

Genome analysis in *Mycoplasma bovis*: a window into pathogenicity

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Clinical forms seen with *M. bovis*

- Pneumonia and polyarthritis
- Pneumonia and middle ear infections
- Mastitis
- Skin abscesses, septicemia, encephalitis
- Infertility

Disease severity is variable

- Pneumonia/polyarthritis [Morb >80%; Mort <30%]
- Mastitis [Morb >30%; Mort <20%]
- Factors affecting severity
 - Stress, management factors
 - Strain pathogenicity
 - Immune status



Intratracheal pathogenicity of *Mycoplasma bovis* strains

Strain	Nr Calves	Clin.Signs		Culture positive				Lung Score
		Fever	Arthr	Nose	Blood	TBLN	Joints	
M23	6	6/6	0/6	0/6	6/6	6/6	1/6	2.4 \pm 1.2
M45	4	1/4	0/4	3/4	0/4	6/6	0/4	0.5 \pm 0.2
428E	5	2/5	1/5	5/5	1/5	6/6	0/5	14.7 \pm 4.7

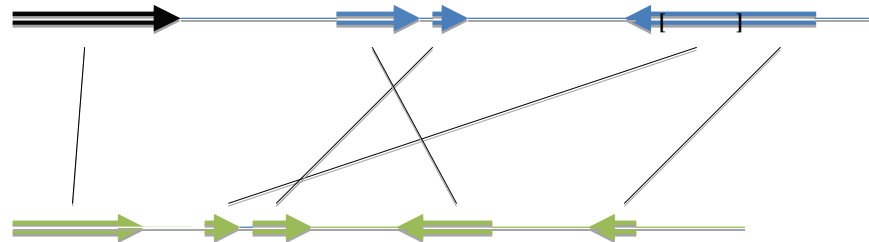
Field Data

Strain	Nr Calves	Morbidity	Mortality (% total)
M23	720	>80%	3.5%
428E	35	100%	34%

STRAIN PHENOTYPE

Translocation to blood
Translocation to LN
Ciliostasis
Pneumonia

Mbov PG45



Maga PG2

MmmSC genes transferred to Maga

(Sirand-Pugnet et al. PLOS Genet, v.3(5);2007)

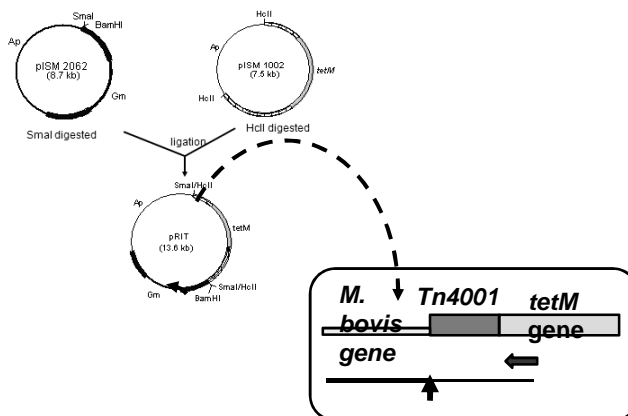
Genome characterization studies using a
random insertion mutant library of
Mycoplasma bovis strain M23

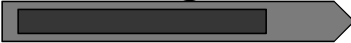


Global mutagenesis of *M. bovis* strain M23

- Generate a large enough number of random insertion mutants to **saturate** the entire genome of the mycoplasma, except for essential genes where disruption is lethal.
- **Antibiotic marker for strain M23**
 - Gentamicin: classic marker, performs poorly with *M. bovis*
 - Tetracycline: Strain M23 is very sensitive (2 µg/ml)

Plasmid pRIT-1

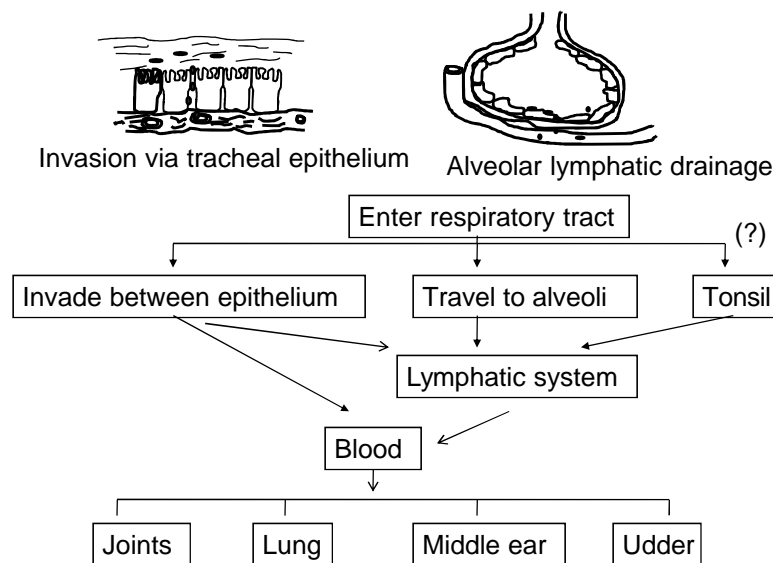


<i>Nr of</i>	
Mutants obtained	3155
Mutant sites determined (sequenced & sited in genome)	1148
Functional gene disruptions 	324
Predicted ORFs in <i>M. bovis</i>	776
Genes assigned in <i>M. bovis</i>	676

Pathogenicity Genes

Identification of *Mycoplasma bovis* genes required for respiratory tract persistence and systemic infection in calves using signature-tagged mutagenesis.

Systemic infection with *M. bovis* (proposed)



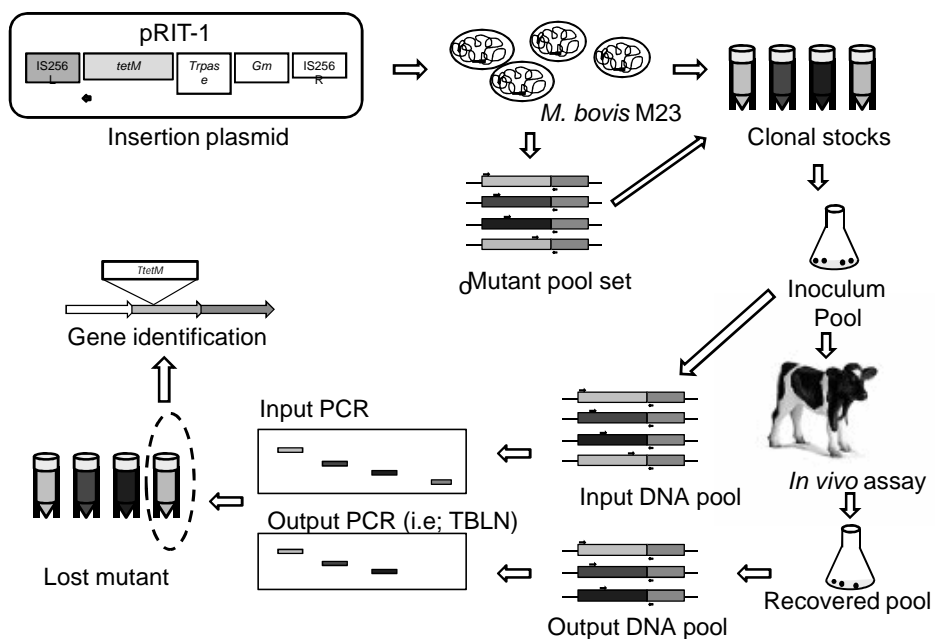
Intratracheal pathogenicity of *Mycoplasma bovis* mutants of strain M23

Strain	Nr Calves	Clin.Signs		Culture positive				Lung Score
		Fever	Arthr	Nose	Blood	TBLN	Joints	
M23	6	6/6	0/6	0/6	6/6	6/6	1/6	2.4±1.2

PHENOTYPES to STUDY

- Persistence on tracheal mucosa
- Translocation to TBLN
- Persistence in tonsil

Signature-tagged mutagenesis application



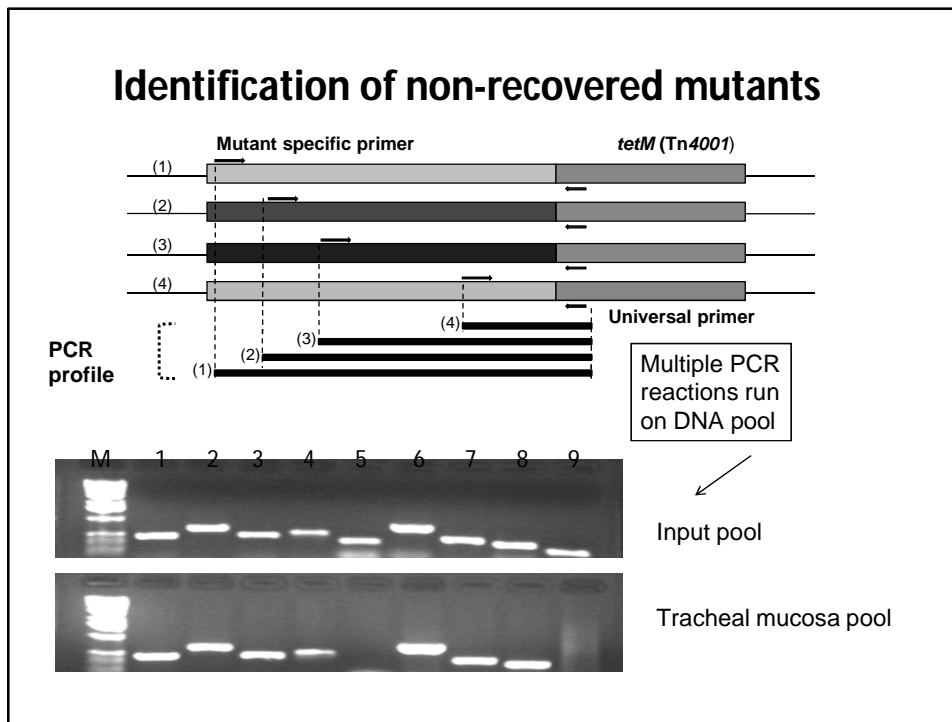
Optimization of *M. bovis* STM in a calf model

Conditions	<i>M. bovis</i> 23
Animal model (natural host)	6 month-old Holstein C/D calf
Challenge dose (route)	10 ⁷ CFU/ mutant (TT)
Number of mutants in mutant pool	10-15 mutants /each pool
Sample collection time	7 days
Best samples to obtain results	TBLN, tracheal mucosa

- **Tracheal mucosa** - persistence
- **TBLN** (tracheo-bronchial lymph node) – the best lymph node to determine systemic dissemination from lung

Selection of candidates from mutant library

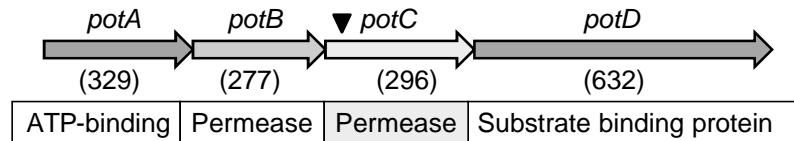
- **Selection of candidates**
 - 50 out of 324 mutants
 - From previous reports with other mycoplasmas (*M. gallisepticum*, *M. mycoides* subsp. *mycoides*), and *Streptococcus* spp.
 - Transporters
 - Critical metabolic pathways
 - Stress-response components
 - Regulatory genes and a putative toxin
- **Assigned into 5 mutant pools**
 - 10-15 mutants/mutant pool
 - 2 calves/mutant pool
- **Inoculation**
 - Trans-tracheal route via catheterization to bronchial bifurcation



Mutant recovery data from STM assays in calf model

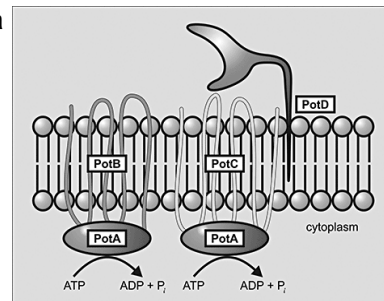
Mutant	Putative function of ORF	Nr positive calves/ Total nr of calves		
		Tonsil	Trachea	TBLN
MboTn293	Endopeptidase O	5/6	6/6	6/6
MboTn627	Spermidine-putrescine ABC transporter permease C (<i>potC</i>)	1/4	2/4	0/4
MboTn1283	Pentose-ribose ABC transporter permease	0/2	0/2	0/2
MboTn1290	Oligopeptide ABC transporter ATP-binding protein (<i>oppF</i>)	0/2	0/2	0/2

potC mutant



Biological role of polyamines in bacteria

- Nucleic acid stabilization
- Transcription regulation
- Translation regulation
- Biofilm formation
- Protect cells from oxidative, nitrosative, acid stress.



Adapted from *Mol. Microb.* 2008

Macrophage uptake assay

- Mouse macrophages (J774.1 line) are grown attached in roller tubes
- Macrophages are stimulated (*E. coli* LPS) or not.
- Mutant is added at moi = 0.1 CFU/macrophage
- After 150 min, quantitate mutant in supernate
- Ratio of mutant uptake by stimulated/non-stimulated macrophages is obtained.

Macrophage uptake results

Mutant	% Captured ±SEM	Gene Product
M23	7±18	wild type
<i>tig</i>	0	trigger factor chaperone
<i>deoA</i>	0	thymidine phosphorilase
<i>lpoA</i>	0	lipoate protein ligase
<i>potC</i>	94±5	polyamine transporter
<i>dnaK</i>	0	stress response chaperone
<i>oppB</i>	73±8	oligopeptide transporter

Respiratory pathogenicity of *Mycoplasma bovis* strains/mutants

- Translocation to blood
- Translocation to lymph node
- Ciliostasis in trachea
- Colonization in trachea
- Pneumonia

CONCLUSIONS AND PROJECTIONS-

- The pathogenic attributes of *Mycoplasma bovis* for the bovine respiratory tract can be dissected from studies with field strains.
- Studies with a library of mutants of *M. bovis* allow assignation of genes to some specific pathogenic attributes.
- Additional *in-vivo* and *in-vitro* models can be tested with mutants and field strains.
- Mutants have potential as modified live vaccine candidates