A target-specific whole cell assay for antibacterial drug discovery

Jun Wang (jun_wang2@merck.com)
Merck Research Laboratories
Lorraine Hernandez
Srinivas Kodali
Doris Cully
Sheo Singh

Method Article

Keywords: FabF, platensimycin, fatty acid synthesis, over-expression, S. aureus

Posted Date: June 23rd, 2006

DOI: https://doi.org/10.1038/nprot.2006.130

Abstract

Introduction

Fatty acids are essential building blocks used in the assembly of many important bacterial components such as phospholipids, lipoproteins and lipopolysaccharides. Because of this type II fatty acid biosynthesis \(FASII) has become an attractive target for mechanism-based drug discovery approaches[1-5]. In the search for FASII inhibitors, both enzymatic and whole cell based screens have been used. Unlike _in vitro_ enzymatic assay screens, whole cell based assays have the advantage of only selecting compounds that are able to penetrate cells and reach intracellular targets. Despite this advantage, most of the compounds found in whole cell screens show poor target selectivity. Here we describe a target selective _S. aureus_ whole cell assay that combines agar-diffusion and protein over expression techniques. This agar based two-plate differential sensitivity assay was used to help confirm the newly discovered antibiotic platensimycin inhibited bacterial growth by specifically targeting the essential FASII enzyme FabF[6].

Reagents

Reagents: For this study Miller's LB \(Invitrogen), select agar \(Invitrogen), chloramphenicol \(Sigma), anhydrotetracycline \(Clontech), single well 86 x 128 mm OmniTrays \(Nunc), and 96-well replicator pin lids \(Nunc) were used. In addition, _S. aureus_ cells \(RN4220) carrying the tet-inducible expression plasmid pTet15 or pTet15-FabF were used.

Equipment

Equipment needed for this assay include a spectrophotometer capable of reading optical densities at 600 nm, and a 37°C shaking incubator.

Procedure

1) _S. aureus_ strains carrying the plasmid pTet15 \(control) or pTet15-FabF were inoculated from frozen cultures into 25 mls Miller's LB containing 34 µg/ml chloramphenicol and grown overnight at 37°C with shaking \(220 rpm). 2) Cultures were diluted in LB to a final OD 600 of 2. For each strain, one ml of diluted culture was added to 25 mls LB/1.2% select agar \(autoclaved and cooled to 50°C) containing 15 µg/ml chloramphenicol and 45 ng/ml anhydrotetracycline, poured into an OmniTray with a 96-well pin lid, and allowed to solidify at room temperature. 3) After solidification, pin lids were removed and 10 µl of various concentrations of platensimycin in DMSO was added to wells on both the control and FabF over expressing plates. 4) Plates were incubated overnight at 37°C and imaged.

Timing

Three days total. \(2 overnight incubations and 1 day with 2-3 hours hands-on time.)

Critical Steps

For this assay it is important to determine the amount of anhydrotetracycline to use for optimal protein expression from the tet-inducible plasmid. This range usually varies between 1 and 100 ng/ml. Additionally the melted LB/agar should not be hotter than 50°C before the bacteria is added. Temperatures greater than 50°C will result in some degree of cell death.

Anticipated Results

When an antibacterial compound is applied to a well of an agar plate seeded with dividing bacterial cells, a circular zone around the well is formed where bacterial growth has been inhibited. Generally, a larger zone indicates high sensitivity of the bacteria to the compound while a smaller zone indicates a lower degree of sensitivity. Due to higher intracellular concentrations of FabF, _S. aureus_ cells over expressing FabF should be less sensitive to compounds that specifically target FabF. Therefore FabF-specific drugs should form smaller zones of inhibition on the FabF overexpressing plate than on the control plate. Zone sizes formed by compounds that are not specific for FabF should be similar for both the control and FabF over expressing plates. In the absence of the inducer anhydrotetracycline, _S. aureus_ cells carrying pTet15-FabF were as sensitive to platensimycin as the control strain as measured by zone of inhibition sizes \(Fig. 1). In the presence of the inducer, however, pTet15-FabF showed increased resistance to platensimycin as measured by smaller or nonexistent zones of inhibition \(Fig. 1). This shows that over expression of FabF specifically leads to platensimycin resistance confirming FabF is the target of platensimycin.

References

1. Campbell, J. W. & Cronan, J. E. Bacterial fatty acid biosynthesis: Targets for antibacterial drug discovery. Annu. Rev. Microbiol. 55, 305-332 \(2001). 2. Zhang, Y.-M., Marrakchi, H., White, S. W. & Rock, C. O. The application of computational methods to explore the diversity and structure of bacterial fatty acid synthase. J. Lipid Res. 44, 1-10 \(2003). 3. Heath, R. J. & Rock, C. O. Fatty acid biosynthesis as a target for novel antibacterials. Curr. Opin. Investig. Drugs 5, 146-53 \(2004). 4. Smith, S., Witkowski, A. & Joshi, A. K. Structural and functional organization of the animal fatty acid synthase. Prog. Lipid Res. 42, 289-317 \(2003). 5. White, S. W., Zheng, J., Zhang, Y. M. & Rock, C. O. The structural biology of type II fatty acid biosynthesis. Annu. Rev. Biochem. 74, 791-831 \(2005). 6. D'Agnolo, G., Rosenfeld, I. S. and Vagelos, P. R. Multiple forms of beta-ketoacyl-acyl carrier protein synthetase in Escherichia coli. J. Biol. Chem. 250, 5289-5294 \(1975).

Figures

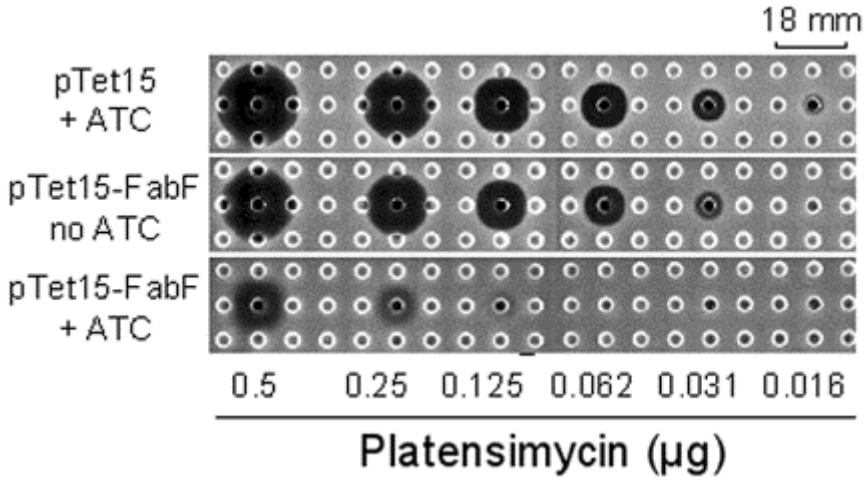

Figure 1

FabF target-specific whole cell assay FabF was expressed in _S. aureus_ strain RN4220 from the plasmid pTet15 under control of a tetracycline-inducible promoter. Under inducing conditions with 45 ng/ml anhydrotetracyclin, the pTet15-FabF strain seeded in LB/agar showed increased resistance to platensimycin compared to the control strain as measured by zone of inhibition sizes. Under non-inducing conditions pTet15-FabF showed similar sensitivity to platensimycin as the control strain.