Supporting Information

Microbiome precision editing: Using PEG as a selective fermentation initiator against methicillin-resistant *Staphylococcus aureus*

Ming-Shan Kao[1, 2], Stephen Huang[3], Wei-Lin Chang[4], Ming-Fa Hsieh[5], Chun-Jen Huang[2], Richard L. Gallo[1], and Chun-Ming Huang[1,6] *

[1]Department of Dermatology, University of California, San Diego, CA, USA
[2]Department of Biomedical Sciences and Engineering, National Central University, Taoyuan, Taiwan
[3]Surface Bioadvances Inc., San Diego, CA, USA
[4]Department of Life Sciences, National Central University, Taoyuan, Taiwan
[5]Department of Biomedical Engineering, Chung Yuan Christian University, Taoyuan, Taiwan
[6]Moores Cancer Center; University of California, San Diego, CA, USA

Running title: Microbiome precision editing using PEG as a SFI against MRSA

*__Correspondence:__ Professor, Chun-Ming Huang, Department of Dermatology, University of California, San Diego. 3525 John Hopkins Court, Rm276, San Diego, CA, 92121, USA
E-mail: chunming@ucsd.edu
Tel: 858-822-4627
Fax: 858-642-1435

Materials and methods

Fermentation in solid media

A *S. epidermidis* strain isolated from human skin with the bankit number (BankIt1661928), sequenceID (Seq1), and GenBank accession number (KF683955) [42] and a *S. aureus* strain isolated from human skin with the bankit number (SUB1437698), sequenceID (SAB), and GenBank accession number (KX156339) were used for study of fermentation in solid media according to the protocol as described in Section 2.3. Materials and methods. Briefly, *S. epidermidis* or *S. aureus* (10^6 CFU/200 µl/well) were mixed with 2% molten (w/v) agar with/without 20 g/l (2%) PEG-DMA in 0.002% (w/v) phenol red-containing rich media in a 96-well V-bottom polypropylene (PP) microtiter plate at 37°C for two days. Controls include 2% molten agar containing rich media plus PEG-DMA or bacteria alone.

PEG-DMA fermentation of bacteria

S. epidermidis (ATCC12228) or USA300 (10^5 CFU/ml) was incubated in aqueous rich media (10 ml) in the absence and presence of 20 g/l (2%) PEG-DMA at 37°C for two days. Control includes rich media plus PEG-DMA without bacteria. The pH values of media were measured after incubation for two days.

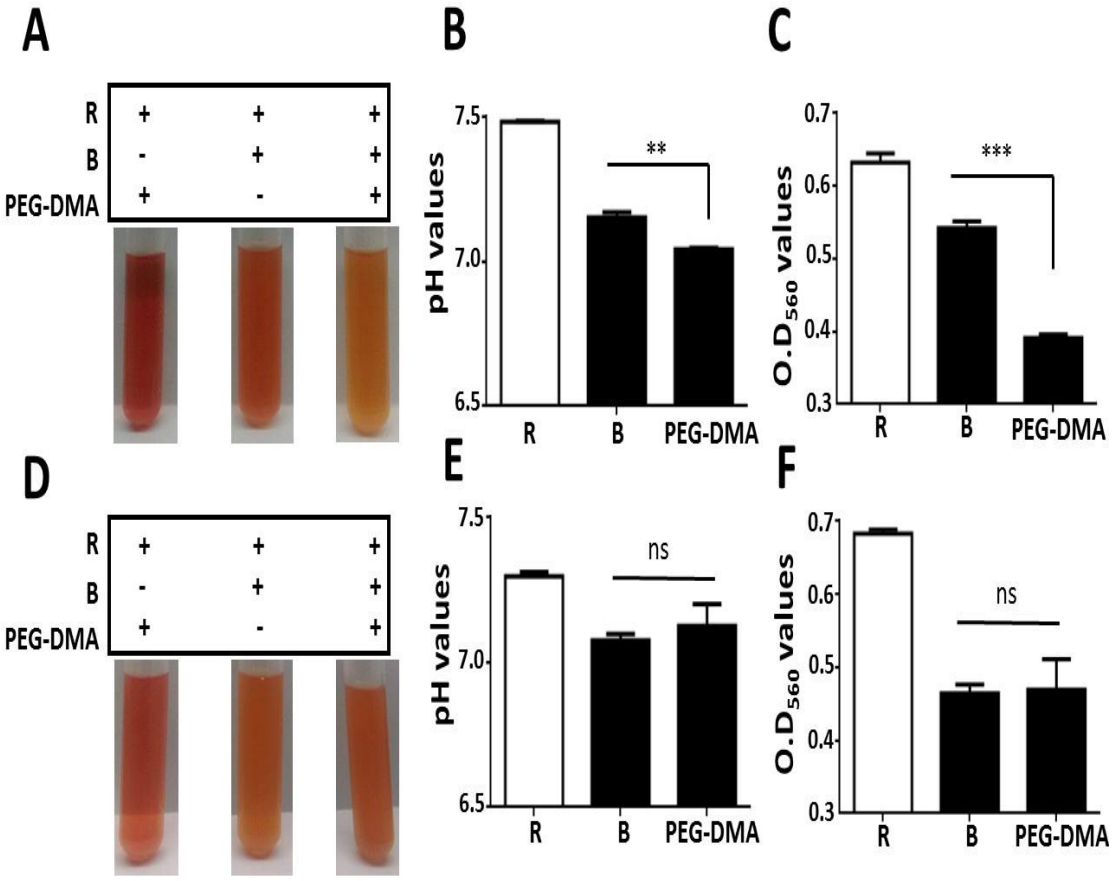
The 0.002 % (w/v) phenol red (Sigma) in rich media with PEG-DMA served as an indicator, changing the color from red-orange to yellow due to fermentation. The color change of phenol red from red-orange to yellow was monitored by the decrease in the OD at 560 nm (OD_{560}) using a UV-scanning spectrophotometer (WTW**,** SpectroFlex 6600**,** Germany).

Supplementary Figure 1. PEG-DMA fermentation of *S. epidermidis* and USA300. The rich media (R) plus PEG-DMA without bacteria (B) were used as a control. (**A**-**C**) *S. epidermidis* (ATCC12228) or USA300 (**D**-**F**) (10^5 CFU/ml) was incubated in rich media in the absence and presence of 20 g/l (2%) PEG-DMA at 37°C for two days. The pH (**B**, **E**) and OD_{560} (**C**, **F**) values of media with PEG-DMA alone, bacteria alone or PEG-DMA plus bacteria are quantified. The pH and OD_{560} values in the media with PEG-DMA plus *S. epidermidis* were significantly lower than those in the media with *S. epidermidis* alone. No decrease in the pH and OD_{560} values were detected when 2% PEG-DMA was present in the culture of USA300. These results indicated that PEG-DMA fermentation occurred in the culture of *S. epidermidis*, but not USA300. Results were shown as the mean ± SD of three independent experiments. $^{**}P<0.01$; $^{***}P<0.001$ (two-tailed t-tests). ns = non-significant.

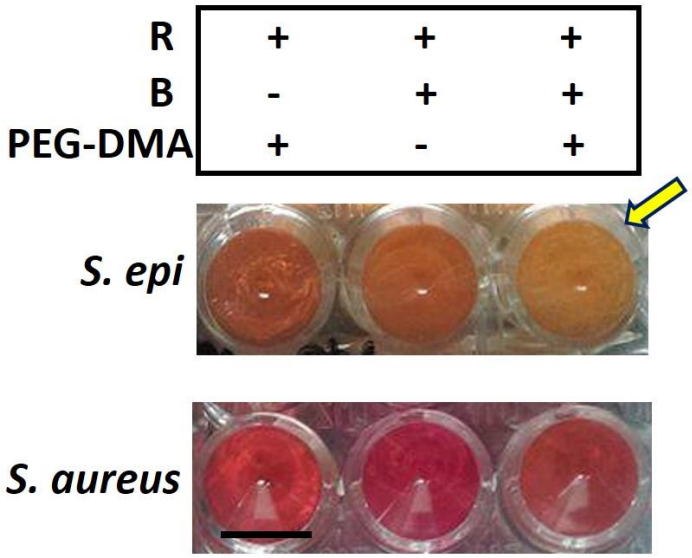
Supplementary Figure 2. PEG-DMA induces the fermentation of *S. epidermidis*, but not *S. aureus*. A *S. epidermidis* strain (*S. epi*) or a *S. aureus* strain (10^6 CFU on 200 µl agar top) isolated from human skin were mixed with 2% molten agar and cultured in rich media (R) in the presence or absence of 2% PEG-DMA in a 96-well V-bottom PP microtiter plate at 37°C for two days. Controls included molten agar containing rich media plus PEG-DMA or bacteria (B) alone. Bacterial fermentation in solid media was indicated by adding 0.002% (w/v) phenol red, which changes its color from red-orange to yellow (arrow).

Supplementary Figure 3. No effect of PEG-DMA hydrogel alone and acrylamide gel laden with or without *S. epidermidis* on the USA300 growth in skin wounds. Ten min after inoculation of USA 300 (10^6 CFU/1 µl PBS), the wounded sites made on the dorsal skin of the ICR mice were applied with (+) or without (-) a PEG-DMA hydrogel (**A**, **B**) or acrylamide gel laden with (*S. epi* +) or without (*S. epi* -) *S. epidermidis* (**C**, **D**). The CFUs in the wound were counted by plating serial dilutions (1:10^0-1:10^5) of the wound homogenates on phenol-red supplemented MSA plates 24 h after USA300 inoculation and displayed as the mean ± SD of three separate experiments with 3 mice per group in each experiment. ns = non-significant.

Supplementary Figure 1.



Supplementary Figure 2.



Supplementary Figure 3.