Detection of Bacterial Gene Expression by mRNA PNA FISH

A New User Friendly and Rapid Molecular Test

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Detection of Bacterial Gene Expression by mRNA PNA FISH –
A New User Friendly and Rapid Molecular Test
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Introduction
• Fluorescence in situ hybridization (FISH) is primarily used for microbial species identification.
• Bacterial mRNA molecules which hold information on expressed functional genes e.g. antibiotic resistance have a much shorter half-life (often only few minutes) than rRNA and are generally much less abundant (1% or less of rRNA abundance).
• Therefore, so far it has only been possible to target and visualize microbial mRNA using very laborious protocols.

Objective
The aim was to develop a PNA FISH technique that provides an alternative to FISH for the mRNA molecules responsible for the antibiotic resistance (mecA) in methicillin resistant Staphylococcus aureus (MRSA) in an end-user friendly and rapid assay format (2 hrs) and combine this with species identification.

Methods

**Induction in tube – 30 minutes**
Add blood culture from ventilation needle to the tube.
Add pre-mixed cefoxitin/TSB solution to the tube.
Incubate at 35 ± 2°C for 40 min.

**Fixation – 10 minutes**
Add 10 μL of induced blood culture to the well on the microscope slide.
Add fixation solution to each well on microscope slide and mix gently to emulsify.
Heat slides at minimum 80°C for 2 min. and transfer slides to PNA FISH Workstation pre-heated to 55°C ± 1°C.
Add 100 μL (or fill slides well) 100% MeOH to the slide well.
Incubate slides for 5 min. on PNA FISH Workstation at 55°C ± 1°C.

**Hybridization – 30 min.**
Same as current PNA FISH

**Rinse, Stringent Wash, Mount and Examination – 35-40 min.**
Same as current PNA FISH for Gram-negative rods.

**Validation**
As a benchmark for the mRNA PNA FISH assay reverse transcriptase quantitative PCR (RT-qPCR) measurements of both 16S rRNA and mecA mRNA were used.

Conclusion
• The mRNA PNA FISH technique is a new molecular test that provides a phenotypic antibiotic resistance answer.
• Great potential for coupling species identity to expression of selected genes on single cell level.
• Can shed light on heterogeneities in gene expression for instance in complex and stratified microbial systems.
• The method has great potential in different clinical applications but also in industrial and environmental settings.

Results

**Fig 1:** Uninduced MRSA show strain dependent mecA expression in PNA FISH.
Red (negative): Strains that do not express the mecA gene without induction.
Green (positive): Strains that express the mecA gene constitutively.
Blue (neg/pos): Strains that both show individual cells with and without mecA expression.
All strains were mecA positive upon induction with the antibiotic cefoxitin.

**Fig 2:** Simultaneous determination of species and gene expression. The 16S rRNA of S. aureus was targeted with a red species identification probe and mecA mRNA with green probes.
Panel A: mecA mRNA in a sample with only methicillin resistant S. aureus (MRSA).
Panel B: Simultaneous detection of a mixture of MRSA and methicillin susceptible S. aureus (MSSA) using both probes. MRSA appear yellow, because MRSA bind both the red probes for S. aureus and the green probes for mecA, while MSSA appear red, because these only bind the red species identification probe.
Panel C: Both types of probes are applied, but in this instance on a mixture of MRSA (yellow) and methicillin resistant coagulase negative staphylococcus (green). The latter expresses the mecA-gene (green probe) but because they are not S. aureus they do not bind the red species identification probe (165 rRNA).

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