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

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Method Article

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Abstract

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.

References

1. Toder, D. S., Ferrell, S. J., Nezezon, J. L., Rust, L. & Iglewski, B. H. lasA and lasB genes of _Pseudomonas aeruginosa_: analysis of transcription and gene product activity. _Infect Immun_. **62**, 1320-1327 \

(1994). 2. Karlsson, A., Saravia-Otten, P., Tegmark, K., Morfeldt, E. & Arvidson, S. Decreased amounts of cell wall-associated protein A and fibronectin-binding proteins in _Staphylococcus aureus_ sarA mutants due to up-regulation of extracellular proteases. _Infect Immun_. **69**, 4742-4748 \(2001).