J. Gen. Appl. Microbiol., **55**, 291–294 (2009)

Full Paper

A new, simple, rapid test for detection of DNase activity of microorganisms: DNase Tube test

Devran Gerceker, Djursun Karasartova, Emel Elyürek, Saeid Barkar, Mehmet Kıyan, T. Murat Özsan, M. Kerem Calgin, and Fikret Sahin*

Microbiology Department, School of Medicine, Ankara University, Basic Science bldg. 3rd floor, Sihhiye-Ankara, Turkey 06100

(Received March 9, 2009; Accepted April 13, 2009)

The DNase test is a simple, economical method that has traditionally been used as a supplemental test to identify pathogenic *Staphylococcus*. This test also aids in the differentiation of closely-related genera within the *Klebsiella–Enterobacter–Serratia* division of *Enterobacteriaceae* and several other pathogens, including screening of *C. diphtheriae*. Currently DNase activity of microorganisms was tested using DNase agar plate methods. These tests have some drawbacks including the necessity of the extensive time to see the results of DNase activity of bacteria. In here, we developed a new method which is simple, rapid, inexpensive and applicable to examine DNase activity of any bacteria. In this method, simply, bacteria is added to the broth medium containing DNA and followed for the DNA degradation caused by the DNase of bacteria by running in agarose gel. This method we called DNase Tube test showed DNA degradation as fast as in half an hour depending on the DNase activity of the bacteria.

Key Words——DNase test; DNase activity; *Staphylococcus*

Introduction

The deoxyribonuclease (DNase) test is used to detect the degradation of deoxyribonucleic acid (DNA) (Fusillo and Weiss, 1959; Jeffries et al., 1957). The test is useful for differentiating *Serratia* spp. from *Enterobacter* spp., *Staphylococcus aureus* from coagulase-negative staphylococci, and *Moraxella catarrhalis* from *Neisseria* species and several other pathogens, including *Pseudomonas aeruginosa*, *Aeromonas* spp., and *Clostridium* spp. (Elston and Elston, 1968; Rothberg and Swartz, 1965; Valu, 1966). In 1957, Jeffries et al. described a rapid agar plate method for demonstrating DNase activity of *Staphylococcus* (Jeffries et al., 1957). This procedure utilized a semi-synthetic medium with nucleic acid solution incorporated in the medium. Enzymatic activity is detected by flooding the plate with 1 M hydrochloric acid (HCl). Intact DNA will be precipitated, making the agar cloudy, but clear zones will appear around colonies which produce DNase. There are some disadvantages which limit the usefulness of this procedure. The 1 M HCl is bactericidal for staphylococci in either isolated colonies or in heavier, more confluent growth. In addition, once the HCl has been applied, the test must be read and cannot be continued by reincubation. Finally, heavy inocula are recommended, and such inocula do not encourage the development of well-isolated colonies. Schreier modified a DNase test agar in which Toluidine Blue is used to indicate DNase activity, but, in the concentration used (0.01%), the medium is inhibitory to gram-positive bacteria (Schreier, 1969).

* Address reprint requests to: Dr. Fikret Sahin, Microbiology Department, School of Medicine, Ankara University, Basic Science Building, 3rd floor, Sihhiye-Ankara, Turkey 06100.

Tel: 011 90-312 492 1029 Fax: 011 90-312 310 6370

E-mail: fsahin29@hotmail.com

Abbreviations: DNA, deoxyribonucleic acid; DNase, deoxyribonuclease; DTT, DNase Tube test

Similarly, an acridine orange deoxyribonucleate-agar is used for detection of nuclease activity, but the procedure is more complex to perform (Lachica and Deibel, 1969). The major drawback of these tests in addition to problems mentioned is the necessity of a long time to see the results of DNase activity of the bacteria. Defining the DNase activity of *S. aureus* or *Corynebacterium* takes 24 to 48 h using the DNase agar test (Pimenta et al., 2008). Here we developed a simple, rapid and inexpensive method to illustrate DNase activity of any microorganism that can grow in any broth medium.

Materials and Methods

Microorganisms. Staphylococcus aureus ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Serratia marcescens* RSSK 579, *Enterobacter aerogenes* RSSK 720, *Escherichia coli* ATCC 35218, *Proteus mirabilis* ATCC 43071, *Proteus vulgaris* ATCC 7829, *Pseudomonas aeruginosa* ATCC 27853, *Corynebacterium pseudodiphtheriticum* RSSK *722*, and *Corynebacterium diphtheriae* RSSK 922/07017 were obtained from Refik Saydam National Hygiene Center–Ankara-Turkey. The stocks were maintained by transfer in Brain Heart Infusion (BHI) broth (Difco—Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

Extraction of DNA used in the DNase Tube test. The DNA used in the experiment was extracted from the overnight growth of *E. coli* using the DNA extraction protocol described by Romero et al. (Romero and Lopez-Goni, 1999). Briefly, bacteria were lysed with a lyses solution consisting of 1% SDS and 2% Triton X-100 followed by proteinase K digestion (100 mg/ml) and an organic extraction with phenol-chloroform. DNA amount was measured by spectroscopy, divided in aliquots of 0.1 μg and stored at −20°C. DNA is thawed and used whenever needed.

DNase Tube test. 0.1 μg of DNA and one loop of growing bacteria from the culture plate were added to the 200 μl BHI broth in an Eppendorf tube. The tube containing bacteria and DNA was incubated at ambient air 37°C with shaking (150 rpm). 20 μl of medium was taken at different time points (1/2, 1, 2, 4, 6, 12, 24 h) and centrifuged at 10,000 × *g* for 1 min and supernatant was run in a 0.9% gel electrophoresis system containing EtBr and visualized with a UV transilluminator. DNA in 200 μl BHI broth without bacteria was used to show the intact DNA.

Results

The DNase tube test (DTT) was first performed to differentiate the DNase activity of the growing culture of *S. marcescens*, *E. aerogenes*, *S. aureus* and *S. epidermidis* (Fig. 1. A and B). *S. marcescens* and *S. aureus* started to degrade DNA in half an hour. No DNA degradation was seen for 24 h in a tube containing *E. aerogenes* or *S. epidermidis* (data not shown). It is noticed that *S. marcescens* degraded DNA faster than *S. aureus* did (Fig 1. A and B). Next, we tested a group of bacteria known as DNase negative including *S. epidermidis*, *E. aerogenes, E. coli, P. mirabilis*, *P. aeruginosa, C. pseudodiphtheriticum* and a group of bacteria known as DNase positive including *S. aureus*, *S. marcescens, P. vulgaris*, *C. diphtheriae* for DNase activity using the DTT at time points 1/2 h, 1 h, 4 h, 6 h, 8 h and 24 h. Most DNase positive bacteria started to degrade

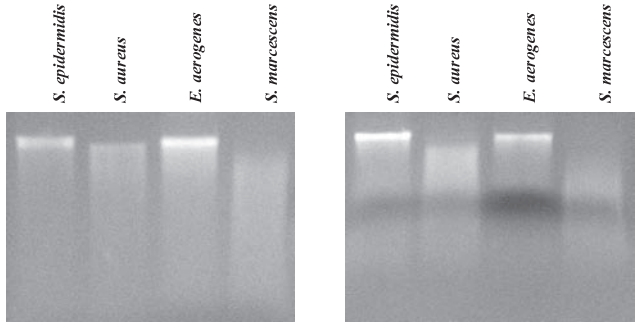


Fig. 1. DNase activity of *S. marcescens*, *E. aerogenes*, *S. aureus* and *S. epidermidis* using the DTT in half an hour (A) and 1 h (B).

Dashed bar shows the degraded DNA and vertical black bar shows the intact DNA.

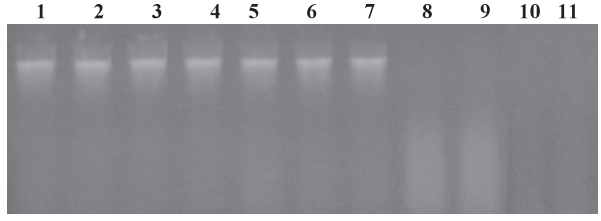


Fig. 2. Testing of DNase activity of the group of bacteria known as DNase negative and the group of bacteria known as DNase positive using the DTT.

Lanes: 1, DNA without microorganism, 2, DNA with *E. coli*, 3, DNA with *S. epidermidis*, 4, DNA with *P. aeruginosa*, 5, DNA with *C. pseudodiphtheriticum*, 6, DNA with *E. aerogenes*, 7, DNA with *P. mirabilis*, 8, DNA with *C. diphtheriae*, 9, DNA with *P. vulgaris*, 10, DNA with *S. aureus*, 11, DNA with *S. marcescens.*

DNA in the first hour (data not shown). All bacteria known as DNase positive had completely degraded DNA at 6 h (Fig. 2).

Discussion

Reliable and rapid methods for the identification of *S. aureus* from different diseases are crucial for control of the disease. Genetic identification methods like sequencing of 16S rRNA genes allow the identification of many bacterial pathogens (Patel, 2001). These methods are still relatively work-intensive and expensive and have not yet made their way into routine diagnostic laboratories. Thus, identification of bacterial pathogens still relies mainly on phenotypic criteria. The high specificity and sensitivity of the coagulase test have made it a standard method for the identification of *S. aureus*. However, only half of the *S. aureus* isolates are positive in this test after a 4 h incubation, and an overnight incubation is necessary to obtain reliable results (Boerlin et al., 2003). This long incubation time represents an important drawback for diagnostic applications, and quicker methods would be preferable. The presence of a DNase activity is often used as a surrogate marker for the identification of coagulase-positive staphylococci. However, the specificity of this criterion or of the more laborious test for DNase with the regular DNase plate agar is not entirely satisfactory (Boerlin et al., 2003).

The procedure we described in this study avoids the disadvantages of the agar plate method. The DTT is simple to perform so as to make its use applicable to the routine laboratory and no any specific expenses are incurred. The whole application including running gel electrophoresis takes 1/2 to 6 h depending on the DNase activity of bacteria using the DTT. From our experience, the detection of the DNA degradation in agarose gel is easier and clearer than the detection of DNA degradation on a DNA agar plate. In the DTT, it is possible to change the medium which might be necessary to achieve better growth of certain microorganisms. Although we tested the DTT in BHI, it is possible to perform the DTT in any other growth medium which might be specific and necessary to other bacteria. Another advantage of the DTT over the other DNase tests is that there is no other factor involved with the DTT that might interfere with growth of the microorganism such as HCl or dyes.

Although the DNase test has been used primarily as a test to determine potentially pathogenic *Staphylococcus* (Cunningham, 1959; Fusillo and Weiss, 1959), it shows potential as a diagnostic tool in identifying any microorganism producing DNase. The DNase test aids in the differentiation of closely-related genera within the *Klebsiella–Enterobacter–Serratia* division of *Enterobacteriaceae* and several other pathogens, including *P. aeruginosa*, *Aeromonas* spp., *Clostridium* spp. and *M. catarrhalis* (Elston and Elston, 1968; Fusillo and Weiss, 1959; MacFaddin, 2000). A short set of routine tests, which includes DNase as a major differentiating factor, would be especially useful as a screening of microorganisms including *Corynebacterium* species (Pimenta et al., 2008).

We showed that it is possible to differentiate *S. marcescens* from *E. aerogenes* in as short as half an hour using the DTT. However, 6 h of incubation of bacteria with the DNA is the most appropriate time to see the DNase activity of any bacteria to avoid a misinterpretation of the results. In summary, the DTT is a new method which is simple, rapid, inexpensive and applicable to examine DNase activity of any bacteria.

Acknowledgments

This study was accomplished using the resources of Ankara University, School of Medicine, Department of Microbiology and Clinical Microbiology and supported by FC Biotek Ltd. AS.

References

Boerlin, P., Kuhnert, P., Hussy, D., and Schaellibaum, M. (2003) Methods for identification of *Staphylococcus aureus* isolates in cases of bovine mastitis. *J. Clin. Microbiol.,* **41**, 767–771.

Cunningham, L. (1959) Micrococcal nuclease and some products of its action. *Ann. NY Acad. Sci.,* **81**, 788–791.

Elston, H. R. and Elston, J. H. (1968) Further use of deoxyribonuclease in a screening test for *Serratia*. *J. Clin. Pathol.,* **21**, 210–212.

Fusillo, M. H. and Weiss, D. L. (1959) Qualitative estimation of staphylococcal deoxyribonuclease. *J. Bacteriol.,* **78**, 520–522.

Jeffries, C. D., Holtman, D. F., and Guse, D. G. (1957) Rapid method for determining the activity of microorganisms on nucleic acids. *J. Bacteriol.,* **73**, 590–591.

Lachica, R. V. and Deibel, R. H. (1969) Detection of nuclease activity in semisolid and broth cultures. *Appl. Microbiol.,* **18**, 174–176.

MacFaddin, J. F. (2000) Deoxyribonuclease (DNase) and thermonuclease (Tnase) tests. *In* Biochemical Tests for Identification of Medical Bacteria*,* 3rd ed., ed. by MacFaddin, J. F.,

Lippincott Williams and Wilkins, Philadelphia, PA, pp. 136–159.

Patel, J. B. (2001) 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. *Mol. Diagn.,* **6**, 313–321.

Pimenta, F. P., Souza, M. C., Pereira, G. A., Hirata, R., Jr., Camello, T. C., and Mattos-Guaraldi, A. L. (2008) DNase test as a novel approach for the routine screening of *Corynebacterium diphtheriae*. *Lett. Appl. Microbiol*., **46**, 307–311.

Romero, C. and Lopez-Goni, I. (1999) Improved method for purification of bacterial DNA from bovine milk for detection of *Brucella* spp. by PCR. *Appl. Environ. Microbiol.,* **65**, 3735–3737.

Rothberg, N. W. and Swartz, M. N. (1965) Extracellular deoxyribonucleases in members of the family *Enterobacteriaceae*. *J. Bacteriol.,* **90**, 294–295.

Schreier, J. B. (1969) Modification of deoxyribonuclease test medium for rapid identification of *Serratia marcescens*. *Am. J. Clin. Pathol.,* **51**, 711–716.

Valu, J. A. (1966) Use of the deoxyribonuclease test as an aid in the differentiation of *Paracolobactrum* (*Hafnia*) from *Serratia*. *J. Bacteriol.,* **91**, 467–468.