

Bioinformatic Characterization of a Novel Lantibiotic Biosynthetic Gene Cluster in Pedobacter heparinus Caleb Leibee and David Essig, PhD

Introduction

Lantibiotics

- Antimicrobial peptides (bacterocins) secreted by gram (+) bacteria.
- Synthesized ribosomally as with a leader peptide
- Are modified post-translationally to form thioether amino acids lanthionine and 3 methylanthionine.
- Have high potential as novel antibiotics

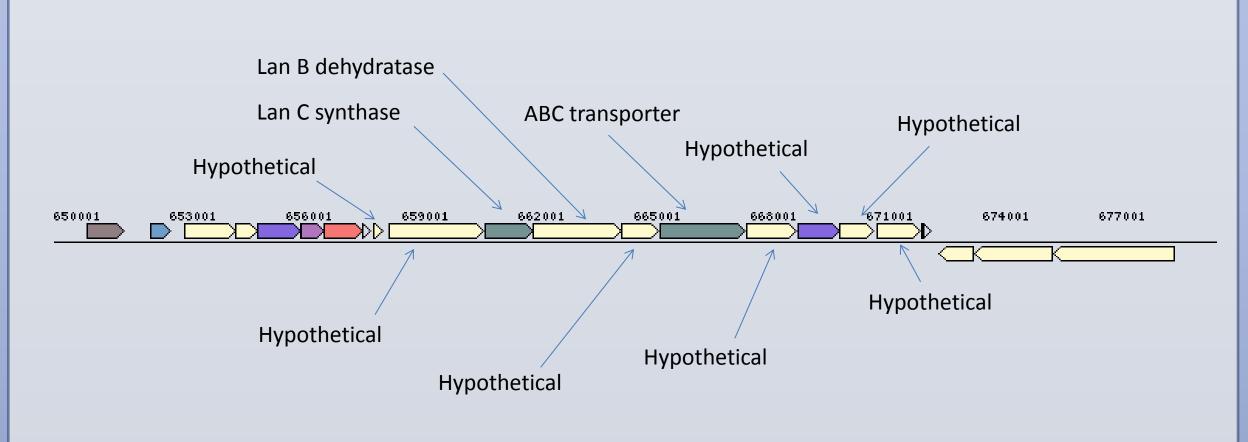
Rationale for Study

- To date all lantibiotics have been found in gram (+) bacteria.
- Pipeline genome annotation of the gram negative Pedobacter heparinus revealed a putative 10 gene lantibiotic gene cluster

Goals:

- Annotate 10 genes in a putative lantibiotic gene cluster found in the gram (-) bacteria Pedobacter heparinus.
- Search for additional lantibiotic gene clusters in other gram negative species

Machine Annotated Lantibiotic Gene Cluster



Method

Annotation was performed using the IMG ACT suite of bioinformatic programs. Predictions of gene product identities and potential pathway roles were based on sequence similarity (BLASTp), protein domains (Pfam) and cellular localization (PSORT, TMHMM, Phobius, and Signal P).

The LanA Pre-Peptide (0553)

Predicted gene locus was suspected to encode the lantibiotic peptide based on

- Small size (93 amino acids)
- Location upstream from genes machine annotated to encode lanithionine synthase and lanithionine dehydratase
- Searched the pedobacter heparinus genome in BAGEL a web server that identifies putative bacteriocin ORFs.
- Phep_0054 was predicted to be a potential bacterocin

Predicted amino acid sequence had

- a canonical leader peptide cleavage site (double glycine or GG)
- 10 cysteine's in the mature peptide!

MKKLRLNKSFISNLTRDEAGKIM<u>GG</u> NDATWEE<mark>C</mark>SDR<mark>C</mark>SDY<mark>C</mark>TTPTYGDDAT<mark>C</mark>YTAGAG<mark>C</mark>PGTGSYG<mark>C</mark>ATDNG<mark>C</mark>QSDLCPDSLQMTE<mark>C</mark>GPIC

Leader peptide

mature peptide

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Macroglobulin (0554)

Macroglobulin genes in bacteria are thought to be involved in limiting host protease damage following antimicrobial induced membrane destruction.

NAME	Organism	E-value
KAOT1_03162	Mucilaginibacter paludis DSM 18603	5e-46
A-macroglobulin complement component	Pedobacter sp. BAL39	2e-36
Large extracellular alpha-helical protein		1.23e-04
A2M_N - MG2 domain	AMN	2.6e-07

- BLASTp and Domain hits: E values less than 1.23X 10 -4 and percent coverage greater than 68%
- Cellular localization: no TMH predicted, a signal peptide is predicted, found to be associated with outer membrane.
- Phep 0554 was found to be homologous with gram (-) Bacteriodetes for macroglobulin proteins.
- Possible role: involved in self immunity?

ABC Transporter (0558)

Many lantibiotics utilize ABC transporter subunit which also contain a peptidase to cleave the leader sequence.

NAME	Organism	E-value
ABC transporter related	Mucilaginibacter paludis	0.0
protein	DSM 18603	
ABC transporter related	Emticicia oligotrophica	0.0
protein	DSM 17448	
Predicted double-glycine		2.08e-
peptidase		11
ABC-type		5.19e-
bacteriocin/lantibiotic		177
exporters		
Peptidase_C39		3.3e-
		38
ABC_membrane		2.1e-
		23
ADC treat		4.6e-
ABC_tran		29

- E-values < 2.08x10⁻¹¹
- Percent coverage > 90.0%
- Cellular localization: five TMH predicted, no signal
- peptide and localized to cytoplasmic membrane. ABC Transporter found to be homologous with gram (-) bacteriodetes ABC genes and localized in the cytoplasmic membrane functioning in transport and as a peptidase.

Lan B Dehydratase (0555) Lan B dehydratase performs the first step in post translational modification of the peptide. nism E-value forsythia 2e-79 cida OT- 5e-68 3.6e-38 ×10⁻³⁸ > 90.0%. – E-values < 1.5x10⁻²¹ Percent coverage • BLAST > 98%. • PFAM -12.68% on N terminus and 68.16% on C terminus membrane.

Lan A peptide.

NAME	Organi
Lantibiotic	Tannerella fo
biosynthesis	ATCC 43037
protein	
Lantibiotic	Kordia algici
biosynthesis	
protein	
Lanthionine	
synthetase	LANC_lil
С	
- E-val	lues <u><</u> 3.6x
- Perc	ent covera

Lan C Cyclase (0556) Lan C cyclase develops the cyclic structure of the - Percent coverage: BLAST & Domain • Cellular localization: one TMH, no signal peptide, associated with cytoplasmic membrane. • Lan C was homologous with other gram (-) Bacteriodetes Lan C proteins and localized in the cytoplasm membrane. "Hemolysin" Transporter (0557) Some lantibiotics and related hemolysins utilize a hemolysin trasnporter as a second subunit transporter protein to associate

with the ABC subunit.

NAME	Organism	E-value
Secretion	Mucilaginibacter	2e-144
protein HlyD	paludis DSM 18603	
family		
protein		
Hypothetical	Spirosoma linguale DSM	1e-113
protein	74	
Slin_0782		
HlyD_3		1.4e-11
		1

- E-values < 1.4x10⁻¹¹
- Percent coverage: BLAST
- & Domain <u>></u> 93.8%.
- Cellular localization: one TMH, no signal peptide, and found in cytoplasmic membrane.
- 0557 protein was homologous with gram (-) Bacteriodetes secretion hemolysin translocator proteins and localized in the cytoplasmic membrane.

"Spa B" Transporter (0556)

Lantibiotic transport complexes are often associated with a Spa B transporter as a third subunit to the channeling/transport process

NAME	Organism	E-value
Lantibiotic biosynthesis	Kordia algicida OT-1	3e-77
protein		
Thiopeptide-type	Odoribacter laneus YIT 12061	5e-68
bacteriocin biosynthesis		
domain-containing		
protein		
SpaB C-terminal domain	SpaB_C	3.6e-38

- E-values < 3.6x10⁻³⁸
- Percent coverage: BLAST & Domain > 85.17%.
- Cellular localization: no TMH, no signal peptide, associated with cytoplasmic membrane.
- 0555 protein was homologous to other gram (-) bacteriodetes proteins and localized in the cytoplasmic membrane. The large Spa B domain has been shown in other species to be a subunit of the transporter.

NAME	Organism	E-
		value
Lantibiotic	Kordia algicida OT-1	0.0
biosynthesis		
protein		
Lantibiotic	Chitinophaga pinensis DSM	0.0
dehydratase	2588	
Lantibiotic		1.5e-
dehydratase,	Lant_dehyd_C	21
N terminus		21
Lantibiotic		2.3e-
dehydratase,		2.se- 59
C terminus		59

- Cellular localization: no TMH, no signal peptide, found in cytoplasm associated with cytoplasmic
- Lab B was homologous with gram (-) Bacteriodetes Lan B genes and localized in the cytoplasm.

Glucosyltranferases (0560)

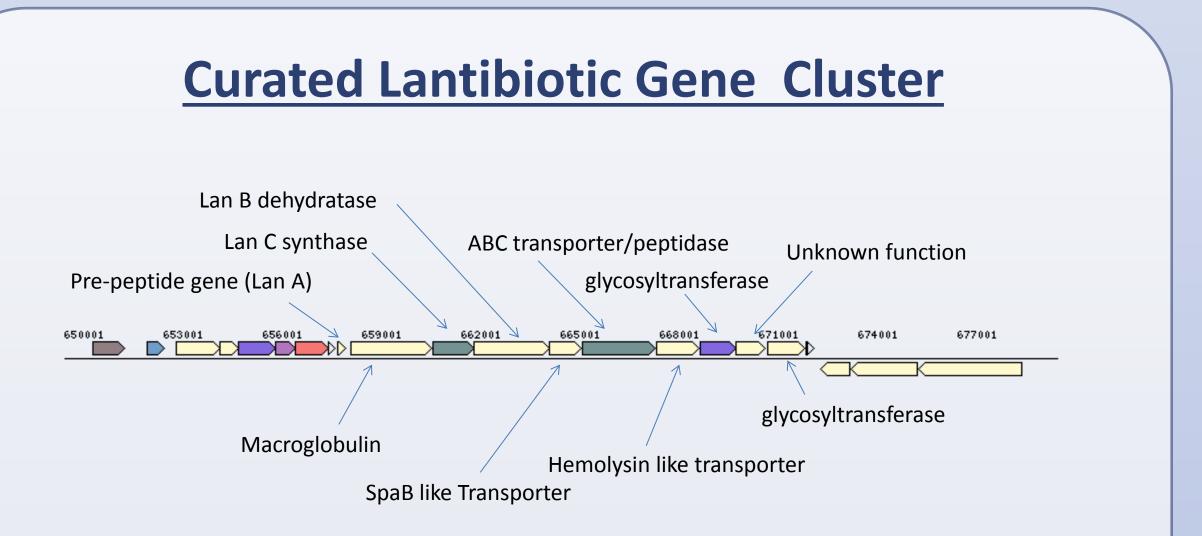
Three genes code for glycosyltransferases.: Phep 0560, 0562, and 0563. 0560 is presented here with the strongest Pfam correlations.

All three were found in the biosynthetic cluster. They are involved in either glycosylation of the lantibiotic peptide or membrane repair. Our bioinformatics point toward membrane repair through conserved domains but cellular location points toward glycosylation of lantibiotic peptide in cytoplasm.

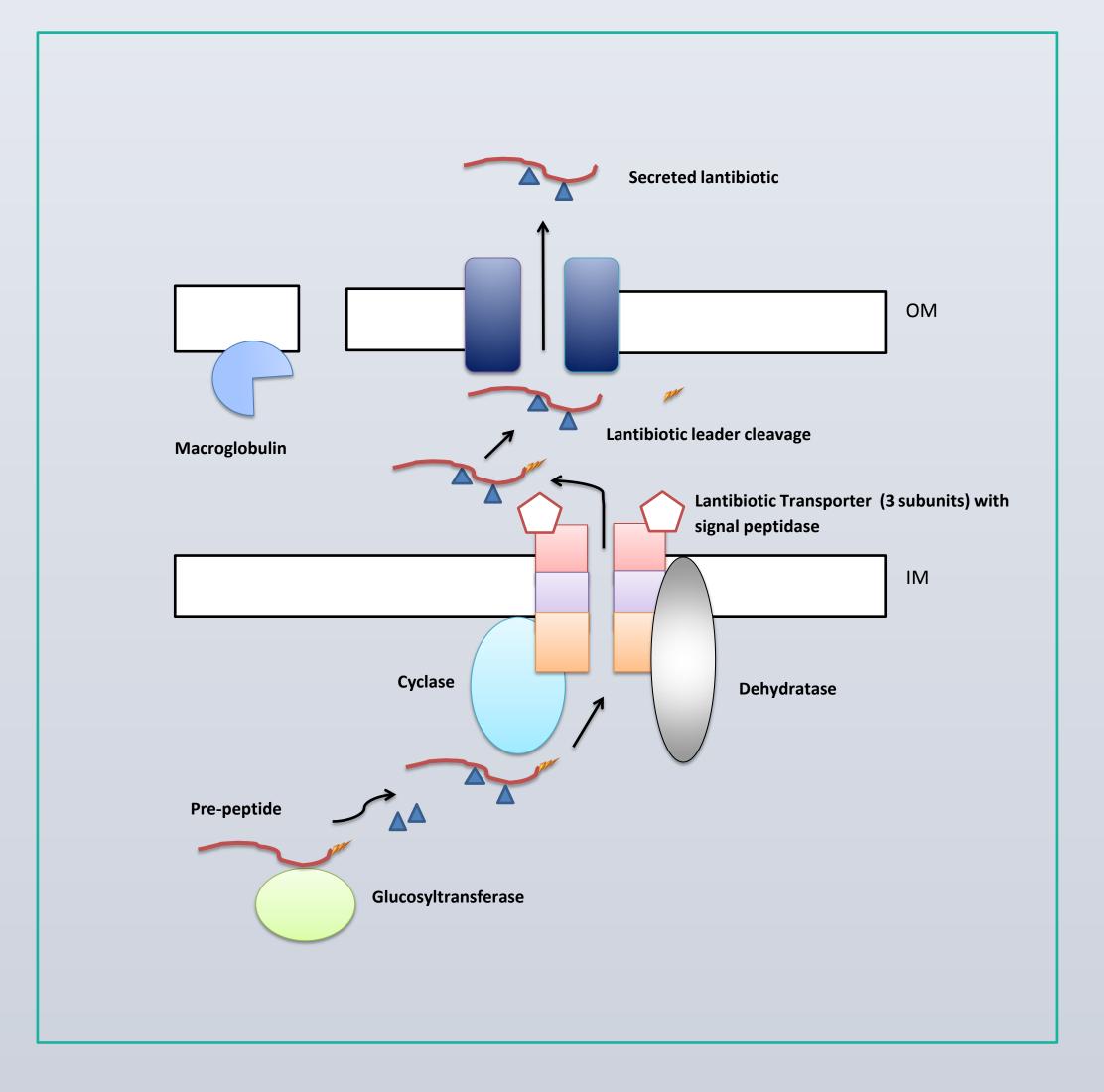
NAME	Organism	E-value
Hypothetical protein	Mucilaginibacter paludis DSM 18603	9e-71
Mucpa_3478		
Hypothetical protein	Pedobacter sp. BAL39	2e-70
PBAL39_15349		
WcaA - Glycosyltransferases		4.11e-06
involved in cell wall biogenesis		
Glycosyltransferases, probably		4.11e-06
involved in cell wall biogenesis		
Glycosyl transferase family 2	Glycos tra	4.8e-08
N-terminal domain		4.5e-08
galactosyltransferase		4.56-00

- E-values < 4.11x10⁻⁰⁶
- Percent coverage > 68.3%
- Cellular localization: no TMH predicted, no signal peptide, found to be in cytoplasm.
- Phep 0560 found to be homologous with gram (-) glycosylation and outer membrane repair proteins but localizes in cytoplasm. Propose that this enzyme may glycolsylate the lantibiotic as shown for a few lantibiotics (give reference here?)





Proposed Lantibiotic Biosynthetic Pathway



Conclusions and Implications

Based upon bioinformatic evidence, a lantibiotic biosynthetic gene cluster was found in the gram (-) Pedobacter heparinus :

- Awaits biochemical confirmation
- predicts other gram (-) bacteria may harbor similar gene clusters.

Preliminary data gathered from gene neighborhood analysis (IMG-ACT) found 10 other gram (-) species to have lantibiotic biosynthetic gene clusters. Therefore,

• lantibiotic peptides may not be unique to gram (+) bacteria.

Gram (-) bacteria may

- utilize lantibiotics for defensive purposes.
- be rich sources for novel antibiotics.