Transcriptional Organization of the O Antigen Biosynthesis Cluster in the GC-Rich Bacterium *Burkholderia cenocepacia*

Ximena Ortega and Miguel A. Valvano*
Department of Microbiology and Immunology, Siebens-Drake Research Institute, Schulich School of Medicine, Faculty of Medicine and Dentistry, The University of Western Ontario, Canada
**Corresponding author: xortega@uwo.ca*

Ximena Ortega

Miguel A. Valvano

Introduction

Infections with members of the *Burkholderia cepacia* complex have become a serious threat for immunocompromised individuals, particularly those who suffer from cystic fibrosis and chronic granulomatous disease [1]. In Canada, *B. cenocepacia* is the *B. cepacia* complex species most commonly found in infected subjects (approximately 83% of all the isolates). The *B. cenocepacia* type strain J2315 has a genome size of 8.056 Mb organized into three chromosomes and one large plasmid (www.sanger.ac.uk/Projects/B_cenocepacia/).

In general, it is difficult to perform molecular investigations in *B. cenocepacia* due to its high GC content (66.9%), the abundance of insertion elements in its genome, and the multiple antibiotic resistance of all strains. Recently, our laboratory reported the isolation of *B. cenocepacia* mutants which were attenuated for survival in a rat agar bead model of lung infection. Four of these mutants had transposon insertions in genes of an O antigen lipopolysaccharide (LPS) biosynthesis cluster, indicating that production of O antigen is important for bacterial survival *in vivo* [2]. Further studies have shown that the O antigen cluster in *B. cenocepacia* is complex and consists of several transcriptional units [3]. We investigated the transcriptional organization of this cluster by RT-PCR analysis using the Transcriptor Reverse Transcriptase with primers that allowed us to detect co-transcription between genes. Using the Transcriptor Reverse Transcriptase, GC-rich RNA with a high content of secondary structure was optimally transcribed.

Materials and Methods

Total RNA isolation

The method described by Glisin *et al.* [4] was used to isolate total RNA from the *B. cenocepacia* strain K56-2. A 25-ml culture of *B. cenocepacia* K56-2 was grown in Luria broth to an OD_{600} of 0.6. Bacterial cells were collected by centrifugation, resuspended in 3.5 ml of RNase-free TESS buffer (20 mM Tris pH 7.6, 10 mM EDTA, 100 mM NaCl, 1% SDS), and lysed by heating (95ºC for 3 minutes). Cesium chloride powder was added to the lysate at a final concentration of 1 g/ml, and the lysate was deposited on top of a 1-ml CsCl cushion prepared with 5.7 M CsCl, 0.1 M EDTA in SW50.1 tubes (Beckman Coulter, Fullerton, California). The cell lysate was centrifuged at 39 000 rpm for 16 hours at 20ºC. After centrifugation, a thick band containing DNA and cell debris was visible in the middle of the tubes, while the RNA was deposited as a clear pellet at the bottom of the tubes. After aspiration of the content, the bottoms of the tubes were carefully cut with a hot scalpel and the RNA pellet was dissolved in 100 µl of 20 mM sodium acetate and 1 mM EDTA. A volume of 200 µl of ethanol was added and the tubes were stored at -20ºC. An aliquot of the precipitated RNA was centrifuged. The pellet was then washed, dried, and resuspended in RNase-free water, and treated with DNase I. DNase I was eliminated by the clean-up protocol of a commercially available RNA isolation kit, and the resulting RNA was used for the RT-PCR analysis.

RT-PCR analysis

The sequence of the primers can be obtained from the authors upon request.

The reverse transcription reaction was carried out as follows: The template, primer, and water were incubated at 65°C for 5 minutes. Then, the mixture containing the 5x RT-PCR buffer, RNase inhibitor, dNTPs, and the Transcriptor Reverse Transcriptase was added. This reaction mixture was incubated at 55°C for 30 minutes to allow the reverse transcription reaction to occur. Finally, the reverse transcriptase was inactivated by incubation at 85°C for 5 minutes.

To ensure the absence of any RNase activity, the Protector RNase Inhibitor was used in all the reverse transcription reactions. The resulting cDNA was subjected to PCR using Taq DNA polymerase. The conditions for the amplification were 10 cycles of 2 minutes at 94°C, 10 seconds at 94°C, 30 seconds at 54°C, and 2 minutes at 72°C, followed by 30 cycles of 10 seconds at 94°C, 30 seconds at 59°C, 2 minutes at 72°C, and a final extension of 7 minutes at 72°C. The PCR products were visualized in a 1.7% (wt/vol) agarose gel.

Results and Discussion

Bacterial mRNAs are usually very unstable due to their short half-lives. The cesium chloride-based method was used to obtain large quantities of RNA, but also because it rapidly inactivates RNases at the lysis step. From a 25-ml culture, RNA was purified at a concentration of 14.2 mg/ml. The RNA was stored in ethanol at -20°C and has a long shelf life. This allowed us to perform all RT-PCR analyses with the same RNA preparation, thereby ensuring uniformity of the results from the various RT-PCR analyses.

For each PCR, the appropriate controls with water and cDNA synthesized in the absence of reverse transcriptase were included. This ensured that the amplifications obtained resulted from the presence of cDNA and not of contaminating genomic DNA. Figure 1 clearly shows that after DNase treatment, no amplification was obtained in the water control and the reaction without reverse transcriptase. This confirms that any products obtained from the subsequent PCRs were due to the presence of the corresponding cDNA and not to chromosomal DNA contamination.

The RT-PCR analysis on the O antigen synthesis cluster showed amplification in cases where neighboring genes were co-transcribed across their boundaries. This occurred for five of the eight regions that were studied. Co-transcription was detected in regions 2, 3, 4, 7, and 8, while regions 1, 5, and 6 did not give amplification for the expected fragments (Figure 1). Based on these results, we propose the following transcriptional units: *rmlBACD-wzm-wzt-vioA-wbcFED*, *wbcC*, *wbiFGHI*, *wzx-wbcAB-galE-wecA*, *manB-waaC-wbcY*, *waaA*. The elucidation of the transcriptional organization of the O antigen cluster in *B. cenocepacia* K56-2 provides us with valuable information or a better understanding of how O antigens are synthesized in *B. cenocepacia*.

The high GC content of the *B. cenocepacia* genome (66.9%) and the contamination with RNase did not cause any problems. Transcriptor Reverse Transcriptase has a very high performance even when working on templates with high GC content: This enzyme can efficiently use RNA templates with up to 70% GC content. Moreover, the use of the Protector RNase Inhibitor at every step of the RT-PCR improved the quality of the templates. ■

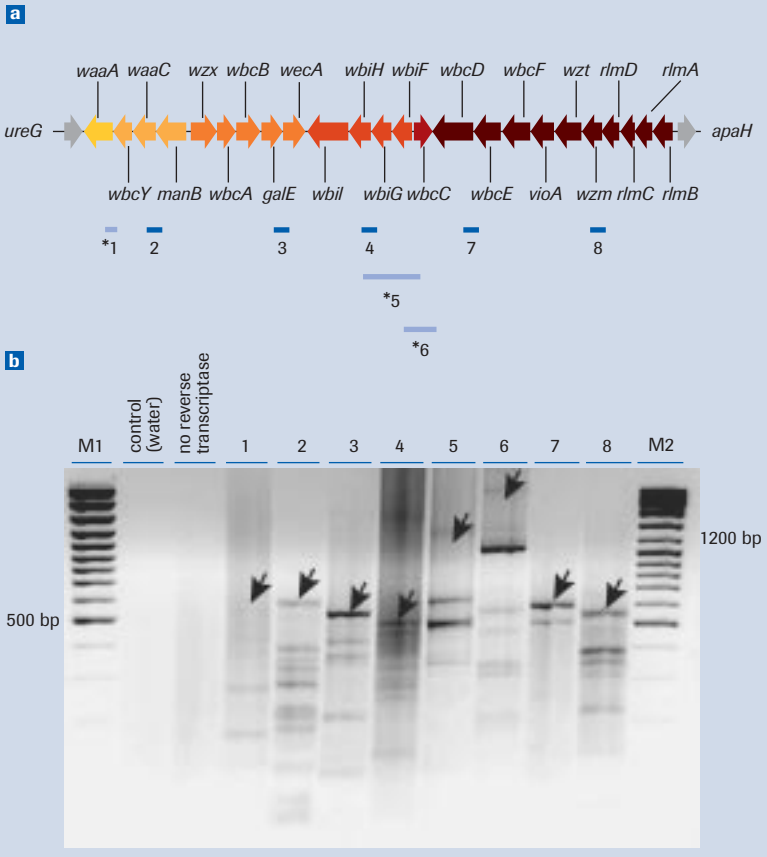

Figure 1: Analysis of the transcriptional organization of the O antigen cluster by RT-PCR. (a) Genetic map of the O antigen cluster of *B. cenocepacia* K56-2: The flanking genes are represented by *gray arrows*. All the genes from the O antigen cluster transcribed as part of the same unit are depicted with the same color. Accordingly, six transcriptional units were found. The *bars* below the map indicate the boundaries of the expected amplification products. Those indicated with *asterisks* denote fragments that were not amplified, and thus correspond to regions that were not co-transcribed. The numbers indicate the lanes in *panel b*. (b) Agarose gel with the appropriate controls and the amplification reactions from the eight regions selected for these experiments. *Arrows* indicate the presence or absence of the expected amplification products.

References

1. Mahenthiralingam E, Urban TA, Goldberg JB (2005) Nat Rev Microbiol 3:144–156
2. Hunt TA, Kooi C, Sokol PA, Valvano MA (2004) Infect Immun 72:4010–4022
3. Ortega X *et al.* (2005) J Bacteriol 187:1324–1333
4. Glisin V, Crkvenjakov R, Byus C (1974) Biochemistry 13:2633–2637