Center for Phage Technology

Characterization of High Molecular Weight Bacteriocins from *Burkholderia cenocepacia*

Victoria E. Berkowitz [1,2], G. W. Yao [1,2], Carlos F. Gonzalez [1,2]

[1] Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843, [2]Center for Phage Technology, Texas A&M University, College Station, TX 77843

Introduction

Members of the *Burkholderia cepacia* complex (Bcc) cause life-threatening respiratory tract infections in persons with cystic fibrosis (CF). Many clinical isolates of the Bcc demonstrate broad-spectrum antibiotic resistance *in vitro*, rendering therapy ineffective (1,2). The clinical observations emphasize the essential need to develop new antimicrobial therapies against CF-associated Bcc infections. High molecular weight bacteriocins (tailocins) are morphologically similar to phage tails and cause depolarization of the bacterial cytoplasmic membrane. Tailocins express "one hit one kill" kinetics that makes them potential antimicrobials and may offer a potential therapeutic control for Bcc infections in CF patients. We have identified six tailocins that have a broad host range and differential activity against members of the Bcc.

Materials and Methods

- To identify tailocin production, eleven *B. cenocepacia* clinical isolates were streaked on Tryptone Nutrient Agar (TNA) and grown for 48 h at 37°C. Cultures were exposed to chloroform vapors (20 min), and then overlayed with TNA-soft agar seeded with indicator hosts. Tailocin production was observed as zones of clearing in the bacterial lawns following a 24 h growth period (Fig 1).
- Isolates exhibiting tailocin activities in the plate assay (AU17799, AU2455, AU23791, AU0969, AU0859, and AU1189) were UV induced (400 µW/cm^2 for 7 sec). Isolates were grown in TN broth to OD_{600} 0.50, centrifuged, resuspended in 0.85% NaCl and UV irradiated. After UV induction, cells were added to an equal volume of 2X TN broth. OD readings were taken every hour until cell lysis occurred. The lysate was centrifuged and filter sterilized(0.22µm).
- Host range was determined by spotting 20 µl of a 1:10 dilution series of each preparation onto bacterial lawns seeded with indicator cells representative of the Bcc. Killing by tailocin was observed as zones of clearing.
- Electron microscopy of 25X concentrated lysates was performed by diluting 1:10 with 1/5 P-buffer (50 mM Tris-HCl pH 7.5, 100 mM NaCl, 8 mM $MgSO_4$). Phage were applied to thin 400-mesh carbon-coated Formvar grids, stained with 2% (wt/vol) uranyl acetate and air dried. Specimens were observed on a JEOL 1200EX transmission electron microscope operating at an acceleration voltage of 100 kV.

Results

Tailocin Plate Assay

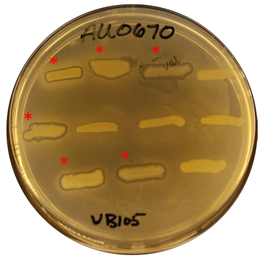

Fig 1. **Tailocin production assay**. A panel of potential tailocin producers were tested using the short streak/overlay method. Tailocin production was indicated by small clear zones (*).

TABLE 1. Matrix of Potential Tailocin Producers

Indicator strain	Potential Tailocin Producer										
	AU1553	AU2455	AU0969	AU8203	AU0859	AU6797	AU25023	AU1189	AU17799	AU23791	AU8006
AU1553	-	-	-	-	-	-	-	-	-	-	-
AU2455	-	-	-	-	+	-	-	-	+	-	-
AU0969	+	-	-	-	+	-	+	+	+	+	-
AU8203	-	-	-	-	-	-	-	+	-	-	-
AU0859	-	-	-	-	-	-	-	-	-	-	-
AU6797	-	-	-	-	-	-	-	-	-	-	-
AU25023	-	-	-	-	-	-	-	-	-	-	-
AU1189	-	+	-	+	+	+	-	-	-	-	-
AU17799	+	+	-	+	+	+	-	-	-	-	-
AU23791	-	-	-	-	-	-	-	+	-	-	-
AU8006	+	+	-	+	-	+	-	-	-	-	-

(+) = presence of clear zone

UV Induction

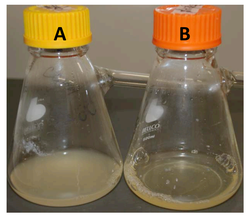

Fig 2. UV induction of tailocin producing isolate. Cultures were grown in broth, with either **(A)** no UV exposure **(**Control) or **(B)** 7 sec of UV exposure, and monitored for cell growth.

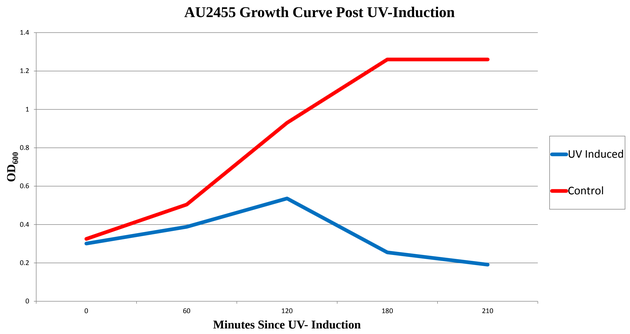

Fig 3. Growth curve of AU2455 with or without UV induction.

Host Range Assay

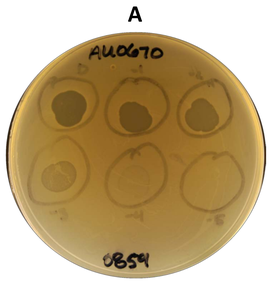

Fig 4. Direct spot assay of UV induced tailocin. To determine host range and relative killing efficiency, stock solutions of UV induced tailocin were tested on a panel of Bcc isolates **(A)** T-0859 tested on AU0670 host**.** A 20 µl aliquot of serial dilution(1:10) of each indicated tailocin (T) was spotted on indicator overlay.

TABLE 2 Tailocin Host Range

Indicator Strain	Producer					
	AU1189	AU17799	AU23791	AU2455	AU0969	AU0859
AU1189	-	-	+	+	+	+
PC184	+	+	-	-	-	+
AU0583	+	-	-	-	-	+
AU1054	+	+	+	+	+	+
AU0670	+	+	+	+	+	+
BCC-1	+	+	+	+	+	+
K56-2	+	+	+	+	+	+
AU0644	+	+	+	+	+	+
AU0279	+	+	-	+	+	+
AU0918	+	+	+	+	+	+
AU17799	-	-	+	+	+	-
AU23791	+	+	-	+	+	+
AU2455	+	+	-	-	-	+
AU0969	+	+	-	-	-	+
AU0859	-	-	+	+	+	-

(+) = sensitive to tailocin; (-) = resistant to tailocin

Electron Microscopy

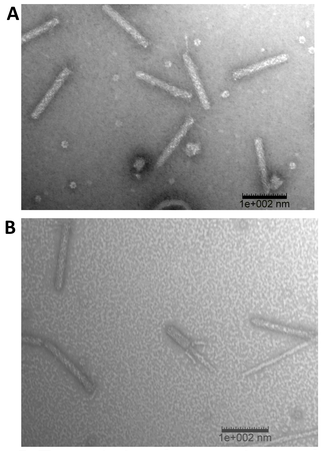

Fig 5. Transmission electron micrographs of tailocins **(A)** T-0859 **(B)** T-23791.

Conclusions

- *B. cenocepacia* of the CM3A group produce broad spectrum tailocins.
- Previously characterized tailocins from CM3A *B. cenocepacia* are chromosomally encoded. Induction studies indicate that tailocins described here are also chromosomally encoded.
- Tailocin production can be induced through UV irradiation. The sudden drop in optical density is the result of cell lysis and indicates tailocin release.
- The similarity in host range activity of tailocins T-2455 and T-0969 indicate that the tailocins adsorb to the same receptor site. Differences in tailocin host range could be the result of differences in receptor sites utilized by tailocins (Table 2).

References

1. **Lipuma, J. J.** (2003). Burkholderia cepacia complex as human pathogens. J Nematol 35, 212-217

2. **LiPuma, J. J**. 2010. The changing microbial epidemiology in cystic fibrosis. Clinical microbiology reviews 23:299-323

Funding

This project was funded by the Bioenvironmental Science Undergraduate Research Scholarship from Texas A&M Department of Plant Pathology and Microbiology.