F-350

Antibacterial Drug Discovery Using Phage-Validated, Novel Targets

Jinzi J. Wu, Francis Arhin, Mario Callejo, Mohammed Dehbi, Daniel Delorme, Dilip Dixit, Nhuan Ha, Tony Kwan, Jing Liu, Greg Moeck, Adel Rafai Far, Suzanne Sirois, Ramakrishnan Srikumar and Dan Williams

PhageTech Inc., Montreal, Canada

7170 Frederick Banting St., 2nd Floor
St-Laurent, Quebec H4S 2A1, Canada

Tel: (514) 332-1008
Cell: (514) 603-9673
Fax: (514) 332-6033
Email: jwu@phagetech.com
Web: www.phagetech.com

1 Abstract

Background: Viruses, and their interactions with their hosts, have played a crucial role in uncovering many fundamental rules of life. Over billions of years of evolution, the viruses of eubacteria, called "bacteriophages" or "phages," have developed unique proteins enabling them to arrest or inhibit critical bacterial cellular processes to augment phage reproduction. To address concerns of the striking global increase in antibiotic resistance, we have exploited the mechanisms of bacterial growth inhibition employed by these phages as tools to discover novel cellular targets for antibiotic discovery and development. **Methods** Using high-throughput bacteriophage genomics and functional genomics, we sequenced the genomes of 47 phages that infect three pathogens (*Staphylococcus aureus*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*). The open reading frames (ORFs) of these phages were identified and screened for those that inhibited bacterial growth when induced in their host. These phage-derived inhibitory ORFs were used as baits to systematically identify their interacting partners in bacteria. **Results**: A variety of targets which were biologically validated by phages and essential for bacterial growth were discovered by our technology platform. High-throughput screening (HTS) campaigns were carried out against some of these targets which are involved in DNA replication and RNA transcription. Small molecule leads were identified. These compounds inhibited bacterial growth and may mimic the effect of the phage ORFs on their cognate bacterial targets. Lead optimization is under way. **Conclusion**: By taking advantage of what we have learnt from nature, our technology platform plays an important role in the battle against increasing antibiotic resistance.

2 Introduction

With its unique proprietary phage genomics platform, PhageTech Inc. identifies and exploits the natural mechanisms developed by bacterial viruses (phages) that lead to the death of their bacterial hosts, including human pathogens. PhageTech has assembled a large collection of phages and a unique proprietary database of the genomes of phages that kill *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. Functional genomics tools are used to identify candidate growth inhibitory proteins (ORFs) from the phages; proteomics tools are then applied to identify the cognate bacterial targets of these phage-derived growth inhibitory proteins. Growth inhibitory protein families and their bacterial targets have been identified. HTS against these targets has identified a number of novel small molecule compounds that may mimic the phage-derived inhibitory proteins in their antibacterial effects. Different from 'phage therapy', this approach supports the development of new classes of small molecule antibiotics against phage-selected bacterial targets involved in essential bacterial functions.

3 Major Approaches to Antibacterial Discovery

- Screening of candidate molecules in whole-cell based assays for antibacterial activity
- Analoging of known drugs or drug candidates with the goal of creating newer or improved versions
- Discovering of novel compounds for known therapeutic targets with established mode of action
- Target-based drug discovery via bacterial targets selected by phages = PhageTech approach
- Key advantages of the PhageTech drug discovery platform include:
  - speed of analysis of small phage genomes
  - access to diversified targets
  - inherent validation of the bacterial targets by phages

4 Summary of PhageTech's Genomics and Functional Genomics Programs

Bacterial pathogen	Phages collected	Genomes sequenced	ORFs screened	Inhibitor families
S. aureus	150	27	964	31
S. pneumoniae	50	8	264	5
P. aeruginosa	70	11	500	13
Total	**270**	**46**	**1728**	**49**

5 ORF Affinity Approach to Target ID

Affinity chromatography of bacterial lysate over immobilized inhibitory ORF

⇩

Tryptic peptide mapping, mass spectrometry of eluted proteins

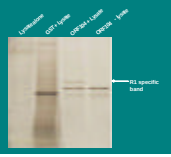
⇩

Target identification

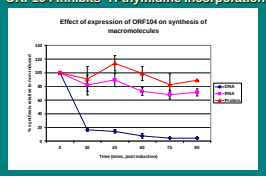
⇩

Target validation { Confirm ORF-Target interaction; Confirm target essentiality; Determine target function

6 Identification of R1 involved in DNA Synthesis as a *S. aureus* Target of ORF104



7 ORF104 Inhibits ^{3}H-thymidine Incorporation



8 Validation of Bacterial Targets from the Proteomics Platform

- Several targets were identified from *S. aureus* lysates using immobilized inhibitory phage ORFs
- Direct interactions between inhibitory phage ORFs and their cognate bacterial targets were confirmed by:
  - Yeast two-hybrid analysis
  - Far western (protein blotting)
  - BIAcore
  - Time-resolved FRET (TR-FRET)
  - Fluorescence polarization (FP)
- These bacterial targets are:
  - essential
  - attractive targets for antibiotic discovery

9 *S. aureus* DNA and RNA Synthesis Proteins Identified by Phage Inhibitory ORFs

Representative of inhibitory ORF family	ORF size (aa)	Bacterial target identified	Essentiality of target
ORF104 ORF016	52 297	R1 (DNA)	Essential
ORF025 ORF168 ORF240	58 74 58	R2 (DNA)	Essential
ORF078	71	R9 (DNA)	Essential
ORF67	198	R12 (RNA)	Essential

10 Summary of Assay Technology

Assay	Players	Type	Principle	Readout
FP	Target / ORF	Protein-protein	Target polarizes ORF tracer	Fluorescence
TR-FRET	Target / ORF	Protein-protein	FRET with proximity of donor to acceptor	Fluorescence
TCA	Target	Enzyme	Label incorporation into product	Radioactivity

11 Screen Strategy

Small molecule compounds

⇩ Primary screening

Actives

⇩ Confirmatory screening

Confirmed actives

⇩ Dose response testing

Hits

⇩ *In vitro* functional assay, if available

Confirmed hits

⇩ Susceptibility testing and compound profiling

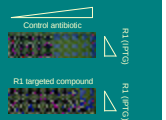
Lead

12 Hit Compounds Identified Against *S. aureus* Targets

Target	Assay	Library	Actives	Confirmed actives	Hits IC_{50} = 10µM or less	Confirmed Hits MIC = 16µg/ml or less
R1 (DNA)	FP	125 K	1311	156	73	16
R9 (DNA)	TR-FRET	125 K	932	464	90	24
R12 (RNA)	TCA	125 K	830	153	73	36

13 Mechanism of Action Studies Using R1 Titrating System

- Use *S. aureus* strain with R1 expression under inducible promoter
- Susceptibility testing for ORF104/R1-derived small molecule compounds
- Assay in the presence of increasing concentrations of inducer IPTG
- Antibiotics against known targets other than R1 as controls



14 Properties of ORF104/R1-Derived Small Molecules

Small molecule compound	ORF104-R1 interaction IC_{50} (µM)	MIC against *S. aureus* (µg/mL)	DNA synthesis IC_{50} (µg/mL)	RNA synthesis IC_{50} (µg/mL)	Fold effect in MIC under R1 regulated expression (100/25 µM IPTG)
1	1.5	0.125	0.8	1.5	4
2	0.6	1	2.4	6.5	8
3	4.8	16	1.5	13.7	8

- Data suggest these compounds kill bacteria by targeting R1
- Confirmed hits are now the focus of SAR / chemistry programs

15 Activities of One Lead Series Against R12

Compound	TCA IC_{50} (µM)	MIC (µg/ml)			
		S. aureus	*S. pneumoniae*	*E. coli*	*E. coli* tolC-
PT11010460	7	0.5	4	>32	0.5
PT99000019	5	4	4	>32	1
PT99000021	2	1	4	>32	1
PT99000029	4	4	4	>32	1
PT99000030	1	1	4	32	0.25
PT99000031	2	2	1	32	0.5

- These compounds kill both Gram positive and Gram negative bacteria

16 Efflux Pumps Lead Compounds Vs Known Antibiotics

Compound	MIC (µg/ml)		
	S. aureus	*S. aureus* norA+	*S. aureus* mdt+
PT11010460	0.5	2	0.5
PT99000030	1	0.5	0.031
PT99000031	2	2	0.062
Norfloxacin	1	32	4
TPP	8	32	32
Erythromycin	0.25	0.25	0.25

- These compounds are not substrates of major efflux pumps

17 MRSA Lead Compounds Vs Known Antibiotics

Compound	MIC (µg/ml)					
	S. aureus	MRSA-1	MRSA-2	MRSA-3	MRSA-4	MRSA-5
PT11010460	0.5	0.25	1	1	8	16
PT99000030	1	0.125	0.5	0.5	4	16
PT99000031	2	0.5	1	2	16	32
Oxacillin	0.25	>32	>32	>32	32	>32
Vancomycin	1	1	1	2	1	8
Erythromycin	0.25	>64	>64	>64	>64	>64
Norfloxacin	1	0.5	0.5	32	0.5	64

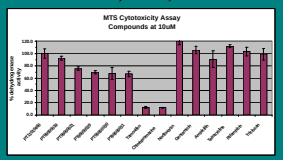
- These compounds are active against MRSA

18 Effect of Serum Proteins on Activity of Compounds

Compound	MIC for *S. aureus* (µg/ml)		
	CAMHB	+25% mouse serum	+25% human serum
PT11010460	0.25	0.5	0.5
PT99000019	0.25	0.5	0.5
PT99000021	0.125	0.25	0.25
PT99000029	0.125	0.25	0.25
PT99000030	<0.125	<0.125	<0.125
PT99000031	<0.125	0.125	0.25

- These compounds are active even in the presence of serum

19 Evaluation of Cytotoxicity in HeLa Cells



- Cytotoxicity was also evaluated in primary hepatocytes

20 Summary

- PhageTech has sequenced the genomes of 47 phages of *S. aureus*, *S. pneumoniae* and *P. aeruginosa*
- 49 families of phage-derived antimicrobial ORFs were identified
- Several biologically validated, essential bacterial targets, including those required for DNA and RNA synthesis in *S. aureus*, have been discovered
- Proprietary ORF-target binding assays and enzymatic assays have been developed around these targets
- Target titration systems have been developed and will be used for demonstration of mechanism of action
- Diverse libraries of small molecules are being screened for inhibitors and selected compounds are being optimized
- Some lead compounds show promising antibacterial profiles against Gram positive and Gram negative bacteria including drug resistant organisms
- Efficacy of lead compounds will be evaluated in animal models