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Global gene expression profile of *Yersinia pestis* induced by streptomycin

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Abstract

Plague, caused by *Y ersinia pestis*, is one of the most dangerous diseases that impressed a horror onto human consciousness that persists to this day. Cases of plague can be normally controlled by timely antibiotic administration. Streptomycin is the first-line antibiotic for plague treatment. In this study, a DNA microarray was used to investigate the changes in the gene expression profile of *Y. pestis* upon exposure to streptomycin. A total of 345 genes were identified to be differentially regulated, 144 of which were up-regulated, and 201 down-regulated. Streptomycin-induced transcriptional changes occurred in genes responsible for heat shock response, drug/analogue sensitivity, biosynthesis of the branched-chain amino acids, chemotaxis and mobility and broad regulatory functions. A wide set of genes involved in energy metabolism, biosynthesis of small macromolecules, synthesis and modification of macromoclecules and degradation of small and macro molecules were among those down-regulated. The results reveal general changes in gene expression that are consistent with known mechanisms of action of streptomycin and many new genes that are likely to play important roles in the response to streptomycin treatment, providing useful candidates for investigating the specific mechanisms of streptomycin action.

Keywords: *Yersinia pestis*; DNA microarray; Transcriptional profiling; Streptomycin

1. Introduction

Plague is a zoonotic disease caused by the gram-negative bacterium *Yersinia pestis* [1]. It has caused social devastation on a scale unmatched by any other infectious agent. Plague is ever present in endemic areas, circulating in various mammalian species. There are hundreds of cases of human plague reported annually, and plague has been recognized as a re-emerging disease by the World Health Organization [2]. *Y. pestis* has recently become of public interest because of its potential as an agent of bioterrorism. Since its first development in 1944, streptomycin has been the antibiotic of choice for the treatment of most forms of plague [3]. When administered in the early phase of the disease, it can effectively reduce the overall human mortality to 5–14%, whereas untreated, the mortality rate is between 50% and 90% [4]. Streptomycin is a water soluble

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aminoglycoside that is marketed as the sulfate salt of streptomycin. Streptomycin interferes with several steps of protein synthesis, its most conspicuous effects being the stimulation of translational errors and a slowing down of translocation resulting in the production of faulty proteins. Streptomycin binds to the decoding center of bacterial 16S rRNA in the absence of ribosomal proteins, and protects a set of bases in the decoding region against dimethyl sulfate attack [5].

Although the development of antibiotics has significantly lowered the mortality rates, there still exists the serious threat to public health posed by pathogenic bacteria that have developed resistance to the traditional antibiotics, which is also the case for plague.

In 1995, a *Y. pestis* strain, resistant to streptomycin, ampicillin, chloramphenicol, kanamycin, sulfonamides, tetracycline and minocycline, was isolated in Madagascar from a 16-year-old boy [6]. Therefore, it is important to extensively understand the molecular mechanisms of action of the traditional antibiotics, and attempts should be conducted as well for the development of new antibiotics. The availability of the genome sequences [7–9] and the subsequent development of DNA microarrays to profile the transcriptome [10–13] have opened a window for monitoring global changes in gene expression patterns in *Y. pestis*.

Here we used the whole-genome DNA microarray to investigate the global transcriptional response of *Y. pestis* triggered by the treatment of streptomycin, giving an overall picture of the molecular mechanisms of action of the antibiotic in vanquishing this deadly pathogen.

2. Materials and methods

2.1. Bacterial strain, medium and MIC determination

Yersinia pestis strain 201 was used in this study. It was isolated from *Microtus brandti* in Inner Mongolia, China. It has major phenotypes as $F1^+$ (able to produce fraction 1 antigen or the capsule), VW^+ (presence of V antigen), Pst^+ (able to produce pesticin) and Pgm^+ (pigmentation on Congo-red media). Strain 201 has an LD_{50} of less than 100 cells for mice by subcutaneous challenge. Strain 201 belongs to a newly established *Y. pestis* biovar, microtus [14]. Biovar microtus strains are supposed to be avirulent to humans, although they are highly lethal to mice [14]. A chemically defined TMH medium [15] was used for cultivating the bacteria.

Minimal inhibitory concentration (MIC) was determined by the broth dilution method [16]. The tests were performed in sterile glass tubes with an initial inoculum of approximately 10^5 CFU of *Y. pestis* cells (overnight cultures) in 1 ml of TMH medium per tube. Streptomycin sulfate (Amresco) was dissolved in each tube and dilution series were made to give a final concentration range from 0.1 to 128 μg/ml. Bacteria were then cultured at 37 °C for 20 h. The MIC was defined as the lowest concentration that prevents the development of visible growth.

2.2. Bacterial growth and RNA isolation

Strain 201 was grown at 26 °C to the middle exponential growth phase (an A_{620} of about 0.6) in the TMH medium. The cell cultures were 1:20 diluted in fresh TMH medium and the cells experienced at least 10 generations in the medium prior to reaching to the middle exponential growth phase. Bacteria were then transferred to grow at 37 °C for 1 h to be ready for antibiotic treatment. Cells were incubated at 37 °C for 30 min under the treatment of 10× MIC (80 μg/ml) of streptomycin; the control culture was allowed to continue growing at 37 °C for the same period of time with adding the same volume of distilled water. Immediately before harvesting for RNA isolation, bacterial cells were mixed with RNA protect Bacteria Reagent (Qiagen) to minimize RNA degradation. Total RNA was isolated by using the MasterPure™ RNA Purification kits (Epicenter). RNA quality was monitored by agarose gel electrophoresis and RNA quantity was measured by spectrophotometer. Two independent bacterial cultures for each test or control condition were prepared as biological replicates for RNA isolation.

2.3. Probe synthesis and mciroarray hybridization

Fifteen to 20 micrograms of RNA were used to synthesize cDNA in the presence of aminoallyl-dUTP, genome directed primers (GDPs) and random hexamer primers with the Superscript II system (Invitrogen). The reverse transcription of bacterial RNA by the mixture of GDPs and random hexamers has been proven to be more effective and reliable than with either GDPs or random hexamers only [17]. The aminoallyl-modified cDNA was then labeled by Cy5 or Cy3 monofunctional dye (Amersham) according to the manufacturer' instruction. Three separated labeled probes were made for each RNA preparation as technical replicates. Pairwise comparisons were made using dye swaps to avoid labeling bias.

Glass slides spotted with PCR amplicons representing about 95% of non-redundant annotated genes or ORFs of *Y. pestis* CO92 and 91001 were used for hybridization [18]. Briefly, based on the genomic sequences of *Y. pestis* CO92 and 91001, a total number of 4015 annotated open reading frames (ORFs) were selected after the exclusion of ORFs encoding IS protein, integrase, and transposase. Specific primer pairs were designed to amplify nearly the full length of each gene. The purified 4005 successful amplifications were spotted on the CSS-1000 silylated glass slides (CEL) by using a SpotArray72 Microarray Printing System (Perkin–Elmer Life