Microbial Pathogenesis 51 (2011) 142–148

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Digestion of extracellular DNA is required for giant colony formation of *Staphylococcus aureus*

Chikara Kaito [1], Takanori Hirano [1], Yosuke Omae, Kazuhisa Sekimizu*

Laboratory of Microbiology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 3-1, 7-Chome, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

ARTICLE INFO

Article history:
Received 1 July 2010
Received in revised form
8 April 2011
Accepted 20 April 2011
Available online 27 April 2011

Keywords:
Staphylococcus aureus
Extracellular DNA
Nuclease
Colony spreading

ABSTRACT

Staphylococcus aureus spreads on soft agar surfaces and forms giant colonies. Here, we examined the inhibitory role of extracellular DNA on the colony spreading activity. The double-deletion mutation of *nuc1* and *nuc2*, which encode secretory nucleases, increased extracellular DNA and showed a decreased ability to form giant colonies. The addition of DNase I or micrococcal nuclease to the soft agar restored the ability of the *nuc1–nuc2* double mutant to form giant colonies. In addition, the promoter activities of *nuc1* and *nuc2* in the wild-type strain were elevated in the peripheral region of the giant colony. These findings suggest that the digestion of extracellular DNA by secretory nucleases is required for the colony spreading activity of *S. aureus*.

1. Introduction

Staphylococcus aureus is a pathogenic bacterium that causes various diseases in humans, such as pneumonia, meningitis, and sepsis [1]. Methicillin-resistant *S. aureus* (MRSA) is a serious clinical problem due to its multi-drug resistance [2,3]. To establish therapeutic strategies against *S. aureus*, a better understanding of the physiologic phenomenon of this pathogen is necessary.

S. aureus is a non flagellated Gram-positive bacterium that was previously believed to not translocate [4]. We recently reported that *S. aureus* spreads rapidly (100 μm/min) on the surface of soft agar plates and forms a giant colony, which we called "colony spreading" [5]. In general, bacterial motility is required for virulence [6–8]. The *psm-mec* gene in the SCC*mec* regions of hospital-associated MRSA chromosomes suppresses both *S. aureus* colony spreading and virulence in mice [9,10]. Absence of the *psm-mec* gene in community acquired MRSA can be a reason for its high virulence and colony spreading ability [9,10]. *S. aureus* virulence gene expression and colony spreading are simultaneously regulated by the virulence regulatory element *agr* [11]. Therefore, *S. aureus* colony spreading is assumed to have a positive effect on its virulence. Elucidation of the molecular mechanism of *S. aureus* colony spreading will facilitate our understanding of the infectious processes of the pathogen. In a previous study, we reported that wall teichoic acids were required for colony spreading [5], but the molecular mechanisms remained unclear.

For most pathogenic bacteria, chromosomal DNA released from dead bacteria is an important environmental factor [12], although it is recognized as a foreign substance by the toll-receptor of host immune systems [13]. Extracellular DNA is required for biofilm formation of various bacteria including *S. aureus* [14,15]. In addition, digestion of extracellular DNA by secretory nucleases reduces *S. aureus* biofilm formation [16,17]. Because the colony spreading of *S. aureus* requires water on the surface of soft agar plates [5], we hypothesized that extracellular environments formed with DNA affect colony spreading. In this paper, we describe our finding that digestion of the extracellular DNA by secretory nucleases is required for colony spreading.

2. Results

2.1. Digestion of extracellular DNA is required for colony spreading

We hypothesized that digestion of extracellular DNA by secretory nucleases is required for *S. aureus* colony spreading. We constructed *S. aureus* secretory nuclease mutants and examined their ability to form giant colonies. *S. aureus* extracellular nucleases are encoded by two genes, *nuc1* and *nuc2* [18]. *nuc1* encodes the 18.8 kDa nuclease B, a precursor of the 16.9 kDa nuclease A, and *nuc2* encodes 14.6 kDa thermonuclease [18]. Although a single gene-disrupted mutant of *nuc2* could form giant colonies, a single

* Corresponding author. Tel.: +81 3 5841 4820; fax: +81 3 5684 2973.
E-mail address: sekimizu@mol.f.u-tokyo.ac.jp (K. Sekimizu).
[1] Contributed equally to this work.

0882-4010/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.micpath.2011.04.007

gene-disrupted mutant of *nuc1* and a double gene-disrupted mutant of *nuc1* and *nuc2* formed smaller giant colonies than did the parent strain (Fig. 1A, B). Introduction of plasmids harboring *nuc1* or *nuc2* into the *nuc1–nuc2* double mutant restored the ability to form giant colonies (Fig. 1A, B). The partial complementation of the *nuc1–nuc2* double mutant with p0746 is probably due to overproduction of *nuc1* from a multi-copy plasmid, since transformation of Newman (WT) with p0746 induced fewer branched arms of the giant colony (data not shown). In the giant colonies formed by the *nuc1–nuc2* double mutant, the amount of extracellular DNA was increased compared to the parent strain (Fig. 1C). Nuclease activities were decreased in the *nuc1* mutant and the *nuc1–nuc2* double mutant in liquid culture (Fig. 1D) and in giant colonies (data not shown). In liquid medium, the growth of these gene-disrupted mutants was indistinguishable compared to the parent strain (Fig. 1E). Moreover, the addition of DNase I or micrococcal nuclease to soft agar plates restored the ability of the *nuc1–nuc2* double mutant to form giant colonies (Fig. 2A, B). Addition of an excess amount of DNase I or micrococcal nuclease to the liquid medium did not affect *S. aureus* growth (Fig. 2C, D). These results suggest that digestion of extracellular DNA by secretory nucleases is necessary for *S. aureus* colony spreading.

Decreased colony spreading in the *nuc1–nuc2* double mutant may be due to inhibition by extracellular DNA or the lack of deoxynucleotides, which is the digested product of DNA, that are required for the colony spreading. We examined the former possibility by adding DNA to soft agar plates. The addition of salmon sperm DNA to the soft agar plates inhibited giant *S. aureus* colony formation (Fig. 3A), whereas the addition of salmon sperm DNA to liquid medium did not affect *S. aureus* growth (Fig. 3B). These results suggest that extracellular DNA inhibits *S. aureus* colony spreading on soft agar plates without disturbing growth.

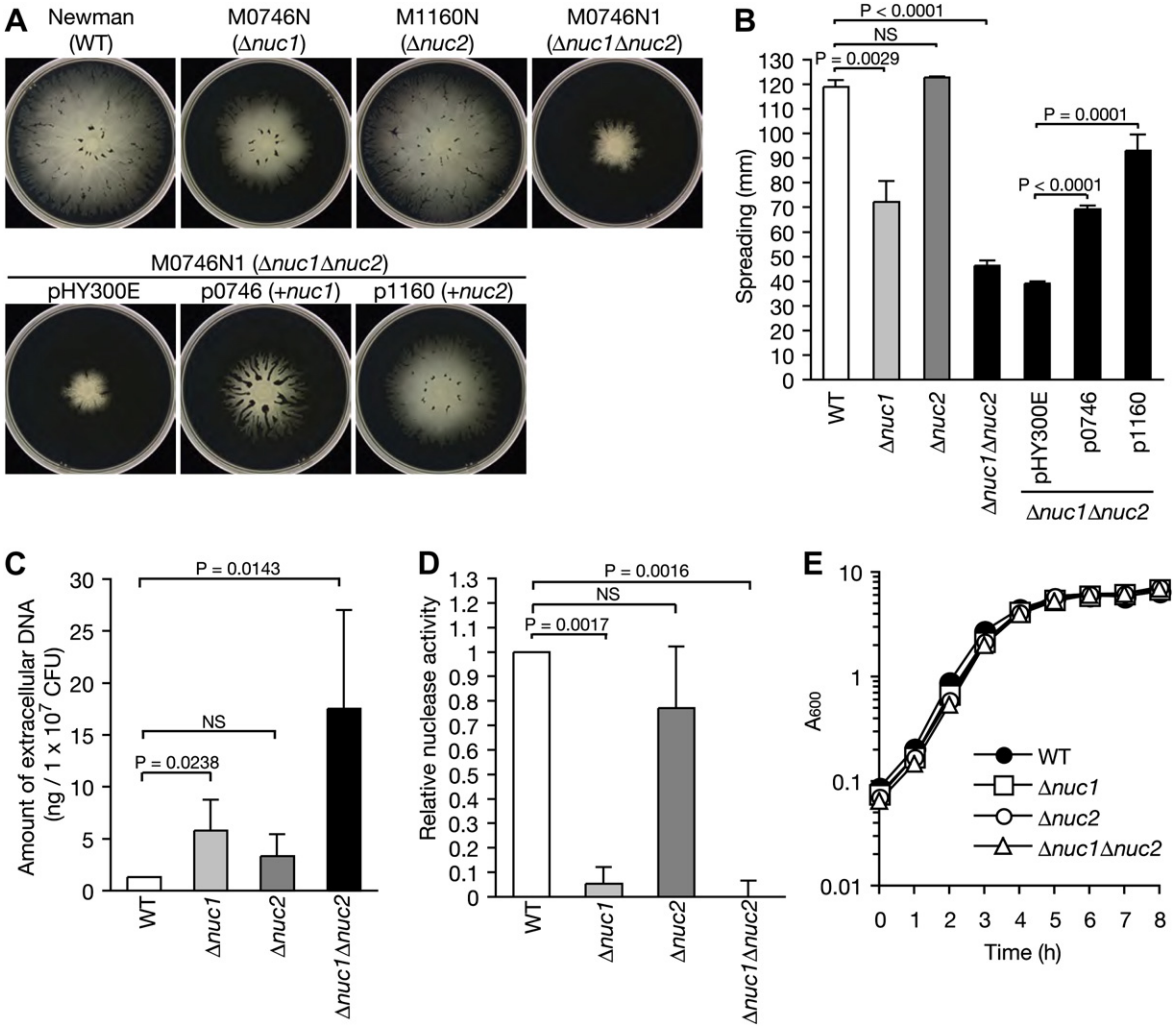

Fig. 1. *nuc1–nuc2* double-disrupted mutant decreases colony spreading. (A) Overnight cultures of *S. aureus* Newman (WT), *nuc1* disrupted-mutant (M0746N, Δ*nuc1*), *nuc2* disrupted-mutant (M1160N, Δ*nuc2*), *nuc1–nuc2* double-disrupted mutant (M0746N1, Δ*nuc1*Δ*nuc2*), and *nuc1–nuc2* double-disrupted mutant (M0746N1) transformed with pHY300E (empty vector), p0746, or p1160 were spotted onto 0.24% agar plates and incubated at 37 °C for 10 h. The CFU of each inoculated strain was as follows: Newman, 1.6×10^6; M0746N, 3.1×10^6; M1160N, 2.9×10^6; M0746N1, 2.0×10^6; M0746N1/pHY300E, 1.0×10^6; M0746N1/p0746, 2.6×10^6; M0746N1/p1160, 1.1×10^6. (B) Halo diameters in panel A. Data are presented as means ± standard deviations of three independent experiments. *P* values calculated by Student's *t* test are shown. (C) Amounts of extracellular DNA in giant colonies of Newman (WT), *nuc1* disrupted-mutant (Δ*nuc1*), *nuc2* disrupted-mutant (Δ*nuc2*), and *nuc1–nuc2* double-disrupted mutant (Δ*nuc1*Δ*nuc2*) were measured using quantitative RT-PCR for 16S rDNA. Data are presented as means ± standard deviations from three independent experiments. *P* values calculated by Student's *t* test are shown. (D) Nuclease activities in culture supernatants of Newman (WT), *nuc1* disrupted-mutant (Δ*nuc1*), *nuc2* disrupted-mutant (Δ*nuc2*), and *nuc1–nuc2* double-disrupted mutant (Δ*nuc1*Δ*nuc2*) were measured. Data are presented as means ± standard deviations of three independent experiments and as relative values against the nuclease activity of Newman. *P* values calculated by Student's *t* test are shown. (E) Growth curves of Newman (WT), *nuc1* disrupted-mutant (Δ*nuc1*), *nuc2* disrupted-mutant (Δ*nuc2*), and *nuc1–nuc2* double-disrupted mutant (Δ*nuc1*Δ*nuc2*).