene. *Biochem. Biophys. Res. Commun.* **181**, 707–713.

Koo, B.-N., Bai, K.-H., Byun, S. M. and Hong, S.-K. (1998) Purification and characterization of *Streptomyces griseus* trypsin overexpressed in *Streptomyces lividans*. *J. Microbiol. Biotechnol.* **8**, 333–340.

Lichenstein, H., Brawner, M. E., Miles, L. M., Meyers, C. A., Young, P. R., Simon, P. L. and Eckhardt, T. (1988) Secretion of interleukin-1 beta and *Escherichia coli* galactokinase by *Streptomyces lividans*. *J. Bacteriol.* **170**, 3924–3929.

Moncheva, P. A., Danova, S. T., Antonova, S. K. and Ivanova. I. V. (1997) Physiological role of extracellular proteases and calcium ions in the processes of differentiation and antibiotic production by *Streptomyces albogriseolus* 444. *Antibiot. Khimioter.* **42**, 14–19.

Okanishi, M., Suzuki, K. and Umezawa, H. (1974) Formation and reversion of streptomycete protoplasts: cultural conditions and morphological study. *J. Gen. Microbiol.* **80**, 389–400.

Olfason, R. W. and Smillie, L. B. (1975) Enzymatic and physicochemical properties of *Streptomyces griseus* trypsin. *Biochemistry* **14**, 1161–1167.

Park, K. W. and Song, C. Y. (1996) Purification and characterization of a serine proteinase from *Acanthamoeba culbertsoni*. *J. Biochem. Mol. Biol.* (formerly *Korean Biochem. J.*) **29**, 455–461.

Sohng, J. K. and Yoo, J.-C. (1996) Cloning, sequencing and expression of dTDP-D-glucose 4,6-dehydratase gene from *Streptomyces antibioticus* Tu99, a producer of chlorothricin. *J. Biochem. Mol. Biol.* (formerly *Korean Biochem. J.*) **29**, 183–191.

Tomich, P. K. (1988) *Streptomyces* cloning: useful recombinant DNA systems and a summation of cloned genes. *Antimicrob. Agents Chemother.* **32**, 1465–1471.

Trop, M. and Birk, Y. (1968) The trypsin-like enzyme from *Streptomyces griseus* (pronase). *J. Biochem.* **109**, 475–476.

Trop, M. and Birk, Y. (1970) The specificity of proteinases from *Streptomyces griseus* (pronase). *J. Biochem.* **116**, 19–25.

Tsuyuki, H., Kajiwara, K., Fujita, A., Kumazaki, T. and Ishii, S. (1991) Purification and characterization of *Streptomyces griseus* metalloendopeptidases I and II. *J. Biochem. (Tokyo)* **110**, 339–344.

Vosbeck, K. D., Greenberg, B. D., Ochoa, M. S., Whitney, P. L. and Awad, W. M. Jr. (1978) Proteolytic enzymes of the K-1 strain of *Streptomyces griseus* obtained from a commercial preparation (Pronase). Effect of pH, metal ions, and amino acids on aminopeptidase activity. *J. Biol. Chem.* **253**, 257–260.