Institut Pasteur

Microbes and Infection 17 (2015) 456–461

www.elsevier.com/locate/micinf

Short communication

Staphylococcus aureus dry stress survivors have a heritable fitness advantage in subsequent dry exposure

Lisa Maudsdotter*, Saki Imai, Ryosuke L. Ohniwa, Shinji Saito, Kazuya Morikawa*

Faculty of Medicine, University of Tsukuba, 305-8575 Tsukuba, Japan

Received 9 September 2014; accepted 24 February 2015
Available online 4 March 2015

Abstract

Staphylococcus aureus is a major cause of hospital-acquired infections. The ability to survive on abiotic surfaces is an important characteristic that facilitates transmission between human hosts. We found that *S. aureus* survivors of dry surface incubation are resistant to subsequent dry stress exposure. Survivors also had reduced sensitivity to the disinfectant chlorhexidine gluconate, but not to ethanol. By using a set of mutants in cardiolipin synthase genes, we further demonstrated that the housekeeping cardiolipin synthase, Cls2, was significant for survival on dry surface. Taken together, this study provides insights into *S. aureus* survival outside of a host.
© 2015 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

Keywords: Staphylococcus aureus; Cardiolipin; Dry stress; Adaptation; Transmission

1. Introduction

Staphylococcus aureus is a major cause of severe hospital-acquired infections such as pneumonia and bacteraemia, associated with significant mortality and morbidity [1]. This bacterium is tolerant of desiccation; its normal colonisation sites, the nasal nares and skin, are relatively dry environments and it can also survive weeks or even months on dry surfaces outside a host [2,3]. Good survival on abiotic surfaces facilitates the spread of bacteria [4,5]. Other bacterial species have been shown to acquire heritable fitness advantage for a certain environment; for example, biofilm and stationary phase culture after pre-exposure to that particular environment [6–8]. In this study, we aimed to test how dry surface incubation influenced the desiccation tolerance of *S. aureus*.

S. aureus has a variety of adaptive mechanisms enabling successful colonisation under harsh conditions [9]. The membrane phospholipid composition is one factor that changes in response to the environment [10,11]. The major phospholipids of *S. aureus* are phosphatitylglycerol, cardiolipin (CL) and lysylphosphatidylglycerol [12]. CL is formed by connection of two phosphatitylglycerols by one of the two cardiolipin synthases (Cls1 and Cls2); Cls2 is the housekeeping CL synthase and Cls1 is functional during stress conditions such as acidic pH and high salinity [10,13,14]. CL protects against membrane permeabilisation by the antibiotic daptomycin and is required for long-term survival under high salinity [14,15]. However, the impact on CL on the desiccation tolerance of *S. aureus* is so far unexplored.

The characterisation of the desiccation tolerance of *S. aureus* has implications for limiting the spread of disease-causing strains. In this study we found that *S. aureus* survivors of dry surface incubation have increased fitness in subsequent exposure to dry stress. Furthermore, we identified the membrane lipid composition as one factor that influences desiccation tolerance.

2. Materials and methods

2.1. Bacterial strains and growth conditions

S. aureus N315 and cardiolipin synthase deletion mutants have previously been described [14]. Bacteria were cultured in

* Corresponding authors. Tel./fax: +81 29 8533928.
E-mail addresses: maudsdotter@md.tsukuba.ac.jp (L. Maudsdotter), morikawa.kazuya.ga@u.tsukuba.ac.jp (K. Morikawa).

http://dx.doi.org/10.1016/j.micinf.2015.02.004
1286-4579/© 2015 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

brain heart infusion (BHI) broth (Becton Dickinson and Company, MD, USA) for approximately 16–20 h at 37 °C with shaking, before experiments. For bacterial enumeration BHI plates with 1.5% agar (Wako Pure Chemical Industries, Ltd. Japan) were used.

2.2. Survival on dry surface

S. aureus overnight cultures containing 10^7 cfu in 10 µl were spread over 0.3 cm^2 in a polystyrene 96-well plate (Thermo Scientific, MA, USA) and dried under airflow for 30 min at room temperature. In some experiments bacteria were washed and resuspended in 0.9% NaCl before drying. The plate was then incubated at room temperature for up to 7 days. After experiments, bacteria were recovered by addition of 100 µl 0.9% NaCl, scraping and extensive rinsing. Survival was analysed by plating serial dilutions.

2.3. Pre-exposure to dry surface

Exposure to dry surface was performed by incubating the bacteria for 7 days at room temperature in a 96-well plate as described above. The bacteria were then recovered, cultured in BHI overnight at 37 °C and frozen glycerol stocks were prepared and stored at −80 °C. These bacteria were termed ‘pre-exposed’. Before survival experiments the ‘pre-exposed’ and wild-type bacteria were cultured in BHI. For each strain, at least three independently pre-exposed bacteria were prepared. In some experiments pre-exposed bacteria passaged five times in BHI were used. The re-culturing was done by diluting overnight cultures 1:1000 and incubating overnight at 37 °C with shaking.

2.4. Bactericidal assay

Bacteria (10^7 cfu) were treated with 100 µl of 2% chlorhexidine gluconate, 0.1% or 0.03% sodium dodecyl sulphate (SDS) or 50% ethanol for 1 min at room temperature. Samples were then diluted in 0.9% NaCl and washed three times before survival was analysed by plating serial dilutions.

2.5. Lipid extraction and thin-layer chromatography

Cells were recovered from overnight culture or dry surface and suspended in PBS. Lipid extraction and thin-layer chromatography were conducted as previously described [14]. Lipids applied on TLC were normalized by OD_{600} values.

2.6. Sequencing analysis

Six colonies were isolated from N315 pre-exposed to dry surface following after the five time passage in BHI (3 colonies from pre-exposed:1, 3 from pre-exposure:2). The dry stress resistance of each colony was as high as pre-exposed N315 (data not shown). Their *cls2* loci were amplified by PCR with primers cls2seqfw1:5′-CTAATAACTTTATCGCAACACTGTTTAA-3′ and cls2seqrv:5′-AAGATAAAAATGACTTCAATTCAACATA-3′. The resultant PCR products were submitted to direct sequencing (Fasmac Co., Ltd. Japan).

2.7. Statistical analysis

Differences between groups were analysed by ANOVA (analysis of variance) followed by Dunnett's multiple comparison of means test, with Graph Pad Prism 6 software. Pairwise comparisons of bacteria before and after additional passaging were analysed by ANOVA with Sidak's test. Statistical analysis of viability or relative viability was performed on log values. A p value below 0.05 was considered statistically significant. Data are expressed as means and error bars represent standard error. Each experiment was repeated at least three times.

3. Results

3.1. Dry stress survivor population is heritably resistant to dry stress

In the present work, we investigated the ability of *S. aureus* to withstand incubation on dry surface. *S. aureus* overnight culture was spread over a 0.3 cm^2 plastic surface (10^7 cfu in 10 µl) and incubated at room temperature. After 7 days the viable counts had decreased to approximately 50% of the initial inoculum (Fig. 1A). To evaluate the ability of *S. aureus* to adapt to a dry surface we next generated a set of pre-exposed bacteria. The pre-exposure was performed by dry surface incubation for 7 days as above. Interestingly, the bacteria previously exposed to a dry surface survived dry stress significantly better than bacteria without pre-exposure (Fig. 1B). Drying bacterial cultures in saline reduced survival by 2–3 log. However, the pre-exposed bacteria had a fitness advantage also in this assay (Fig. 1C). To test whether the pre-exposed phenotype was maintained by re-cultivation, the pre-exposed bacteria were passaged in BHI five times. Re-cultured pre-exposed bacteria retained their fitness advantage over the wild-type (Fig. 1D). We next tested survival after 1 min incubation in the disinfectant chlorhexidine gluconate, the surfactant SDS or ethanol. Strikingly, bacteria pre-exposed to dryness had reduced sensitivity to chlorhexidine gluconate and SDS (Fig. 1E–F). Previous exposure to dryness did however not influence sensitivity of *S. aureus* to ethanol (Fig. 1G). Overall, exposure to dryness increases fitness of the surviving *S. aureus* population in subsequent dry exposure.

3.2. Cardiolipin synthase 2 promotes survival on dry surface

The phospholipid CL protects *S. aureus* against certain stress conditions [14,15]; however, little is known about the impact of CL on dry stress tolerance. Thus, we analysed survival of deletion mutants deficient in one or both of the cardiolipin synthases (Cls1 and Cls2) after 7 days incubation on a

Download English Version:

https://daneshyari.com/en/article/6135663

Download Persian Version:

https://daneshyari.com/article/6135663

Daneshyari.com