

Biological and biochemical modulation of *Trichomonas vaginalis* KT9 isolate after shifting of culture medium from TPS-1 into TYM

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Abstract: To evaluate the biological and biochemical characteristics of *Trichomonas vaginalis* KT9 isolate, the growth and size of trichomonads, pathogenicity in mouse, protein profiles and proteinase activity were examined after shifting the medium from TPS-1 into TYM. Generation time of trichomonads in TYM medium was 4.5 hr in comparison to TPS-1 with 7.1 hr. Size of trichomonads cultured in TPS-1 medium ($8.5 \pm 0.9 \times 6.0 \pm 0.9 \mu\text{m}$) was significantly smaller than those in TYM medium ($10.9 \pm 1.4 \times 8.2 \pm 0.9 \mu\text{m}$). Trichomonads cultured in TYM medium produced subcutaneous abscess in 9 out of 10 mice, whereas those in TPS-1 medium produced abscesses in 2 out of 10 mice. In SDS-PAGE, trichomonad lysates from both media showed ten common bands. However, trichomonads in TYM medium showed additional bands of 136 kDa, 116 kDa and 40 kDa in comparison to those in TPS-1 with 100 kDa. By immunoblot with *T. vaginalis*-immunized rabbit sera, *T. vaginalis* cultivated in both TYM and TPS-1 media showed 5 common bands, and unique bands of 116 kDa, 105 kDa, and 86 kDa were observed in trichomonads in TYM while a 140 kDa band in those in TPS-1. In gelatin SDS-PAGE, trichomonads in TYM degraded gelatin stronger than those in TPS-1. Also protease activity of trichomonads in TYM was significantly higher than that of trichomonads in TPS-1 using Bz-Pro-Phe-Arg-Nan as a substrate. According to the results, it is assumed that the shift from TPS-1 into TYM medium for cultivation of *T. vaginalis* might modulate the biological and biochemical properties of *T. vaginalis* *in vitro*.

Key words: *Trichomonas vaginalis*, TYM medium, TPS-1 medium, pathogenicity, proteinase

INTRODUCTION

The growth condition has been known to affect the biological function and the pathogenicity of various protozoa such as *Naegleria fowleri*, *Trichomonas vaginalis* and *Entamoeba histolytica*. The proliferation of *E. histolytica* varies according to the batch of Panmede and

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bovine serum and components of TPS-1 culture medium (Chávez-Dueñas *et al.*, 1989). Trophozoites of *N. fowleri* grown in Cline medium and Nelson medium show differences in growth rate, locomotion rate, resistance to complement lysis, and the ability to destroy the nerve cell (Marciano-Cabral and Toney, 1994).

Several reports had shown that variation of pH or ingredients of media affects size and pathogenicity of *T. vaginalis*. Winston (1974) reported that variation in size of *T. vaginalis* was due to the effect of the host environment on a single species. Moon *et al.* (1983) and Kim *et al.* (1984) reported that trophozoites cultured under unfavorable pH conditions of TPS-1 medium were significantly larger than those cultured at an optimum pH (pH 7.0), and showed lower level of pathogenicity. Also, iron-sulfur protein including ferredoxin and pyruvate-ferredoxin oxidoreductase increased in *T. vaginalis* grown in iron-rich medium compared to that in low-iron medium (Gorrell, 1985).

We have cultivated *T. vaginalis* using TPS-1 medium for several years, originally devised for axenic cultivation of *Entamoeba* (Diamond, 1968). TYM medium is commonly used to cultivate *T. vaginalis* (Diamond, 1957). It was by mere chance that TPS-1 medium has been used for isolation of *T. vaginalis* instead of TYM medium in our laboratory. It was not known about alteration of *T. vaginalis* after changes of growth condition including shifting of media from TPS-1 to TYM. Therefore, this study was performed to observe the differences of biological and biochemical characteristics of *T. vaginalis* KT9 after medium change from TPS-1 into TYM medium.

MATERIALS AND METHODS

Trichomonas vaginalis and media

The KT9 isolate of *T. vaginalis* was obtained from a Korean woman with vaginitis (Ryu and Lloyd, 1995). Trophozoites were maintained in TPS-1 media for 3 years. For the change of media, trophozoites in TPS-1 media were transferred into TYM media (Table 1), and cultivated for about one year. Companies and catalog numbers of the ingredients of TPS-1

Table 1. Comparison of Diamond's TPS-1 and TYM media for *Trichomonas vaginalis*

Ingredients	TPS-1	TYM
Trypticase	5.0 g	10.0 g
Panmede	10.0 g	
Yeast extract		5.0 g
D-glucose	2.5 g	
Maltose		2.5 g
L-cystein HCl	0.5 g	0.5 g
Ascorbic acid	0.2 g	0.5 g
NaCl	2.5 g	
K ₂ HPO ₄	0.3 g	0.5 g
KH ₂ PO ₄	0.5 g	0.5 g
FeSO ₄ ·7H ₂ O		0.05 g
TC medium		0.25 g
Bovine serum	50 ml	
Horse serum		50 ml
Distilled water	435 ml	435 ml

and TYM media were as follows: BBL® Trypticase® pepton pancreatic digest of Casein (BD, USA), Panmede (Paines & Byrne limited, Greenford-England), yeast extract (Difco, 0123-17-3), maltose (Jassen chimica, B-2440, Geel Belgium), L-cysteine and ascorbic acid (Junsei Chemical Co.), Bovine serum (Hyclone), and Horse serum (Gibco BRL). Also D-glucose (G-8270), NaCl (S-7653), K₂HPO₄ (P-3786), KH₂PO₄ (P-5379), and FeSO₄·7H₂O (F-8263) were purchased from Sigma.

Iron concentrations of TYM and TPS-1 media measured by automatic chemical analyzer (Hidazi 747, Japan) were 2,200 µg/dl and 484 µg/dl, respectively.

Generation time and sizes of trophozoites

To measure generation time, trophozoites (3×10^4) at an early log phase were inoculated into 5 ml of TPS-1 and TYM media, respectively and the number of live trophozoites was quantitated using a hemocytometer twice a day.

For comparison of the size of trichomonads in each media, trophozoites were smeared on slide glasses and stained with Giemsa solution. The lengths and widths of each trophozoites were measured with measuring device attached at a personal computer (PC 8001, NEC). The Mann-Whitney *U*-test was

used for comparison of data.

Animal assay

The 6-day subcutaneous lesion assaying method was employed in order to figure out the degrees of pathogenicity of *T. vaginalis* cultured in both media (Ryu *et al.*, 1995). BALB/c mice were anesthetized with ether and *T. vaginalis* (1×10^6) from each media were inoculated into the subcutaneous tissue of the mice. After 1 week, mice were sacrificed and the size of abscesses were measured.

SDS-PAGE and immunoblot

SDS-PAGE and immunoblot were undertaken to observe the protein and antigenic profile of trichomonads. *Trichomonas vaginalis* in each media were sonicated and centrifuged at 10,000 *g*. Forty μ g of the supernatant was loaded into the well. SDS-PAGE was carried out on a 7.5% running gel under discontinuous buffer system as the method of Laemmli (1970). Immunoblot was carried out according to the method of Tsang *et al.* (1983). Rabbit sera (1:500 diluted) immunized with *T. vaginalis* and peroxidase-conjugated anti-rabbit IgG (1:1000 diluted) were used for the reaction.

Gelatin SDS-PAGE

The ability of trophozoites to degrade gelatin was observed by gelatin SDS-PAGE in 7.5-15% gradient gel (Lockwood *et al.*, 1987). After electrophoresis, gels were soaked in 2.5% Triton X-100 for 1 hr to remove SDS. Proteinase was activated by incubation for 4 hr in sodium phosphate buffer (pH 6.0) containing 1 mM dithiothreitol (DTT) at 37°C. The gels were stained in Coomassie brilliant blue R.

Proteinase activity

Proteinase activity was measured by modified methods of Lockwood *et al.* (1984). Fifteen μ g of trichomonads supernatant was reacted with 15 μ l DTT (10 mM) for 15 min, and 400 μ l sodium phosphate buffer (pH 6.0) and 20 μ l benzoyl-proline-phenylalanine-arginine-nitroanilide (Bz-Pro-Phe-Arg-Nan; 1 mM) were added to the reaction mixture. Enzyme activity was measured by optical density at 405 nm after incubation for 90 min

at 37°C.

RESULTS

Generation time and sizes of trophozoites

The generation times of *T. vaginalis* cultivated in TYM and TPS-1 media were 4.5 hr and 7.1 hr, respectively ($P=0.1$). Growth of the trophozoites in TYM media did not show significant differences from that in TPS-1 (Fig. 1).

Mean lengths and widths of trophozoites cultivated in TYM and TPS-1 media were $10.9 \pm 1.4 \times 8.2 \pm 0.9 \mu\text{m}$ and $8.5 \pm 0.9 \times 6.0 \pm 0.9 \mu\text{m}$, respectively. Trichomonads of TYM media were significantly larger than those of TPS-1 media ($P<0.0005$).

Animal assay

Among 10 mice tested for pathogenicity of *T. vaginalis*, trophozoites in TYM media produced abscesses in 9 mice whereas trichomonads in TPS-1 produced abscess in 2 mice. The mean abscess size ($11.7 \pm 7.47 \text{ mm}^2$) caused by trophozoites cultured in TYM was larger than that formed by trichomonads in TPS-1 ($7.7 \pm 0.55 \text{ mm}^2$).

SDS-PAGE and immunoblot

In SDS-PAGE, common bands were observed at 75 kDa, 60 kDa, 58 kDa, 52 kDa,

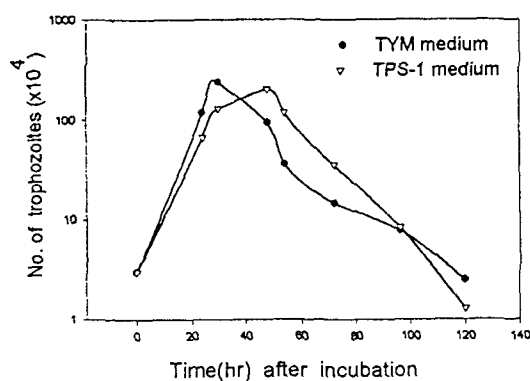


Fig. 1. Propagation of *Trichomonas vaginalis* KT9 by inoculum dose (3×10^4) in TYM (●) and TPS-1 (▽) media. Trophozoites in TYM media grew faster than that in TPS-1, especially at an early log phase of growth.

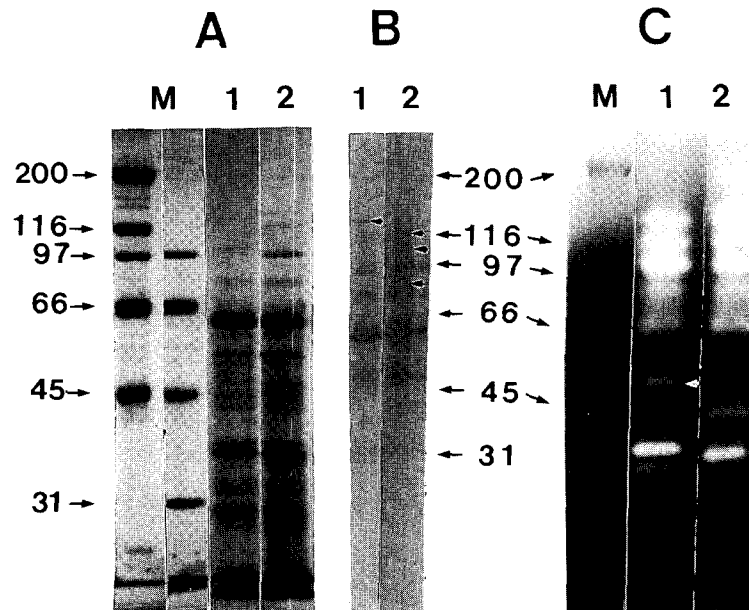


Fig. 2. SDS-PAGE (A), immunoblot (B) and gelatin SDS-PAGE (C) findings of lysates of *Trichomonas vaginalis* KT9 isolates. Lane 1, trichomonads cultured in TPS-1 media; lane 2, trophozoites in TYM media. Arrows (→), standard size marker (M); arrowheads (◄), specific bands due to different cultivation media.

47 kDa, 45 kDa, 43 kDa, 38 kDa, 37 kDa, and 32 kDa, and trichomonads in TYM and TPS-1 media showed additional bands at 136 kDa, 116 kDa and 40 kDa, and at 100 kDa, respectively (Fig. 2A). By immunoblot, *T. vaginalis* in both TYM and TPS-1 media showed 5 common bands at 95 kDa, 75 kDa, 60 kDa, 47 kDa, and 32 kDa, and trichomonads in TYM revealed unique bands at 116 kDa, 105 kDa and 86 kDa. By contrast, trophozoites in TPS-1 showed a band at 140 kDa (Fig. 2B).

Gelatin SDS-PAGE

Trichomonads in TYM media degraded gelatin stronger than those in TPS-1 media. Ten bands were observed in common and a band at 52 kDa was detected in trichomonads cultured in TPS-1 media only (Fig. 2C).

Proteinase activity

Enzyme activity using Bz-Pro-Phe-Arg-Nan was measured by optical density at 405 nm. Optical density (0.27 ± 0.07) of trichomonads in TYM media was significantly higher than that of trichomonads (0.11 ± 0.03) in TPS-1 ($P < 0.01$).

DISCUSSION

It has been known that small size of *T. vaginalis* might be related with stronger pathogenicity or more severe symptoms than large trophozoites (Winston, 1974; Moon *et al.*, 1983; Kim *et al.*, 1984). On the contrary, trichomonads in TYM media showed more stronger pathogenicity and larger size than those in TPS-1 in the present study.

Shim *et al.* (1993) reported that neutral and acid proteinase activities in pathogenic group of *T. vaginalis* were significantly higher than those of weak pathogenic group. In the present study, trichomonads in TYM showed higher proteinase activity and stronger pathogenicity than those in TPS-1. Therefore, it is supposed that the pathogenicity of *T. vaginalis* could be related with proteinase activity rather than the size of trophozoites.

The importance of iron for growth of *T. vaginalis* was demonstrated by several investigators (Gorrell, 1985; Lehker *et al.*, 1991; Lehker and Alderete, 1992). Iron-sulfur protein for energy production required for

survival of trichomonads increased in *T. vaginalis* grown in iron-rich medium compared with that in low-iron medium (Gorrell, 1985). Also surface expression and synthesis of adhesins, which is related with adhesion of trichomonads to epithelial cell, was mediated by iron concentration (Lehker *et al.*, 1991). In the present study, iron concentrations of TYM and TPS-1 media were 2,200 µg/dl and 484 µg/dl, respectively. Although iron concentration of TPS-1 medium was lower than that of TYM, this value seems to be enough for *T. vaginalis* because trichomonads cultivated at a concentration of about 200 µg/dl of iron manifested strong pathogenicity (data not shown). Nonetheless it is desirable to compare TYM with TPS-1 supplemented with iron to confirm importance of iron concentration for cultivation of *T. vaginalis*.

TPS-1 medium was originally devised for axenic cultivation of *Entamoeba* (Diamond, 1968). Major ingredient of TPS-1 medium is Panmede, which is manufactured by enzymatic hydrolysis of bovine liver homogenates (Diamond, 1968). Although not precisely quantified, Panmede's quality is known to vary, and most lots cannot support amoebal growth (Diamond *et al.*, 1978). Chávez-Dueñas *et al.* (1989) reported that it can improve the growth of *E. histolytica* by selecting appropriate lots of Panmede.

Even though it is suggested that the difference of medium component, such as serum, Panmede, yeast extract and ferrous sulfate, between TYM and TPS-1 might affect the various pathophysiologic effects of *T. vaginalis*, it is difficult to find out which one or complex of medium components cause those differences.

Trophozoites in TYM media showed shorter generation time, larger size, stronger proteinase activity and virulence in comparison with those of TPS-1 media in the present study. Therefore, TYM media may be better than TPS-1 media for maintenance of various characteristics related with pathogenicity of *T. vaginalis*.

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=초록=

TPS-1 배지 및 TYM 배지에서 배양된 질편모충의 생물학적 특성의 변화

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질편모충의 배양배지인 Diamond의 TPS-1 배지에서 3년간 배양한 질편모충 KT9 국내 분리주를 TYM 배지로 옮겨 1년간 배양하고 양쪽 배지에서 자란 질편모충의 증식, 병원성, 영양형의 크기, 단백질 성장 등을 비교하였다. 각 배지 원충의 generation time은 TYM 배지의 원충은 4.5시간이었고 TPS-1 배지의 원충은 7.1시간으로 TYM 배지에서 더 빨리 자랐다. TYM 배지에서 자란 원충의 크기가 TPS-1 배지에서의 영양형에 비해 유의하게 작았다. TYM 배지에서 배양한 원충은 10마리 마우스 중 9마리에서 피하 농양을 형성한 반면 TPS-1 배지에서 배양한 원충은 2마리에서 피하 농양을 형성하였고, 농양의 크기도 작았다. SDS-PAGE에서 10개의 단백분획이 공통적으로 관찰되었으며 TYM 배지의 원충에서는 136 kDa, 116 kDa 및 40 kDa의 분획이, TPS-1 배지의 원충에서는 100 kDa 분획이 특징적으로 관찰되었다. Gelatin SDS-PAGE에서는 200 kDa과 70 kDa 사이에 10개의 gelatin 분획이 공통적으로 관찰되었으며, TYM 배지에서 배양한 원충이 TPS-1 배지에서 배양한 원충에 비해 gelatin을 많이 분해하였다. Bz-Pro-Phe-Arg-Nan를 기질로 사용하여 단백질분해효소활성을 측정하였는데 TYM 배지에서 배양한 원충의 단백질분해효소활성은 TPS-1 배지에서 배양한 원충에 비해 유의하게 높았다. 이 연구결과 질편모충의 배양에서 TPS-1배지를 TYM 배지로 전환하였을 때 원충의 크기가 커졌고 단백질분해효소 활성이 증가하며 독력 (병원성)이 증가됨이 관찰되었다.

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