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A compendium of antibiotic-induced transcription profiles reveals broad regulation of *Pasteurella multocida* virulence genes

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Abstract

The transcriptional responses of *Pasteurella multocida* to eight antibiotics with known mode of actions (MoAs) and one novel antibiotic compound with an unknown MoA were collected to create a compendium of transcriptional profiles for MoA studies. At minimal inhibitory concentration the three bactericidal compounds enrofloxacin, cefquinome and the novel compound had a minor impact on gene regulation with approximately 1% of the *P. multocida* genome affected, whilst the bacteriostatic compounds florfenicol, tilmicosin, rifampin, trimethoprim and brodimoprim regulated 20% of the genome. Novobiocin was special in that it regulated 40% of all *P. multocida* genes. Regulation of target genes was observed for novobiocin, rifampin, florfenicol and tilmicosin and signature genes were identified for most antibiotics. The transcriptional profile induced by the novel compound was unrelated to the compendium profiles suggesting a new MoA. The transcription of many *P. multocida* virulence factors, particularly genes involved in capsule synthesis and export, LPS synthesis, competence, adherence and iron transport were altered in the presence of antibiotics. Virulence gene transcription was mainly negatively affected, however the opposite effect was also observed in the case of rifampin where the up-regulation of the *tad* locus involved in tight adherence was seen. Novobiocin and trimethoprim caused a marked reduction in the transcription of capsule genes, which correlated with a concomitant reduction of the capsular layer on the surface of *P. multocida*. The broad negative impact on virulence gene transcription supports the notion that the therapeutic effect of some antibiotics could be a combination of growth and virulence inhibition.

Keywords: *Pasteurella multocida*; Antibiotic; Microarray; Virulence

1. Introduction

Bacteria respond to antibiotic stress with a transcriptional reflex to counteract the assault on essential processes such as cell wall synthesis,

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translation, transcription and replication. A very intuitive response to an antibiotic attack is the up-regulation of target gene transcription to compensate the inhibited target molecules. As the inhibition of target proteins causes alterations in the metabolic network of the cell, antibiotic-induced gene regulation is not limited to target genes, but triggers a complex secondary transcriptional response in an attempt to balance stressed physiology. The extent of regulation in response to an antibiotic attack is time- and dose-dependent (Lin et al., 2005; Shaw et al., 2003) and leads to an avalanche of regulatory responses often affecting the transcription of hundreds of genes. Global analysis of transcriptional data showed that antibiotics can leave transcriptional traces that reveal the inhibited target and often the transcriptional profile can be used as a characteristic transcriptional fingerprint for a particular antibiotic, substance class or inhibited target. Examples are ribosomal inhibitors like macrolides, which induce the transcription of ribosomal genes (Ng et al., 2003), or the fluoroquinolones, which induce the transcription of SOS response genes (Gmuender et al., 2001; Kaldalu et al., 2004). When the transcriptional profiles of antibiotics with known mode of actions (MoAs) are compiled into a database, a compendium of transcriptional profiles is created, which allows the comparison with transcriptional responses of novel compounds with unknown MoAs. A match in profiles suggests that the novel compound inhibits a target also exploited by a compendium antibiotic, while a distinct profile would therefore point to a new MoA. Compendiums of transcriptional profiles from gram-positive and gram-negative bacteria have increasingly been used to obtain first indications of the MoAs of novel compounds (Boshoff et al., 2004; Hutter et al., 2004; Freiberg et al., 2005). However, these studies differed with respect to the organisms, the antibiotics, the dose and the duration of treatment, making it difficult to identify common regulatory mechanisms across species. It does appears though, that besides the inhibition of the ribosome and the DNA gyrase, little consistency exists in the transcriptional responses of different bacteria to the same antibiotic class, indicating that to some extent the response to an antibiotic attack is species-specific. Therefore, when querying a compendium the profiles of the compendium antibiotics and the novel compound should have been generated using the same organism.

Bovine shipping fever is a severe inflammation of the bovine lung caused primarily by the gram-negative bacteria *Mannheimia haemolytica* and *Pasteurella multocida* (Mosier, 1997). Despite the success of antibiotic treatment, the disease is far from controlled in feedlots (Duff and Galyean, 2007), warranting the development of more efficient drugs. In order to aid the development of novel compounds to treat shipping fever, we created a compendium of transcriptional profiles in *P. multocida* to commonly used antibiotics in bovine pneumonia and queried it with the transcriptional profile of a novel compound with unknown MoA and excellent activity on *P. multocida*.

2. Materials and methods

2.1. Bacterial strains, antibiotics and growth conditions

P. multocida L386 is a serovar A14 bovine isolate provided by Prof. Wieler of the Free University of Berlin. *P. multocida* L386 was cultured on brain heart infusion (BHI, AES Laboratoire, France) agar plates for 18 h at 37 °C. BHI broth cultures were incubated at 37 °C with rotary aeration at 220 rpm. Bacterial densities were determined by measuring the optical density (OD) at 578 nm. For the microarray experiments growth was done as follows: for each antibiotic a mid-log grown culture was split into 3 × 250 ml Erlenmeyer flasks to an OD_{578} of 0.01 with a final volume of 40 ml BHI. One flask served as non-treated control, the other two for the 10 and 30 min antibiotic-treated time points. The antibiotics were added at minimal inhibitory concentration (1 × MIC, Table 1) 10 and 30 min before the untreated culture reached an OD_{578} of ∼0.5. Cells were harvested for 5 min at 5000 × *g* at 4 °C and were shock frozen and stored at −80 °C prior to RNA isolation. For each antibiotic and time point at least three replicate cultures were prepared. Novobiocin sodium salt, enrofloxacin, florfenicol, trimethoprim, rifampin and tilmicosin were purchased from Sigma–Aldrich (USA). The cefquinome sulphate and the novel compound from the thiazin class were from Intervet Innovation, Germany. Solutions for these antibiotics were prepared in H_2O. The trimethoprim, brodimoprim (Intervet Innovation, Germany) and rifampin solutions