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Efficient non-enzymatic cleavage of *Staphylococcus aureus* plasmid DNAs mediated by neodymium ions

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ABSTRACT

Staphylococcus aureus plasmids are the main factor in the spreading of antibacterial resistance among bacterial strains that has emerged on a worldwide scale. Plasmids recovered from 12 clinical and food isolates of *S. aureus* were treated with 10 mM free lanthanide Nd^{3+} ions (non-enzymatic cleavage agent) in Hepes buffer (pH 7.5) at 70 °C. Topological forms of plasmids—closed circular (ccc), open circular (oc), and linear (lin)—produced by cleavage at different times were separated using pulsed-field agarose gel electrophoresis. The method is proposed to detect and differentiate several plasmids in the same bacterial strain according to their size.

Staphylococcus aureus is a versatile bacterial pathogen that infects or colonizes a variety of different mammal host species, causing different types of illnesses ranging from minor superficial skin infections to life-threatening systemic diseases, syndromes caused by exotoxins and food poisoning. This species is characterized by rapid evolution leading to substantial strain variability and to the appearance of novel virulent and antibiotic-resistant clones from which methicillin-resistant *S. aureus* (MRSA) strains are found worldwide and most of which are multidrug resistant [1,2]. Mobile genetic elements (MGEs), including plasmids that carry diverse virulence and antimicrobial resistance traits, are investigated with respect to evolution of important *S. aureus* lineages [3–6]. It is generally accepted that *S. aureus* plasmids are the main factor of the spreading of antibacterial resistance among bacterial strains by horizontal gene transfer mediated by bacteriophages [7–9]. According to the size ranging from 1.2 kb to more than 100 kb and the replication mechanism, staphylococcal plasmids are classified into three classes [10]. Recently, an approach for the classification of plasmids in *S. aureus*, which assigns plasmids to rep families based on polymerase chain reaction (PCR) detection of specific *rep* gene sequences, was described [11].

The rapid and simple technique of agarose gel electrophoresis is commonly applied to the study of different topological forms of plasmid DNA [12]. This method is complemented by restriction analysis of plasmids in epidemiological studies [13]. The cleavage of one of the two strands of the covalently closed circular (ccc) form of plasmid DNA is accompanied by its conversion to the open circular (oc) form and, after cleavage of the second strand, to the linear (lin) form. Without digestion, supercoiled plasmids migrated at electrophoretic mobilities that are not a simple function of their molecular weights, thereby making size determinations problematic. The difference in migration rates of topological forms of plasmid DNA could be solved by converting all plasmid forms present in the sample into the lin form. However, there is difficulty in interpreting the plasmid content in uncharacterized strains frequently carrying several plasmids of different types and sizes. The development of a simple and universal method for rapid detection and characterization of plasmid DNAs in epidemiological studies, therefore, is desirable.

Rare earth ions and their complexes derived from a wide range of ligands of varied skeletal structures are known by high efficiency

Abbreviations: MRSA, methicillin-resistant *S. aureus*; MGE, mobile genetic element; PCR, polymerase chain reaction; ccc, closed circular; oc, open circular; lin, linear; EDTA, ethylenediaminetetraacetic acid; PFGE, pulsed-field gel electrophoresis.

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in the phosphate ester bond hydrolysis due to the lack of redox chemistry and a strong Lewis acidity [14,15]. The advantage of lanthanide ions is that they cleave the phosphodiester bonds via a non-oxidative mechanism [16]. It was shown that different lanthanide complexes [17–21] and free lanthanide ions (Eu^{3+}, La^{3+}, Nd^{3+}, Pr^{3+}, and Gd^{3+}) [22] were suitable as non-enzymatic cleavage agents for plasmid DNAs. We pronounced a hypothesis that the time-dependent plasmid DNA cleavage and the creating of DNA forms of different intensities on agarose gel can enable us to distinguish different plasmids present in one strain.

The aim of this work was to evaluate the above-mentioned hypothesis by using the application of lanthanide Nd^{3+} ions for the cleavage of plasmid DNAs isolated from different *S. aureus* strains. Hydrolytic reactions between Nd^{3+} ions and plasmid DNAs were studied by monitoring the conversion of the closed-circular supercoiled DNA (ccc) form to the nicked (oc) and linear (lin) ones.

Materials and methods

Bacterial strains

Plasmid DNAs were isolated from 10 strains of *S. aureus* (MRSA) originated from hospital sources and 2 strains originated from food samples deposited at the Department of Experimental Biology, Faculty of Science, Masaryk University (Brno, Czech Republic) [3,23–25]. The strains contained 38.0 kb conjugative plasmid, 30.0 kb penicillinase plasmid, 27.6 kb enterotoxin D plasmid, 26.0 kb penicillinase plasmid, 4.0 kb tetracycline resistance plasmid, 2.9 kb chloramphenicol resistance plasmid, and 2.4 kb erythromycin resistance plasmid. These plasmids were presented individually in some of the studied strains, whereas they were found in combination with small cryptic plasmids ranging in size from 2.0 to 3.0 kb in the other strains (Table 1).

Plasmid DNA extraction

Control plasmid DNAs pUC19 and pBR322 were isolated from the bacterial strain *Escherichia coli* JM109 (pUC19) and *E. coli* DH5α (pBR322) grown overnight in LB medium by alkaline lysis [26]. Linearized pUC19/*Sma*I (2686 bp) and pBR322/*Sma*I (4322 bp) were prepared according to the restriction endonuclease producer's recommendation (Roche Diagnostics, Indianapolis, IN, USA). Analyzed plasmid DNAs were isolated from *Staphylococcus* strains grown overnight in meat peptone broth prepared from 13.0 g of nutrient broth CM1 (Oxoid, Basingstoke, UK), 3.0 g of yeast extract powder L21 (Oxoid), and 5.0 g of peptone L37 (Oxoid) dissolved in distilled water to 1000 ml (pH 7.4) using a NucleoSpin Plasmid (NoLid) Kit (Macherey–Nagel, Düren, Germany) with the following modification: cell lysis was induced by adding lysostaphin (Dr. Petry Genmedics, Reutlingen, Germany) to a final concentration of 30 μg/ml to the cell suspension and tempered for 180 min at 37 °C [3]. The subsequent steps of isolation were made according to the recommendation of the kit producer. Spectrophotometric measurements were carried out on a NanoPhotometer (Implen, München, Germany).

Cleavage of plasmid DNA by Nd^{3+} ions

Hydrolysis of plasmid DNAs was carried out using a cleavage mixture containing 2 μl of 100 mM solution of $NdCl_3$ (Sigma, St. Louis, MO, USA), 4 μl of 50 mM Hepes buffer (pH 7.5), DNA, and water up to a reaction mixture volume of 20 μl. The amount of each plasmid DNA was checked to approximately 500 ng according to the intensity of bands on agarose gel. All components of the cleavage mixture were centrifuged briefly. Cleavage was carried out in time dependence on intervals of 0, 30, 60, 120, and 240 min at temperatures of 65, 70, and 75 °C. The reaction was stopped by adding 2 μl of 0.5 M ethylenediaminetetraacetic acid (EDTA, pH 8.0). The linear plasmid DNA length sizes were calculated using the software program BioNumerics version 5.1 (Applied Maths, Kortrijk, Belgium).

Gel electrophoresis

Conventional gel electrophoresis was carried out with an Enduro Power Supply, model E0303 (Labnet International, Edison, NJ, USA) in 0.6 or 1.0% agarose (Serva, Heidelberg, Germany) at 2.5 V/cm for 3 h using TBE buffer (45 mM boric acid, 45 mM Tris base, and 1 mM EDTA, pH 8.0). Pulsed-field gel electrophoresis (PFGE) was carried out with a CHEF Mapper electrophoresis system

Table 1
Plasmid patterns of *Staphylococcus* strains characterized in this study.

S. aureus strain	Origin	Source	Number of plasmids	Plasmid size (kb)		Reference
				Determined	Literature	
NRL/St 07/968	National Institute of Public Health Praha (CZ)	Blood culture[a]	1	3.3 ± 0.1	3.0	[3]
NRL/St 05/670	National Institute of Public Health Praha (CZ)	Wound swab[a]	2	3.1 ± 0.1 28.0 ± 0.4	3.0, 27.6	[3]
E46	Faculty Hospital Brno–Bohunice (CZ)	Wound swab[a]	2	4.5 ± 0.1 37.5 ± 0.1	4.0, 38.0	[3]
E60	Faculty Hospital Brno–Bohunice (CZ)	Pus[a]	2	2.7 ± 0.1 2.5 ± 0.1	2.2, 2.4	[3]
E19	Faculty Hospital Brno–Bohunice (CZ)	Wound swab[a]	3	2.5 ± 0.1 2.9 ± 0.1 25.9 ± 0.6	2.4, 2.9, 26.0	[3]
S. carnosus TM300 (pC194)[b]	University of Tübingen (Germany)	Laboratory strain	1	3.1 ± 0.1	2.9	[23]
COL (pT181)	Rockefeller University (USA)	Laboratory strain	1	4.5 ± 0.1	4.4	[24]
UMCG-M4	University Medical Center Groningen (NL)	Pleural fluid[a]	3	2.7 ± 0.17.3 ± 0.1 28.2 ± 0.1	2.0, 7.0, 30.0	[25]
SA1471	University of Veterinary and Pharmaceutical Sciences Brno (CZ)	Food[c]	1	3.4 ± 0.1	Newly characterized	
SA1679	University of Veterinary and Pharmaceutical Sciences Brno (CZ)	Chinese cabbage[c]	1	3.2 ± 0.1	Newly characterized	

Note. CZ, Czech Republic; NL, The Netherlands.
[a] The strain was isolated from a hospital source.
[b] Plasmid bearing resistance to chloramphenicol.
[c] The strain was isolated from a food sample.