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Letter to the Editor

A Cheap and Simple Method for DNA Isolation from Endonuclease Producing *Serratia marcescens*

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Dear Editor-in-Chief

After its first description in 1819, *Serratia marcescens* has been considered a harmless saprophytic microorganism until the mid-1900s, when reports about its role as a human pathogen have started to appear. Nowadays, numerous cases of *Serratia* associated infections, including nosocomial outbreaks, have been described (1, 2). Of great interest in these cases are the increased rates of antimicrobial resistance and the associated genes as well as the epidemiological relationship between isolates responsible for intra-hospital outbreaks. However, to study these characteristics, DNA is necessary to be obtained – an issue due to the ability of *S. marcescens* to produce an endonuclease. The protein, with an approximate mass of 26 kDa (EC 3.1.30.2), is part of a family comprising homologous non-specific nucleases with widespread distribution and found in different microorganisms and even mammals. A unique feature of this enzyme is the ability to break down both DNA and RNA molecules. Thus, the catalytic nuclease activity compared to that of *Staphylococcus* spp. is 4-fold higher and more than 30 times more potent than that of DNase I (3, 4).

In addition, cases of Tris-dependent activity of the enzyme have also been described. This phenomenon further complicates the isolation of DNA, as Tris is part of the standard protocols for DNA extraction and elution (5). A study was conducted to test the activity of the enzyme at different Tris concentrations and conditions. It became clear that even at low Tris concentration (0.08 M Tris-HCl), the activity of the enzyme increased 100 times (4).

Numerous methods to obtain *S. marcescens* DNA, which include inactivation of the DNase activity of the enzyme and elimination of the auto degradation process, have been introduced: steps including SDS, phenol-chloroform-isoamylic alcohol, proteinase K, formaldehyde fixation, commercial extraction kits, etc. (6). All these methods under different conditions lead to DNA isolation, but also have their drawbacks: time-consuming protocols, expensive reagents, toxicity, need for additional appliances, etc. (7).

We proposed a cheap, relatively fast and save method to obtain DNA from *S. marcescens*, which can be used in small laboratories with limited budget and resources.

Five isolates of *S. marcescens* that demonstrated a positive double-disc synergy test with amoxicillin/clavulanic acid, ceftazidime and ceftriaxone

for ESBL production were tested. The DNA isolation was done as follows: 4-5 identical bacterial colonies, previously cultivated on McConkey agar for 24 h, were selected and suspended in 200 µl DNase-free water and centrifuged at 10 000 rpm for 5 min. The supernatant fluid was carefully pipetted and discarded. The pellet was resuspended in 200 µl of DNase-free water and heated at 100 ˚C for 20 min. It was centrifuged for 1 min at 10 00 rpm and the supernatant was transferred to a new microcentrifuge tube. Ice cold 95% ethanol (600 µl) and 20 µl sodium acetate (3M) were added and mixed well. The mix was stored for 1 h at – 20 ˚C for DNA precipitation. Then the tubes were spinned for 30 min at 10 000 rpm. The supernatant was discarded and the pellet was washed twice with 70 % ice cold ethanol (500 µl) and spinned for 10 min each time. The alcohol was pipetted and discarded. The pellet was incubated at room temperature for a min with open cap. The air dried pellet was resuspended in 200 µl DNase-free water and stored at – 20 ˚C until used.

Simultaneously with this protocol, a thermal extraction with a standard protocol for our laboratory was carried out. Briefly, bacterial colonies were suspended in 200 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), centrifuged, washed with TE buffer and boiled at 100˚C for 10 min. The supernatant was transferred and stored at -20 ˚C.

To test the new extraction method, a multiplex PCR for *bla*$_{SHV}$ and *bla*$_{CTX-M}$ was done as previously described (8). The PCR products were resolved on 1.5% gel with TAE buffer at 120 V for 45 min and stained with 0.5 µg/ml EtBr. DNA ladder 100 bp (BioLabs, New England) was used. The results are presented on Fig. 1. When using the standard protocol, DNA degradation and smear with barely noticeable (585 bp) or missing bands were observed. Following the new protocol, no degradation and clearly visible specific bands were found. All five isolates were positive for *bla*$_{CTX-M}$ gene (585bp).

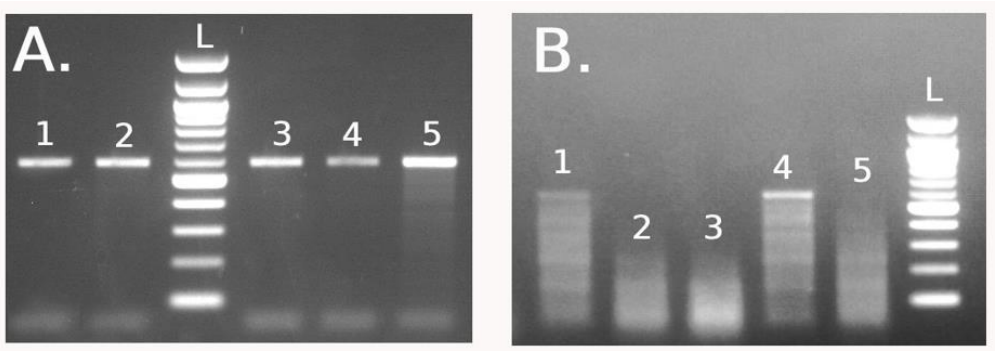

Fig. 1: Detection of *bla*$_{SHV}$ and *bla*$_{CTX-M}$ by Multiplex PCR. **A.** New protocol, Lines 1 - 5 - isolates positive for *bla*$_{CTX-M}$ (585 bp) with no degradation; **B.** Standard protocol, Lines 1 - 5 - isolates with smears, degradation and barely visible or no bands; L – 100 bp DNA Ladder

The proposed protocol, including steps for enzyme heat inactivation and DNA precipitation, is inexpensive and reliable method to obtain a purified DNA from *S. marcescens* isolates.

Conflict of interest

The authors declare that there is no conflict of interest.

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