Journal of Life Science 2009 Vol. 19. No. 5. 676~679 ©JLS / ISSN 1225-9918

- Note -

Anti-cancer Activity of Supernatant of *Rahnella aquatilis* AY 2000 Cocultured with *Streptomyces griseus*

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Received February 4, 2009 / Accepted March 16, 2009

In order to produce a new antibiotic material against Jurkat T cells using horizontal gene transfer among microbes, co-cultures between soil bacteria AY2000 and the multiple antibiotic producer *S. griseus* was carried out. It showed that the highest active substance against Jurkat T cells was produced at 48 hr of co-culture time with MTT assay. Moreover, a morphological change of nuclear of Jurkat T cells treated with co-cultured substance was observed in DAPI staining. This result suggests that a new material was produced with co-culture supernatant, and that co-culture between microboes can develop new antibiotic materials.

Key words : Coculture, horizontal gene transfer, AY2000, *S. griseus*, antibiotic material

Introduction

Rahnella aquatilis strain AY2000 was isolated from soil samples near plant roots for the production of anti-yeast substance against *Candida albicans* [11]. *Rahnella* families are Gram negative rod bacteria and worldwidly distributed in nature. Generally, *Rahnella* strains exist in soil near plant roots [14] or water [8]. So far, this strain has been only evaluated for the production of antibiotic activity against *C. albicans*.

Antibiotic production by horizontal gene transfer has not been well known, although there is indirect evidence that is occurs [2,3,5,16]. Recently, a new methodology was intruduced via coculture between a strain of *Rhodococcus fascians* that does not produce an antibiotic and a strain of *S. padanus* that is a highly stable actinomycin producer [7]. The *Rhodococcus* strain 307CO isolated from one such coculture produced novel antibiotics (rhodostreptomycin A and rhodostreptomycin B), which are isomers different from the configuration of the carbon atom at C6 in streptose [7]. Genetic analysis showed that a strain of *Rhodococcus* strain 307CO contained a large segment DNA derived from the *Streptomyces* strains, and there is correlation between antibiotic production and the presence of the *Streptomyces* DNA in the *Rhodococcus* [7].

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In the present study, we tried to review the development of cytotoxic effect reagents against Jurkat T cells through co-culture between *R. aquatilis* AY2000 Strain and *S. griseus*.

Materials and Methods

Microbe culture

AY2000 was cultured in YM broth [malt extract 0.3%, yeast extract 0.3%, peptone 0.5%, sucrose 0.5% (pH5.4)] at 28°C for 48 hr. *S. griseus* was also cultured in the same condition. The both cultured broths were collected by centrifugation (12,000 rpm, 30 min, Micro 17TR, Hanil Science Industrial, Korea). After centrifugation, the broth did freezing dry and resultant material (1 mg/ml) was resuspended with RPMI 1640 medium. 50 μl suspension solution (50 μg) was subjected into Jurkat T cells for MTT assay. Other cultured supernatant was also followed with the same protocol.

Coculture condition

For coculture between AY2000 strain and *S. griseus*, *S. griseus* was pre-cultured for 24 hr and then innoculated with 1% volume of *S. griseus* culture volume of AY2000 strain present of OD 0.5 value. After innoculation, coculture was carried out at 28°C for 48 hr. While coculture was progressing, culture broth was sampled by each 6 hr, pH value was measured, the resulting broth was collected by centrifugation (12,000 rpm, 30 min). Obtained broth was solified by a freezing dryer, and resultant material (1 mg/ml) was

resuspended in RPMI 1640 medium and then 50 µl suspension solution (50 µg) of coculture between AY2000 and *S. griseus* (SCAS) was used for MTT assay.

Cytotoxicity assay with Jurkat T cell (MTT assay)

Cytotoxicity of prepared each sample against Jurkat T cell was determined by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay as described previously [1]. Briefly, cells were innoculated at a density of $4x10^4$cells/well and incubated for 24 hr with each sample. After 24 hr, cell viability was determined by using 50 µl MTT (1.0 mg/ml) further incubated for 4 hr. After removal of medium, cells were dissolved in 150 µl DMSO and measured at 540 nm using micro plate reader (70474-AXQI, Molecular device, US).

DAPI staining

DAPI staining was described previously [10]. Samples cocultured for 48 hr were treated with Jurkat T cells for 24 hr. Cells were washed 2 times with PBS and were treated with 95% ethanol for 1 hr at 4°C as a fixative. After washing the sample, RNase (12.5 µg) in 1.12% sodium citrate buffer (pH 8.45) was added at 37°C or 30 min. Sample treated Jurkat T cells were stained with DAPI (4 µg/ml) and observed under the microscope (Microphot-FX, Nikon, Tokyo, Japan).

Results

Effect of substance produced by AY2000 strain against Jurkat T cells

To evaluate cytotoxicity of substance secreted by AY2000 (SAY) strain into the medium, supernatant were sampled by 6 hr interval. After freezing dryer, resultant material was resuspended with RPMI 1640 medium. Jurkat T cell was subjected with SAY. As shown in Fig. 1, each sample revealed that no influence on cytotoxicity against Jurkat T cells was when compared with control.

Effect of cytotoxicity by SAY, SCAS and SSG

In order to detect cytotoxicity of the component of each cell supernatant including coculture supernatant, Jurkat T cells was treated with samples cultured for 48 hr.

As shown in Fig. 2, SCAS treated cells showed decrease in cell viability compared with control and other two componets. This suggested that coculture condition with

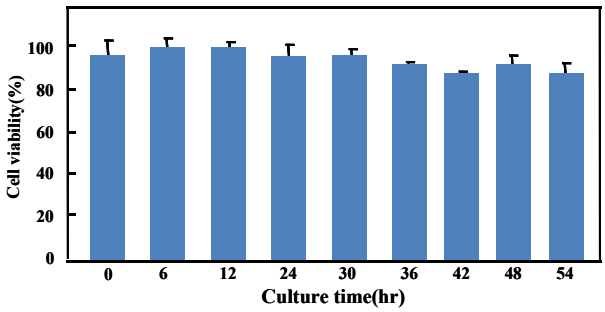

Fig 1. Cell viability of supernatant of AY2000 on Jurkat T cells. AY2000 strain was cultured in YM broth and centrifuged (12,000 rpm, 30 min). After centrifugation, the broth did freezing dry and resultant material (1 mg/ml) was resuspended with RPMI 1640 medium. 50 µl suspension solution (50 µg) was subjected into Jurkat T cells for MTT assay. Samples were prepared indicated time interval. Vertical bar represents standard error

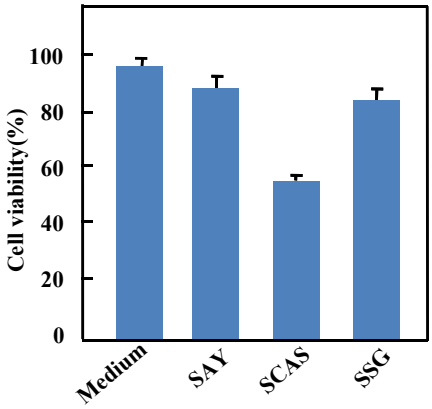

Fig 2. Cell viability by cocultured supernatant between AY2000 and *S. griseus*. *S. griseus* was pre-cultured for 24 hr and then innoculated with 1% volume of *S. griseus*. After innoculation, coculture was carried out at 28°C for 48 hr. Obtained broth was solified by a freezing dryer, resultant materials (1 mg/ml) were resuspended in RPMI 1640 medium and 50 µl solution (50 µg) were used for MTT assay. Substance secreted by AY2000: SAY, substances of coculture between AY2000 and *S. griseus*: SCAS, substances of *S. griseus*: SSG. Vertical bar represents standard error

AY2000 and *S. griseus* can produce new cytotoxicity substances against human acute cancer cell line, Jurkat T cells.

Effect of cytotoxicity among coculture times

To determine cytotoxicity of the substances of coculture between AY2000 and *S. griseus* (SCAS) with human cancer cell line, Jurkat T cells were assayed by MTT assay. As shown in Fig. 3, the growth of the Jurkat T cells was gradually inhibited when treated with SCAS cultured until 60 hr. However, it seemed that 48 hr time was suitable for

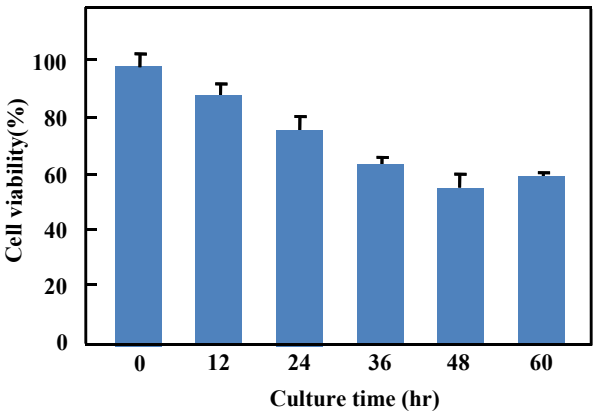

Fig 3. Cell viability by coculture time between AY2000 and *S. griseus*. *S. griseus* was pre-cultured for 24 hr, innoculated with 1% volume of *S. griseus*. After innoculation, samples were prepared at indicated time. Obtained broth was solified by a freezing dryer, resultant materials (1 mg/ml) were resuspended in RPMI 1640 medium and 50 µl solution (50 µg) was used for MTT assay. Vertical bar represents standard error

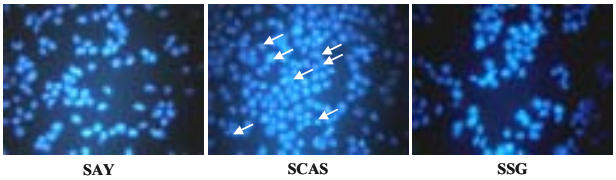

Fig. 4. Nuclear morphological change observed by SAY, SCAS and SSG. Samples cocultured for 48 hr were treated with Jurkat T cells for 24 hr. Cells were washed and were treated with 95% ethanol. After washing, sample-treated Jurkat T cells were stained with DAPI (4 µg/ml) and observed under the microscope (Microphot-FX, Nikon, Tokyo, Japan).

coculture time.

Nuclear morphological changes induced by SCAS

To evaluate cytotoxicity of SCAS, the morphological change was examined by subjecting to nuclear DAPI staining for 24 hr. When the cells were exposed to SAY, SCAS and SSG, SCAS treated cells represented apoptotic bodies (Fig. 4). In regard with MTT assay, SCAS contained cytotoxicity agent against human Jurkat T cells (arrow).

Discussion

It has become increasingly evident that horizontal gene transfer among microbes is important to microbial activities that influence our health and the environment [6,9,12,13]. In order to survive microbes from environments, they produces several kinds of antibiotics to kill other microbes with soluble factors. To the best of our knowledge, there are *Streptomyces* species capable of producing numerous antibiotics. Example of such strains include *S. antibiotocus* [15], *S. chrysomallus* [4], *S. lanatus* [4], *S. michiganensis* [4]. We performed competitive coculture between *S. griseus* that is a multi-antibiotic producer and the newly isolated strain AY2000 that is unique in that it is capable of producing anti-yeast substance. Bioassay of the supernatant cocultured between AY2000 and *S. girseus* showed the ability of attack against jurkat T cell compared to each cultured substance. It seems that coculture condition makes an influence on synthesis of new antibiotic materials. In coculture, as coculture time went up, the growth of *S. griseus* inhibited and decreased (data not shown). In contrast, the growth of AY2000 increased. In this report, we demonstrated the possibility for new drug development method. However, we did not tell any genomic study for AY2000 or *S. griseus* to detect gene segment for horizontal gene transfer. Now, we are preparing genome analysis for cocultured microbes and coculture optimal condition such as pH, carbon source, temperature, initial innoculation concentration of cell, *etc*. When coculture condition is optimized, a bulk of antibiotic material is produced and this new method is applied to new antibiotic material development.

Acknowledgement

This research was financially supported by Dong Eui university in 2008 year (2008AA194).

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초록 : *Rahnella aquatilis* AY 2000과 *Streptomyces griseus*의 공배양 상등액의 항암활성

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미생물들 사이에 존재하는 수평적 유전자 전달을 이용하여 Jurkat T cell에 대하여 새로운 항생물질을 생산하기 위해 토양박테리아 AY2000과 여러 종류의 항생물질 생산 균주인 *Streptomyces griseus*의 공배양을 수행하였다. MTT assay를 수행하여 세포 독성을 실험을 하였을 때 공배양 상등액은 각각 배양 하였을 때보다 높은 세포독성을 보였고 또한 48시간 배양 하였을 때 가장 높은 활성을 나타내는 것으로 나타났다. 더욱이 DAPI 염색을 하였을 때 Jurkat T cell 세포의 세포핵의 변화도 관찰 되었다. 이런 결과는 공배양 상등액에 새로운 항생물질이 생성되었음을 보여주었고 이런 방법으로 새로운 항생물질 생산에 이용되어 질수 있음을 의미한다.