한국가축위생학회지 제41권 제4호 (2018)
Korean J Vet Serv, 2018, 41(4), 239-244
ISSN 1225-6552, eISSN 2287-7630
https://doi.org/10.7853/kjvs.2018.41.4.239

Korean Journal of
Veterinary Service
Available online at http://kjves.org

< Original Article >

Effects of selenate and L-glutamate on the growth of *Mycobacterium tuberculosis* complex

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(Received 29 March 2018; revised 4 October 2018; accepted 1 December 2018)

Abstract

Mycobacterium tuberculosis (*M. tuberculosis*) complex is the causative agent of tuberculosis (TB) in humans and bovine TB in mammalian hosts and grows very slowly. Selenium is a central molecule in nitrogen metabolism and an essential ingredient for all living cells and glutamic acid. The effects of selenium on the growth of *M. tuberculosis*, a representative slow-growing Mycobacterium species, were investigated and measured using the BacT Alert 3D System (MB/BacT System). Sodium selenate, at a final concentration of 10 μg/mL, reduced the average time-to detection (TTD) to 197.2 hours (95% confidence interval (CI), 179.6～214.8) from 225.1 hours (95% CI, 218～232.0) in the control culture media ($P < 0.05$). The TTD did not increase with L-glutamate concentrations up to 10 μg/mL, but a significant reduction in the TTD was observed in the presence of 20 μg/mL L-glutamate in culture media ($P < 0.05$). In conclusion, selenate and L-glutamate enhance the growth of *M. tuberculosis*.

Key words : *M. tuberculosis* complex, Sodium selenate, L-glutamate

INTRODUCTION

Tuberculosis (TB) is caused by members of the *Mycobacterium tuberculosis* (*M. tuberculosis*) complex, a group of closely related species and subspecies that include *M. tuberculosis*, *M. bovis*, *M. bovis* bacillus Calmette-Guérin (BCG), *M. africanum*, *M. orygis*, *M. microti*, *M. canetti*, *M. caprae*, *M. pinnipedii*, *M. suricattae*, and *M. mungi* (Ernst et al, 2007). *M. tuberculosis* is the most common cause of TB in humans (Getahun et al, 2015) and *M. bovis* causes bovine TB in a wide range of mammalian hosts, livestock (including cattle, pigs, and goats), wildlife, and humans (Pesciaroli et al, 2014). TB in humans is a global health problem and bovine TB is widespread and responsible for major agricultural economic losses (Pesciaroli et al, 2014; Getahun et al, 2015).

M. tuberculosis is an aerobic, non-motile rod-shaped and acid-fast bacterium with a lipid-rich cell wall that is mainly composed of mycolic acids, which are key virulence factors and render *M. tuberculosis* resistant to drying and chemicals (Abrahams and Besra, 2016). *M. tuberculosis* has a very slow generation time of 15～20 hours and takes about 4～6 weeks to form visible colonies. The culture media that are commonly used to grow *M. tuberculosis* include egg-based solid media, such as Löwenstein-Jensen medium, and agar-based solid media, such as Middlebrook 7H10 or 7H11 (Ji et al,

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2014). Liquid culture systems, such as the Mycobacteria Growth Indicator Tube (MGIT; Becton Dickinson, Franklin Lakes, NZ, USA) and BacT Alert 3D System (MB/BacT; BioMerieux, Marcy-Etoile, France), have been adopted for the rapid detection of *M. tuberculosis*; these are based on liquid media such as Middlebrook 7H9 or Middlebrook 7H12 (Kim et al, 2016).

However, there is still an urgent need to increase the growth of *M. tuberculosis*, a typical slow-growing Mycobacterium, and to shorten the culture period for its isolation.

Selenium is essential for all living cells, as it is present in active sites of crucial oxidoreductase enzymes; however, it is toxic at high concentrations (Bock et al, 1991). Under aerobic conditions, selenium is present predominantly in the high valence oxidized forms of selenite (SeO_3^{2-}, +IV) and selenate (SeS_4^{2-}, +IV) (Bebien et al, 2001). The oxidized forms of selenium are highly soluble and more bioavailable and toxic. Smalley et al. reported that the addition of sodium selenate to media enhanced the growth of *Legionella pneumophila* (Smalley et al, 1980).

Glutamine and glutamate are central molecules in nitrogen metabolism. Glutamine, which is synthesized from L-glutamine, ammonia, and ATP by glutamine synthetase (Reitzer, 1996), functions as a nitrogen donor for many nitrogen-containing molecules in the cell. The *glnA1* gene of *M. tuberculosis* encodes a glutamine synthetase type I enzyme, a form of glutamine synthetase (Tullius et al, 2003). A *glnA1*-deleted *M. tuberculosis* mutant requires a high level of L-glutamine for growth. Additionally, *M. tuberculosis* has a higher metabolic requirement for L-glutamine than *Salmonella enterica* Typhimurium (Klose and Mekalanos, 1997). Some researchers have suggested that the high L-glutamine requirements of *M. tuberculosis* might be related to the synthesis of peptidoglycan, which is a major component of the cell wall and a poly-L-glutamate/glutamine cell wall structure (Hirschfield et al, 1990). Therefore, it is necessary to investigate the effects of sodium selenate and L-glutamate on the growth of *M. tuberculosis*.

In the present study, we examined the effects of selenate and L-glutamate on the growth of *M. tuberculosis* using MB/BacT, a mycobacterial liquid culture system.

MATERIALS AND METHODS

Bacterial strains

M. tuberculosis H37Rv (ATCC 27294) was grown in Middlebrook 7H9 medium (Becton Dickinson, Franklin Lakes, NZ, USA) supplemented with 10% Middlebrook OACD enrichment medium (Becton Dickinson) and 0.5% glycerol at 37°C. The cells were frozen at −70°C until use. The viable amount of *M. tuberculosis* stock was measured by plating serial dilutions onto a Middlebrook 7H10 agar plate.

Mycobacterial culture using the MB/BacT System

M. tuberculosis stock was diluted to a volume of 0.5 mL with sterile saline to reach the desired number of bacteria and inoculated into an MB culture bottle that contained liquid medium (Middlebrook 7H9) with casein, serum bovine albumin and catalase, and antibiotic supplement for the MB/BacT System (amphotericin B, azlocillin, nalidixic acid, polymyxin B, trimethoprim, and vancomycin) according to the manufacturer's guidelines to reduce bacterial contamination. The final volume of the MB culture bottle was 10 mL, including 0.5 mL of diluted *M. tuberculosis* and 0.5 mL of additive ingredient. MB culture bottles were incubated in the MB/BacT System incubator cabinet, and the time-to-detection (TTD) was monitored until a positive signal was detected. Each test was performed using five culture bottles.

To determine the inoculum dose of *M. tuberculosis*, serially diluted *M. tuberculosis* suspensions were inoculated into the bottles and the growth of *M. tuberculosis* was monitored via the reflectance units, the index for the growth of bacteria in the MB/BacT System, and viable bacterial counting by plating serial dilutions onto the Middlebrook 7H10 agar plate. When a positive signal was detected through a built-in computer terminal, the bottle was unloaded and the TTD was documented.

To examine the effects of sodium selenate and L-glutamate on the growth of *M. tuberculosis*, sodium selenate and L-glutamic acid (Sigma-Aldrich, St. Louis, MO,

USA) were used. The sodium selenate and L-glutamic acid were diluted using sterile saline to a volume of 500 μL and added into the MB bottle. The final concentrations of sodium selenate were 5, 10, and 20 μg/mL. The final concentrations of L-glutamate were 5, 10, and 20 μg/mL. The growth of *M. tuberculosis* was monitored and the TTDs were used to compare the effects of the ingredients on the growth of *M. tuberculosis*.

Statistical analysis

All data were analyzed using IBM SPSS Statistics for Windows (IBM Co., Armonk, NY, USA). One-way ANOVA was used to determine significant differences among the treatments; differences were considered significant at $P<0.05$.

RESULTS

Detection of the growth of *M. tuberculosis* in the MB/BacT System

We examined changes in reflectance and TTD in the MB/BacT System and counted the viable bacteria after the inoculation of *M. tuberculosis*. The optimum inoculum dose in the MB/BacT System was nearly 10 days, which is close to the TTD of the smear-positive specimens in the MB/BacT System (Pfyffer et al, 1997). When 5×10^5 CFU/mL of *M. tuberculosis* was inoculated, the reflectance was approximately 1200 at the starting point (Fig. 1A). The reflectance increased slightly until approximately 9 days after inoculation and then suddenly shifted to approximately 2000 when a positive signal was detected in the MB/BacT System at 10 days post-inoculation (average TTD, 225.1 hours, 95% confidence interval (CI), 218.2～232.0). The bacterial number slightly increased until 144 days post-inoculation and steeply incremented until 288 days post-inoculation, when bacterial growth began to slow. The bacterial number was 1×10^7 CFU/mL when the positive signal was detected in the MB/BacT System (Fig. 1B).

Effects of sodium selenate on the growth of *M. tuberculosis*

To investigate the effects of selenate on the growth of *M. tuberculosis*, sodium selenate was added to the broth culture media and the growth of *M. tuberculosis* was monitored using the MB/BacT System (Fig. 2). When *M. tuberculosis* was grown in the media without sodium selenate, the average TTD was 225.1 hours (95% CI, 218.2～232.0). When sodium selenate was added to the culture media (final concentration of 10 μg/mL), the TTD significantly decreased to 197.2 hours (95% CI, 179.6～214.8, $P<0.01$) compared to the control culture

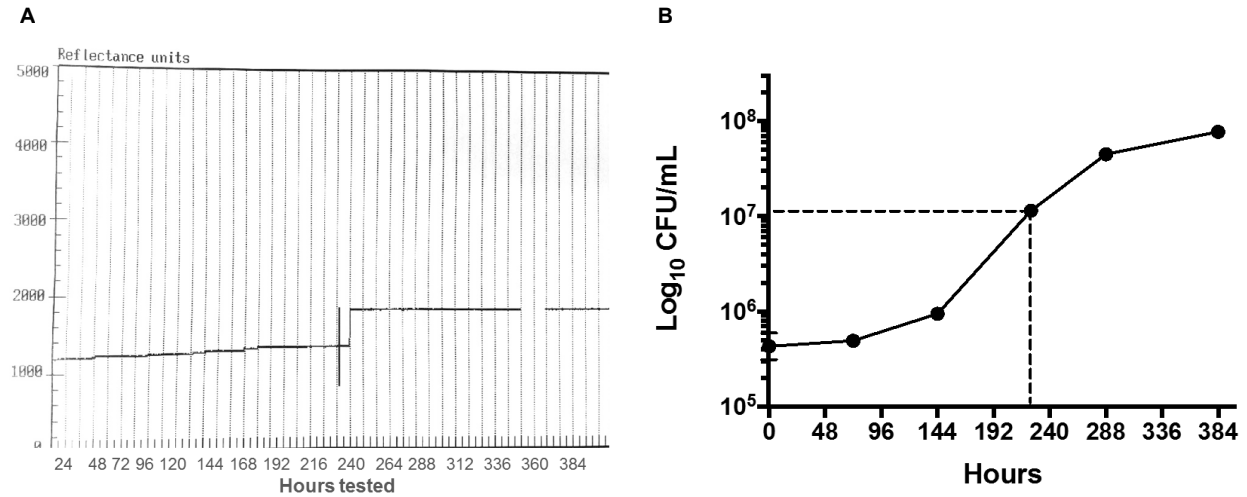

Fig. 1. Growth of *Mycobacterium tuberculosis* in the MB/BacT System. The growth of *M. tuberculosis* was monitored via the reflectance units of the MB/BacT System (A) and by viable bacterial counting of *M. tuberculosis* (B).

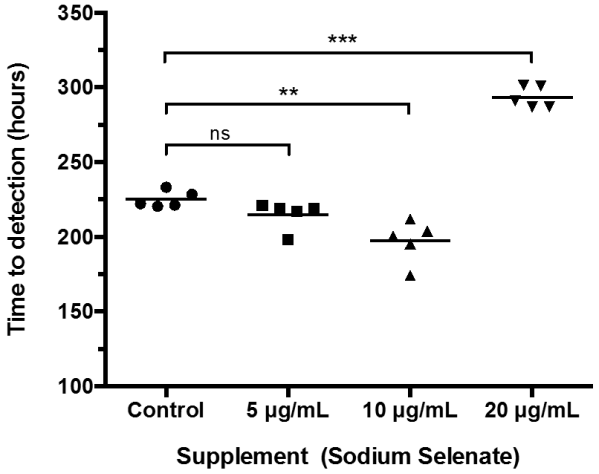

Fig. 2. Effects of sodium selenate on the growth of *M. tuberculosis*. Sodium selenate was added to the culture media (Middlebrook 7H9 broth media supplemented with 10% OADC) at final concentrations of 5, 10, or 20 μg/mL. The growth of *M. tuberculosis* was measured using the time-to-detection (TTD). Each treatment group was tested using five MB/BacT culture bottles.

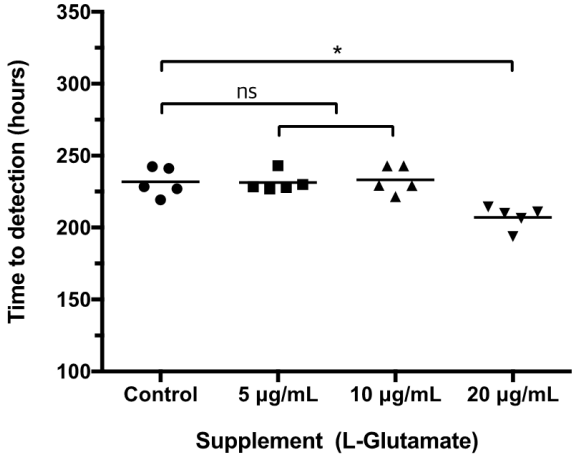

Fig. 3. Effects of L-glutamate on the growth of *M. tuberculosis*. L-Glutamate was added to the culture media at final concentrations of 5, 10, or 20 μg/mL. The growth of *M. tuberculosis* was measured using the TTD. Each treatment group was tested using five MB/BacT culture bottles.

media. Interestingly, the TTD increased when the concentration of sodium selenate was increased to 20 μg/mL, which was higher than the control culture media ($P<0.001$). These results suggest that sodium selenate enhances the growth of *M. tuberculosis* within a limited range of concentrations.

Effects of L-glutamate on the growth of *M. tuberculosis*

The effects of L-glutamate, a nitrogen source, on the growth of *M. tuberculosis* were examined using the MB/BacT System (Fig. 3). When L-glutamate was added to the culture media at either 5 or 10 μg/mL, there was no significant change in the TTD compared to the control culture media. However, when the culture media contained 20 μg/mL L-glutamate, the TTD decreased compared to the control media ($P<0.05$). These results indicate that L-glutamate enhances the growth of *M. tuberculosis* and shortens the TTD.

DISCUSSION

In the present study, we investigated the effects of sodium selenate and L-glutamate on the growth of *M. tuberculosis* using the MB/BacT System. We demonstrated that selenate enhanced the growth of *M. tuberculosis* at a low concentration, but growth was inhibited at a high concentration (i.e., 20 μg/mL). These results were generally consistent with previous studies. Smalley et al. reported that sodium selenate enhanced the growth of *L. pneumophila* at an optimum concentration between 5 and 10 μg/mL, but the growth of *L. pneumophila* was inhibited at a higher concentration (Smalley et al, 1980). Li et al. reported that sodium selenite enhanced the growth of *Spirulina platensis* at a low concentration, but toxic effects were observed at higher concentrations (Li et al, 2003). In our study, sodium selenate at a concentration of 20 μg/mL delayed the growth of *M. tuberculosis*, which might be due to its toxic effects, as described by Barceloux (Barceloux, 1999). These results suggest that selenate enhances the growth of bacteria at low concentrations but is toxic at higher concentrations.

When L-glutamate, a nitrogen source for the growth of *M. tuberculosis*, was added to the culture medium, increased growth was observed at a concentration of 20 μg/mL L-glutamate. This result is, to some extent, concordant with previous reports. Rehm et al. reported that L-glutamine was an excellent nitrogen source for *Corynebacterium glutamicum* (Rehm, 2010). Additionally, Tullius et al. reported that the requirement of *M. tuberculosis* for glutamine may be modulated by the *glnA1*

gene of *M. tuberculosis*, which encodes glutamine synthetase type I enzyme (Tullius et al, 2003). Therefore, an additional supply of L-glutamine was, to a certain degree, beneficial for the growth of *M. tuberculosis*.

CONCLUSION

In conclusion, selenate and L-glutamate were effective on the growth of *M. tuberculosis* in the MB/BacT System. Selenate enhanced the growth of *M. tuberculosis* at a limited concentration, whereas L-glutamate effectively enhanced the growth of *M. tuberculosis* at a high concentration.

CONFLICTS OF INTEREST

All authors have no conflicts of interest to declare.

ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology, Republic of Korea (No. NRF-2013R1A1A2059687 and No. NRF-2016R1A6A3A11933077).

REFERENCES

Abrahams KA, Besra GS. 2016. Mycobacterial cell wall biosynthesis: a multifaceted antibiotic target. Parasitology 1-18.

Barceloux DG. 1999. Selenium. J Toxicol Clin Toxicol 37: 145-172.

Bebien M, Chauvin JP, Adriano JM, Grosse S, Vermeglio A. 2001. Effect of selenite on growth and protein synthesis in the phototrophic bacterium *Rhodobacter sphaeroides*. Appl Environ Microbiol 67: 4440-4447.

Bock A, Forchhammer K, Heider J, Leinfelder W, Sawers G, Veprek B, Zinoni F. 1991. Selenocysteine: the 21st amino acid. Mol Microbiol 5: 515-520.

Ernst JD, Trevejo-Nuñez G, Banaiee N. 2007. Genomics and the evolution, pathogenesis, and diagnosis of tuberculosis. J Clin Invest 117: 1738-1745.

Getahun H, Matteelli A, Abubakar I, Aziz MA, Baddeley A, Barreira D, Den Boon S, Borroto Gutierrez SM, Bruchfeld J, Burhan E, Cavalcante S, Cedillos R, Chaisson R, Chee CB, Chesire L, Corbett E, Dara M, Denholm J, de Vries G, Falzon D, Ford N, Gale-Rowe M, Gilpin C, Girardi E, Go UY, Govindasamy D, D Grant A, Grzemska M, Harris R, Horsburgh CR Jr, Ismayilov A, Jaramillo E, Kik S, Kranzer K, Lienhardt C, LoBue P, Lönnroth K, Marks G, Menzies D, Migliori GB, Mosca D, Mukadi YD, Mwinga A, Nelson L, Nishikiori N, Oordt-Speets A, Rangaka MX, Reis A, Rotz L, Sandgren A, Sañé Schepisi M, Schünemann HJ, Sharma SK, Sotgiu G, Stagg HR, Sterling TR, Tayeb T, Uplekar M, van der Werf MJ, Vandevelde W, van Kessel F, van't Hoog A, Varma JK, Vezhnina N, Voniatis C, Vonk Noordegraaf-Schouten M, Weil D, Weyer K, Wilkinson RJ, Yoshiyama T, Zellweger JP, Raviglione M. 2015. Management of latent *Mycobacterium tuberculosis* infection: WHO guidelines for low tuberculosis burden countries. Eur Respir J 46: 1563-1576.

Hirschfield GR, McNeil M, Brennan PJ. 1990. Peptidoglycan-associated polypeptides of *Mycobacterium tuberculosis*. J Bacteriol 172: 1005-1013.

Ji M, Cho B, Cho YS, Park SY, Cho SN, Jeon BY, Yoon BS. 2014. Development of a quantitative sandwich enzyme-linked immunosorbent assay for detecting the MPT64 antigen of *Mycobacterium tuberculosis*. Yonsei Med J 55: 746-752.

Kim SC, Jeon BY, Kim JS, Choi IH, Kim J, Woo J. 2016. Performance of the BacT Alert 3D System Versus Solid Media for Recovery and Drug Susceptibility Testing of *Mycobacterium tuberculosis* in a Tertiary Hospital in Korea. Tuberc Respir Dis (Seoul) 79: 282-288.

Klose KE, Mekalanos JJ. 1997. Simultaneous prevention of glutamine synthesis and high-affinity transport attenuates *Salmonella typhimurium* virulence. Infect Immun 65: 587-596.

Li ZY, Guo SY, Li L. 2003. Bioeffects of selenite on the growth of *Spirulina platensis* and its biotransformation. Bioresour Technol 89: 171-176.

Pesciaroli M, Alvarez J, Boniotti MB, Cagiola M, Di Marco V, Marianelli C, Pacciarini M, Pasquali P. 2014. Tuberculosis in domestic animal species. Res Vet Sci 97 (Suppl): S78-S85.

Pfyffer GE, Welscher H, Kissling P, Cieslak C, Casal MJ, Gutierrez J, Rusch-Gerdes S. 1997. Comparison of the Mycobacterial Growth Indicator Tube (MGIT) with Radiometric and solid culture for recovery of acid-fast bacilli. J Clin Microbiol 35: 364-368.

Rehm N, Georgi T, Hiery E, Degner U, Schmiedl A, Burkovski A, Bott M. 2010. L-Glutamine as a nitrogen source for *Corynebacterium glutamicum*: derepression of the *AmtR* regulon and implications for nitrogen sensing. Microbiology 156: 3180-3193.

Reitzer LJ. 1996. Ammonia assimilation and the biosynthesis of glutamine, glutamate, aspartate, L-alanine, D-alanine. In: Neidhardt FC, Curtiss R, ed. *Escherichia and Salmanella typhimurium*. 2nd ed. Washington, D.C.: ASM Press 391-407.

Smalley DL, Jaquess PA, Layne JS. 1980. Selenium-enriched medium for *Legionella pneumophila*. J Clin Microbiol 12: 32-34.

Tullius MV, Harth G, Horwitz MA. 2003. Glutamine synthetase GlnA1 is essential for growth of *Mycobacterium tuberculosis* in human THP-1 macrophages and guinea pigs. Infect Immun 71: 3927-3936.