Culture of extracellular protease-producing and non-producing bacterial strains for antimicrobial activity assay

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Introduction

To determine the specificity of the microbial proteases in triggering the respiratory proteins to produce bactericidal ROS, we compared the level of bacterial clearance of protease-producing and non-producing strains of typical Gram-negative and Gram-positive bacteria. For Gram-negative bacteria, we chose *Pseudomonas aeruginosa* strain PAO-Iglewski which produces PAE (the major extracellular protease virulence factor), and the PAE-knockout mutant PAO-B1A1 which does not produce PAE[1]. For Gram-positive bacteria, we cultured *Staphylococcus aureus* PC1839 strain which produces active V8-protease, and AK3 strain which is a V8 protease-mutant[2]. The genes controlling PAE in *P. aeruginosa* and the V8-protease in *S. aureus* are under the control of quorum-sensing signals. Therefore the culture conditions which facilitate the production of active extracellular proteases are utilized.

Procedure

1. Inoculate single colony of *P. aeruginosa* and *S. aureus* into 10 ml each of Luria-Bertani (LB) broth and Tryptone Soy Broth (TSB), respectively, and shake the culture at 200 rpm for 16 h at 37 ºC.
2. For *P. aeruginosa*, in order to induce the production of PAE, dilute the overnight cultures of PAO-Iglewski and PAO-B1A1 with LB broth to 10^3 cfu/ml, and incubate as standing cultures at 37 ºC for 48 h[1].
3. For *S. aureus*, dilute the overnight cultures of each strain in TSB until OD_{600nm} reaches 1.0, and shake the culture at 220 rpm, 37 ºC for 4 h.
4. For the naturally occurring *Bacillus* species, dilute the overnight culture with marine broth until OD_{600nm} reaches 0.5, and then shake at 220 rpm, 37 ºC for 2 h.
5. Collect the bacterial cultures by centrifugation at 8000 x g for 10 min.
6. To evaluate the extracellular protease production, filter the supernatant through 0.22 µm pore-sized membrane-filter and measure the soluble protease activity in the filtered bacterium-free culture medium using the Azocoll protease assay (see

protocol #12).

Anticipated Results

1. For *P. aeruginosa*, the typical readout of the extracellular protease activity is: A550=0.4-0.7 for PAO-Iglewski, and A550<0.05 for PAO-B1A1.
2. For *S. aureus* PC1839 and ATCC49775, the typical readout of the extracellular protease activity assay is A550= 0.2-0.3, whereas for AK3 strain and the clinical MRSA strains, the typical readout is A550 <0.05.
3. For the naturally occurring *Bacillus* strains, the typical readout of the protease-producing strains is A550=0.3-0.5, while that of the protease non-producing strains is A550< 0.05.

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