Evidence for a Role of the Polysaccharide Capsule Transport Proteins in Pertussis Pathogenesis

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Overview

- *Bordetella pertussis* is a deadly bacteria that is abundant around the world and thus is a great target for research.
- The tables from the article depict the individual strains and primers manipulated throughout the experiment.
- The figures provided articulate the methods and results demonstrated in the study.
- A comparison between the current findings and previous experimental findings allows for further insight on the conclusions.

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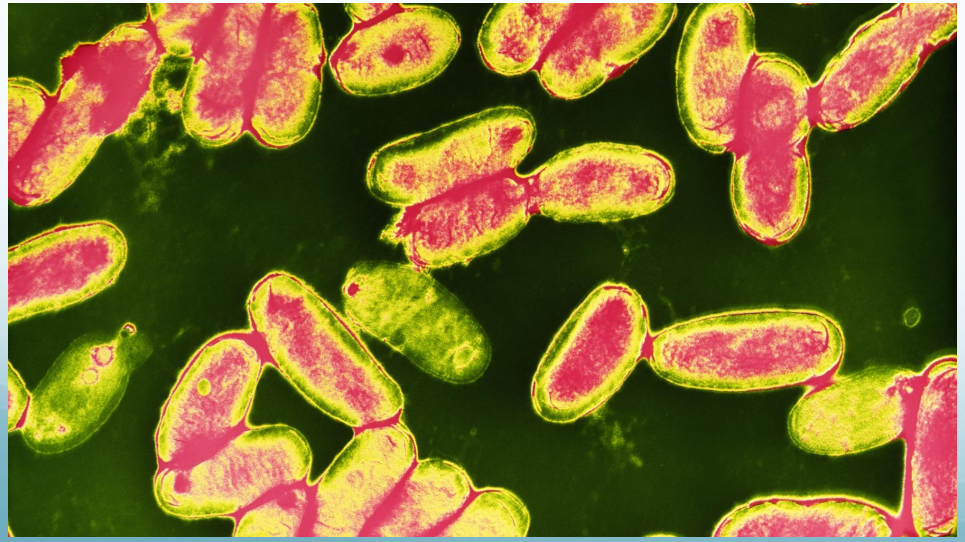
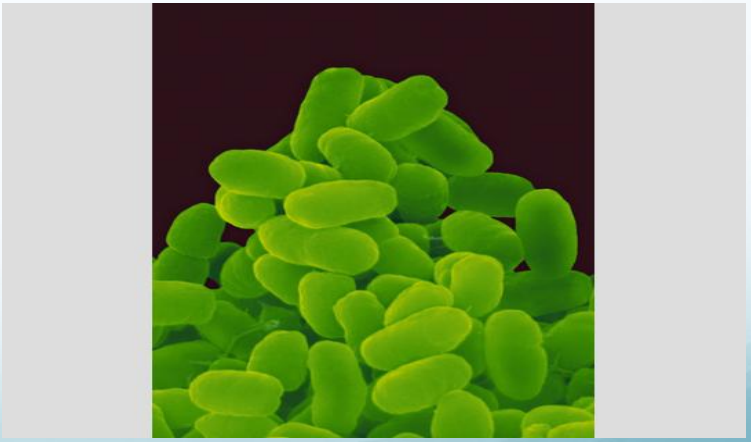
Significance of *B. pertussis* in the world

- The Gram-negative bacterium *Bordetella pertussis* is the causative agent of pertussis or whooping cough.
- Pertussis is responsible for 300,000-400,000 deaths each year as it is one of the top ten most infectious diseases worldwide.





Pictures by electron micrograph

Polysaccharide capsules in *B. pertussis* to determine virulence

- *B. pertussis* produces an intact polysaccharide (PS) microcapsule on the surface of its bacteria in response to environmental stimuli.
- This study identified PS capsules as important virulence determinants for bacterial pathogens, as well as KpsT as a membrane protein involved in the transport of PS polymers across the cellular envelope in *B. pertussis.*
- To determine the impact of PS capsules on the virulence of *B. pertussis,* a microarray experiment was run testing a ΔKpsT mutant against the wild-type.

Producing the Replicates

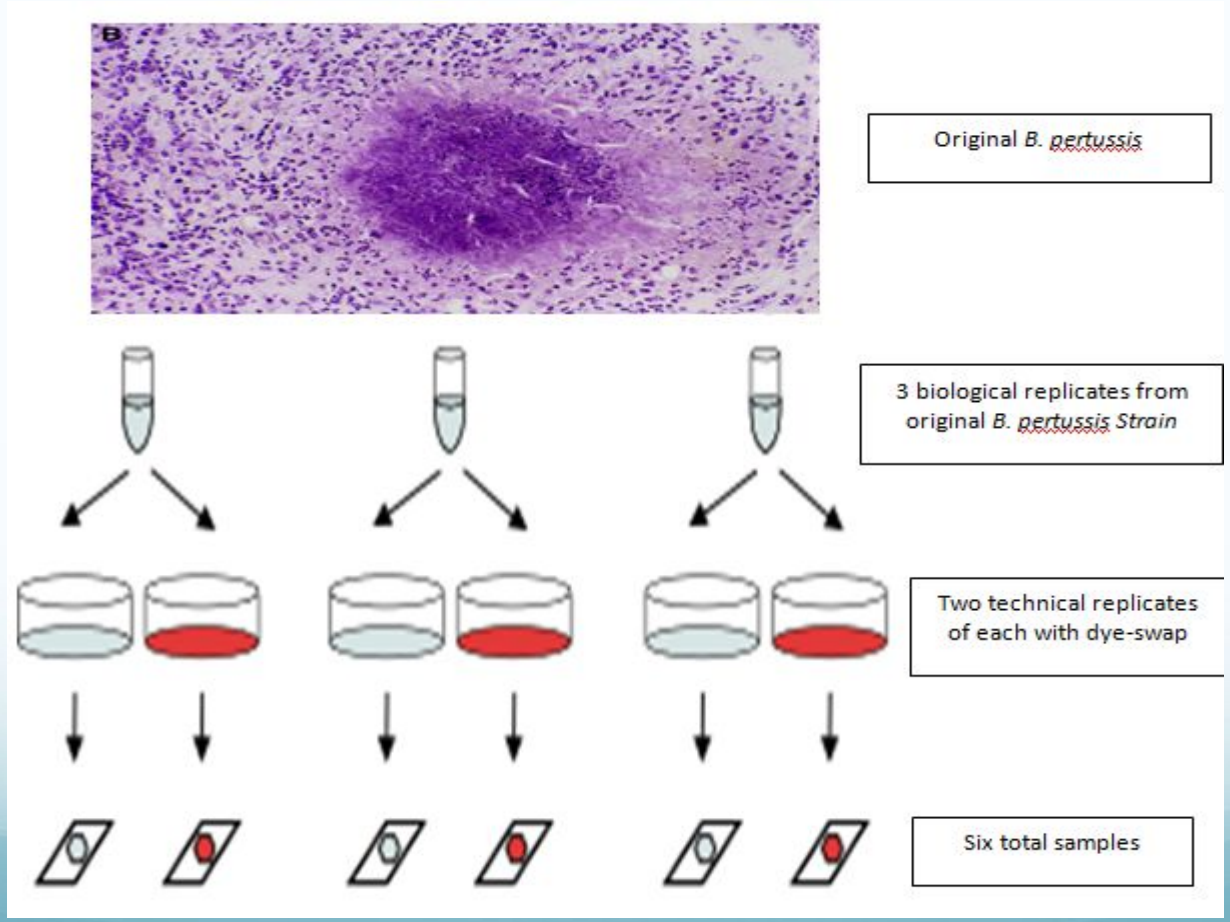


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Table 1: Bacterial strains and growth conditions

B. pertussis strains	Genotype/Relevant features	Source
BPSM	Tohama I derivative, mutant rpsL	[72]
ΔkpsT	BPSM carrying an in-frame deletion in kpsT ORF	This study
ΔkspE	BPSM carrying an in-frame deletion in kpsE ORF	This study
ΔvipC	BPSM carrying an in-frame deletion in vipC ORF	This study
KOcaps	BPSM carrying an in-frame deletion from kpsM to wcbO ORFs	[17]
ΔkpsTcom	BPSM carrying an in-frame deletion in kpsT ORF containing vector pBBR::Pcaps-kpsT	This study
BvgS-VFT2	BPSM carrying amino acid substitution at F375E and Q461E at the periplasmic VFT2 domain	[31]
BvgS-VFT2-ΔkpsT	BvgS-VFT2 carrying an in-frame deletion in kpsT ORF	This study
KOcaps:kpsT	KOcaps containing vector pBBR::Pcaps-kpsT	This study
KOcaps:kpsMT	KOcaps containing vector pBBR::Pcaps-kpsMT	This study
BPSH	BPSM derivative expressing his-tagged BvgS at the N-terminal	This study
BPSH-KOcaps	BPSH carrying an in-frame deletion from kpsM to wcbO ORFs	This study
BPSH-ΔkpsT	BPSH carrying an in-frame deletion in kpsT ORF	This study
BPSH-ΔkpsTcom	BPSH carrying an in-frame deletion in kpsT ORF containing vector pBBR::Pcaps-kpsT	This study

doi:10.1371/journal.pone.0115243.t001

B. pertussis strains used in this study.

Table 2: Construction of *B. pertussis* capsule-deficient mutant strains

Oligo	Sequence (5′ to 3′)	Description
kpsM1F	ttggatcctgtccaccaccatctacgtggtgt	Forward primer to amplify PCR1-*kpsT*
kpsM2R	ttgctagccagctccatgccgcagatca	Reverse primer to amplify PCR1-*kpsT*
kpsE1F	ttgctagccttggacgaaaccatcgcgc	Forward primer to amplify PCR2-*kpsT*
kpsE2R	ttaagcttgccagctgcagattggcctc	Reverse primer to amplify PCR2-*kpsT*
kpsT1F	ttgaattccgcatgatctgcggcatcga	Forward primer to amplify PCR1-*kpsE*
kpsT2R	ttaagcttgacatactggtcggacgcaat	Reverse primer to amplify PCR1-*kpsE*
wbpT7F	ttaagcttgaggccaatctgcagctggc	Forward primer to amplify PCR2-*kpsE*
wbpT6R	ttggatcctatgcccgcggcgcggctt	Reverse primer to amplify PCR2-*kpsE*
wbpTF	ttgaattccatgccgccggtggaccg	Forward primer to amplify PCR1-*vipC*
wbpTR	ttaagcttacggcacatgcccagcacg	Reverse primer to amplify PCR1-*vipC*
wzaF	ttaagcttgagttcgagccggtgctgg	Forward primer to amplify PCR2-*vipC*
wzaR	ttggatccttgctggtaaggaatgcgctg	Reverse primer to amplify PCR2-*vipC*
kpsTcomF	ttggatcccgttgatggagacggccatg	Forward primer to amplify full length *kpsT*
kpsTcomR	ttaagctttcaggattgctcagcgtcgac	Reverse primer to amplify full length *kpsT*
BvgA-BamHI-F	ttggatcctgtactgagattcgccgtc	Forward and reverse primer to amplify PCR1 from 3′ end of *bvgA* ORF to 5′ end of *bvgS* signal peptide ORF
BvgS-XbaI-R	tttctagagcttgcctgcgcgggc	
BvgS-XbaI-6His-F	tttctagacatcatcaccatcaccaccaggagctgaccctg	Forward and reverse primer to amplify PCR2 downstream of *bvgS* signal peptide sequence; forward primer carries nucleotides encoding 6x histidines
BvgS-HindIII-R	ttaagcttggcgactacgcgaacgtcattgaa	

Restriction sites are underlined.

doi:10.1371/journal.pone.0115243.t002

List of forward and reverse primers used in cloning.

Table 3: Real-time polymerase chain reaction

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
bvgA	TCCTCATCATTGACGATCACCC	CGATGACTTCCAGCCCGTCCA
bvgR	AACAGCTGCTGGCGCAGGTT	GCCGCAGGCTATGCAGGCTT
brkA	GTATCTCGATAGATTCCGTCAAT	CGTGTTGTCCCGTGGTCG
fhaB	TGTCCGCCATGGAGTATTTCAA	CCCAAATGTACTCGTAGCGATTC
ptx	GCGTTGCACTCGGGCAATTC	CAGATGGTCGAGCACATTGTC
sphB1	TGCTGCAGGACAACCTGTATTC	TCAGGCCGGCCGAGACTTCG
recA	GACGACAAAACCAGCAAGGCC	CGTAGACCTCGATCACGCGG

doi:10.1371/journal.pone.0115243.t003

List of forward and reverse primers (from 5′ to 3′) used in Real-time PCR analysis.

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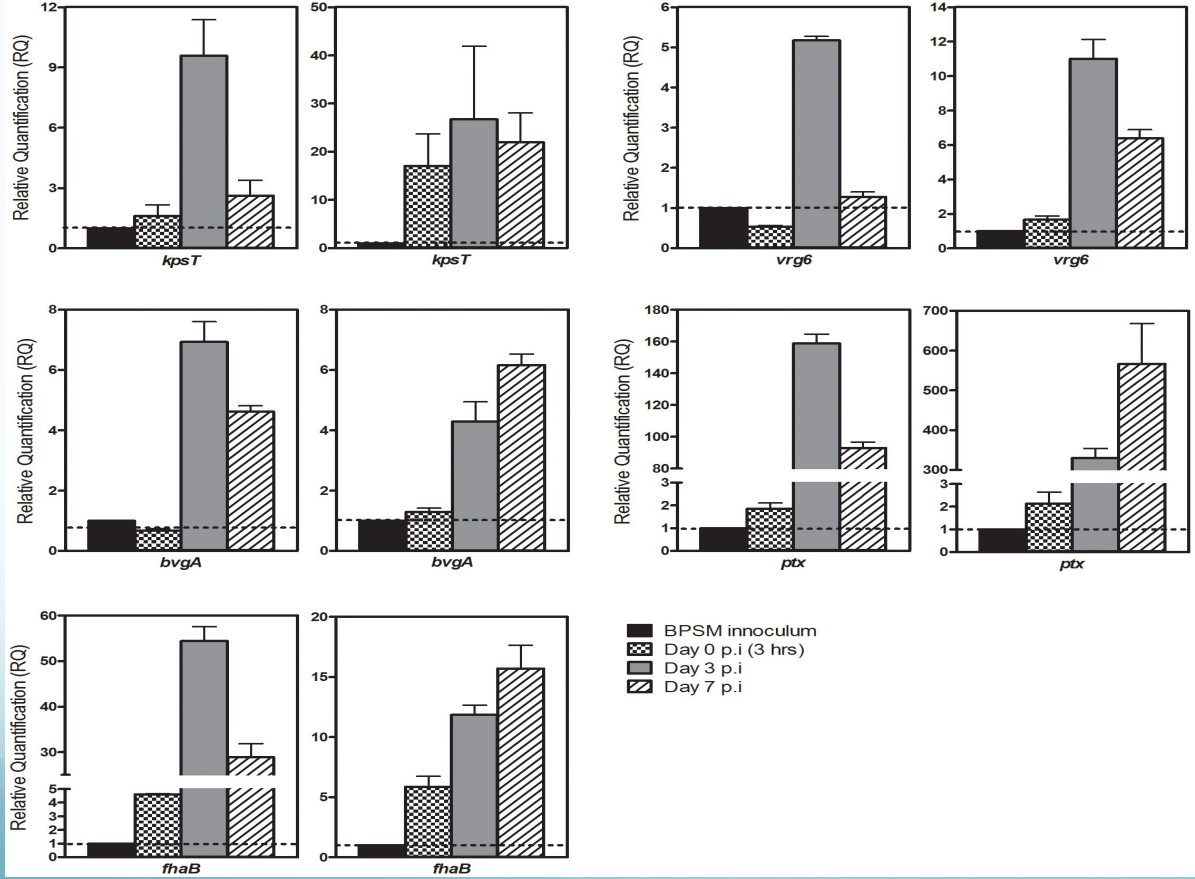
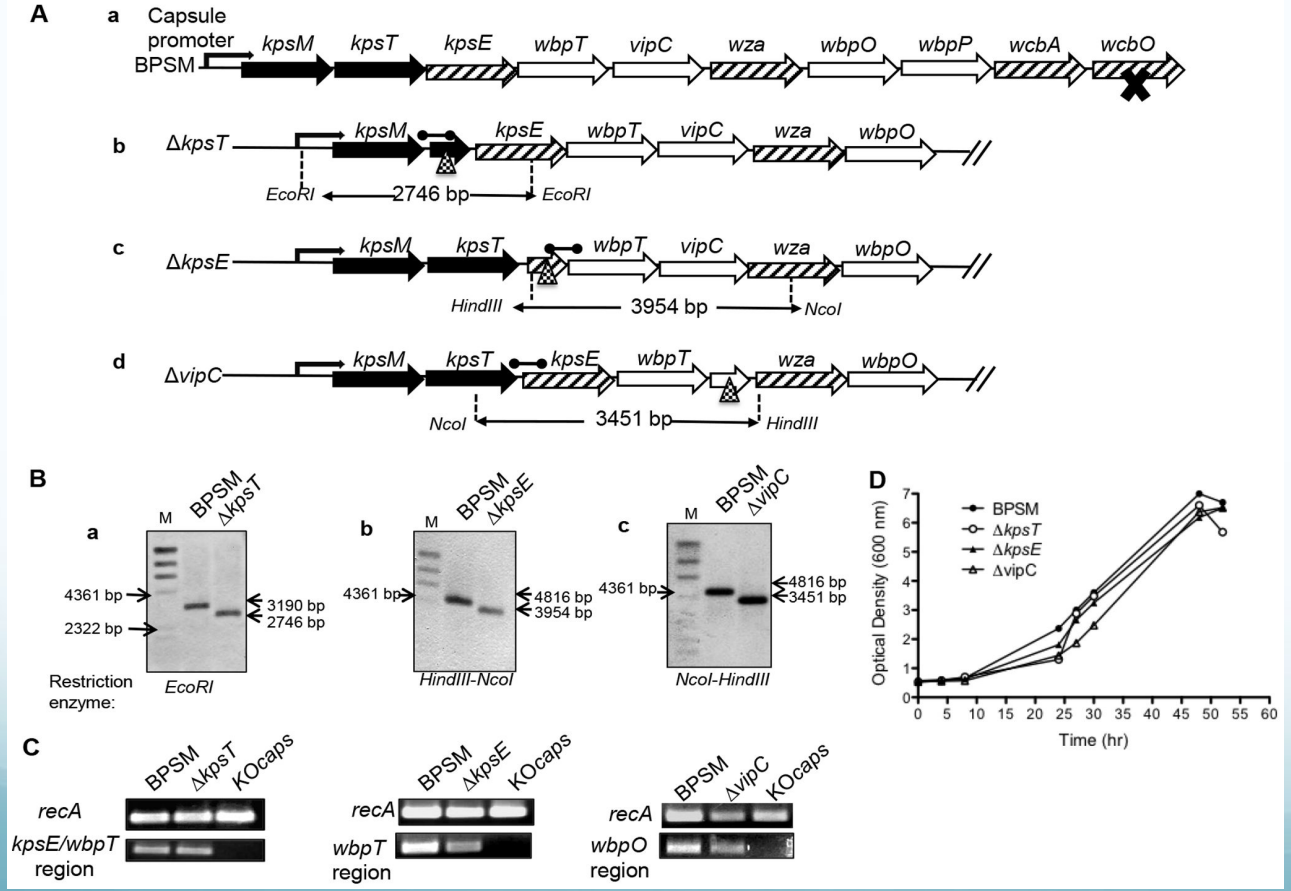
Figure 1: The capsule locus expression is modulated during pertussis infection



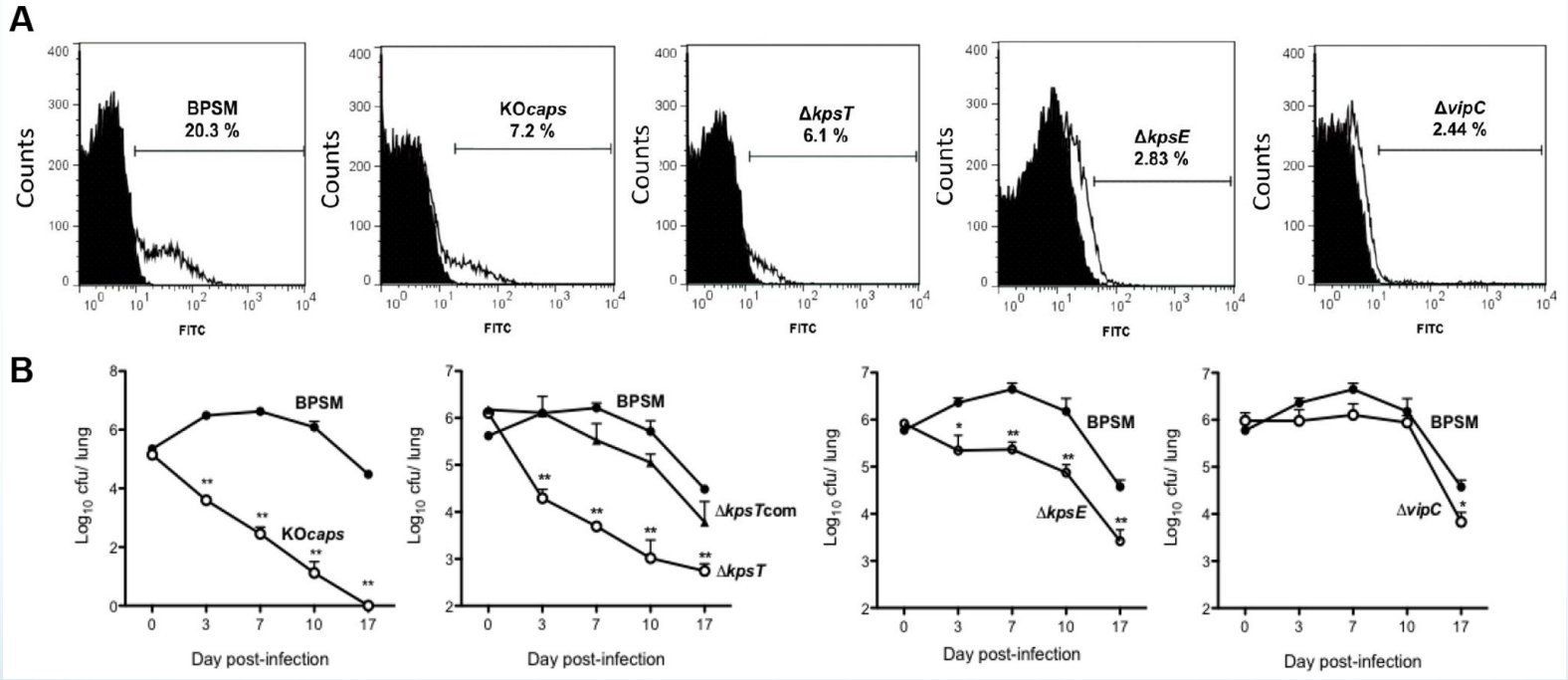
Relative transcriptional activity of *vrgs* and *vags* in BPSM bacteria recovered from mice lungs versus *in vitro* BPSM grown in virulent phase.

Figure 2: Construction and characterization of *B. pertussis* capsule-deficient mutants



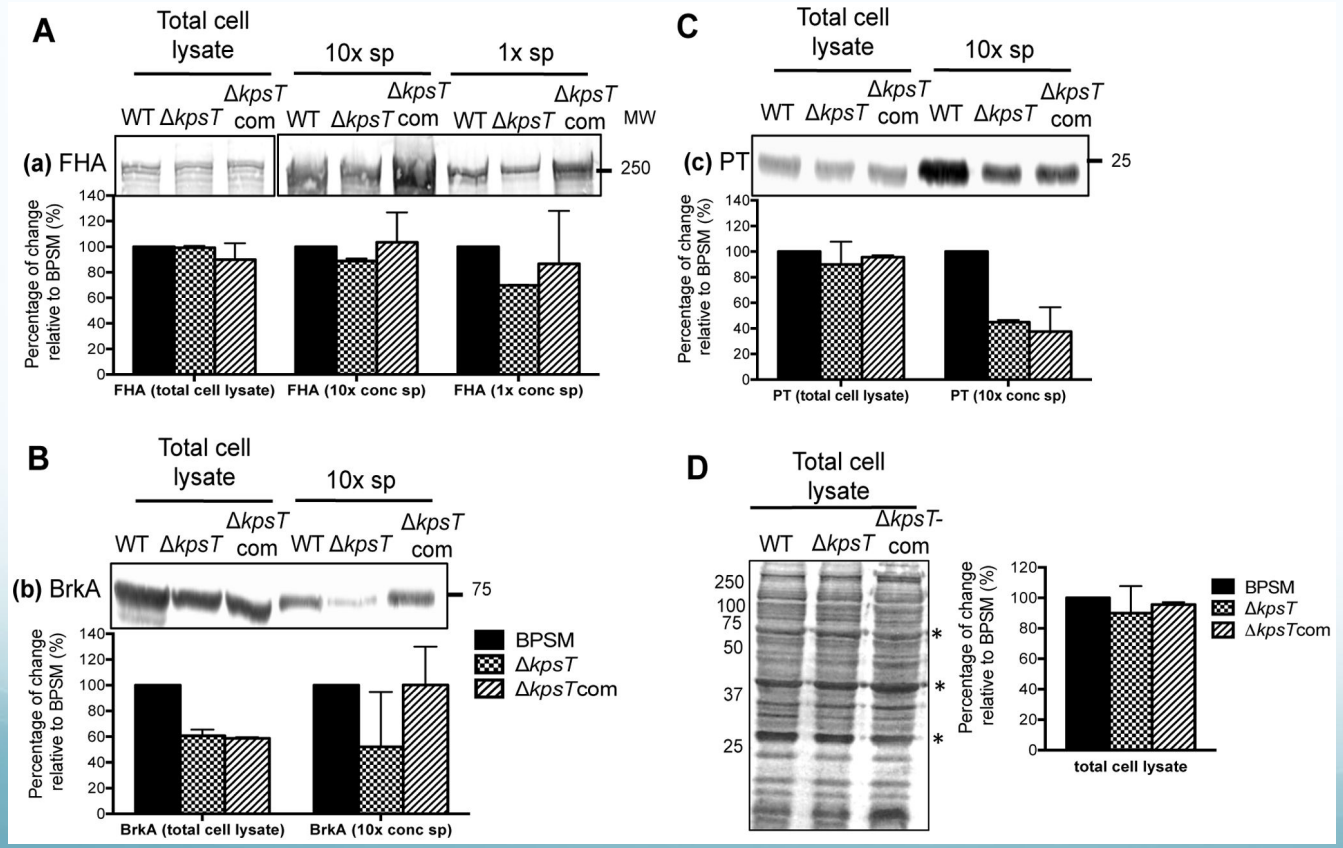
Construction of Δ*kpsT*, Δ*kpsE* and Δ*vipC B. pertussis* mutants.

Figure 3: The PS capsule transport-export proteins are involved in pertussis pathogenesis



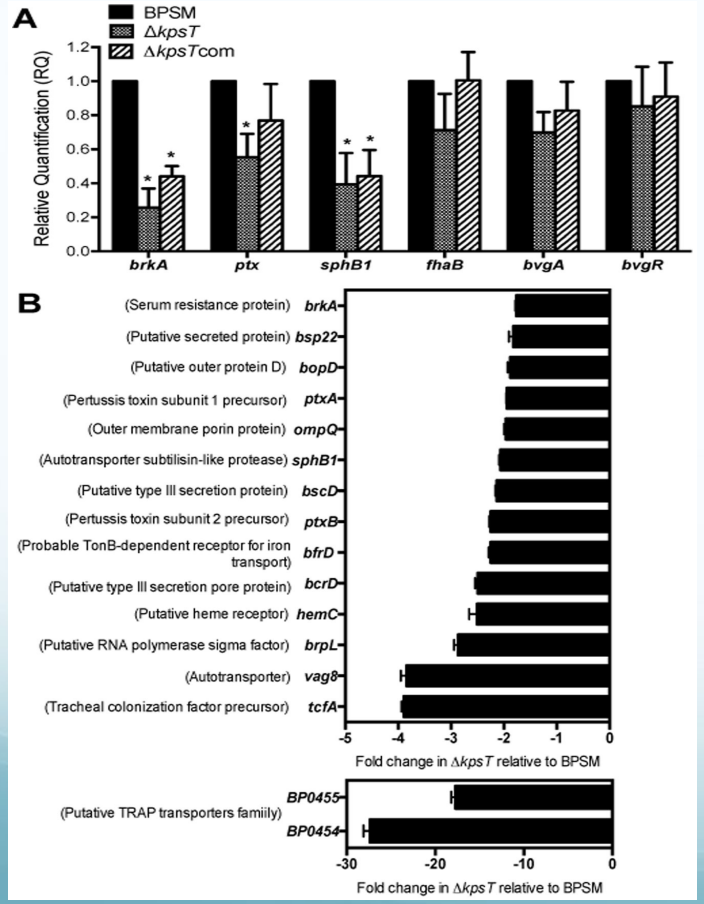
Phenotypic characterization of the Δ*kpsT*, Δ*kpsE* and Δ*vipC* mutants.

Figure 4: Absence of KpsT in *B. pertussis* results in mild reduction in the production and/or secretion of major *bvg*-regulated virulence factors



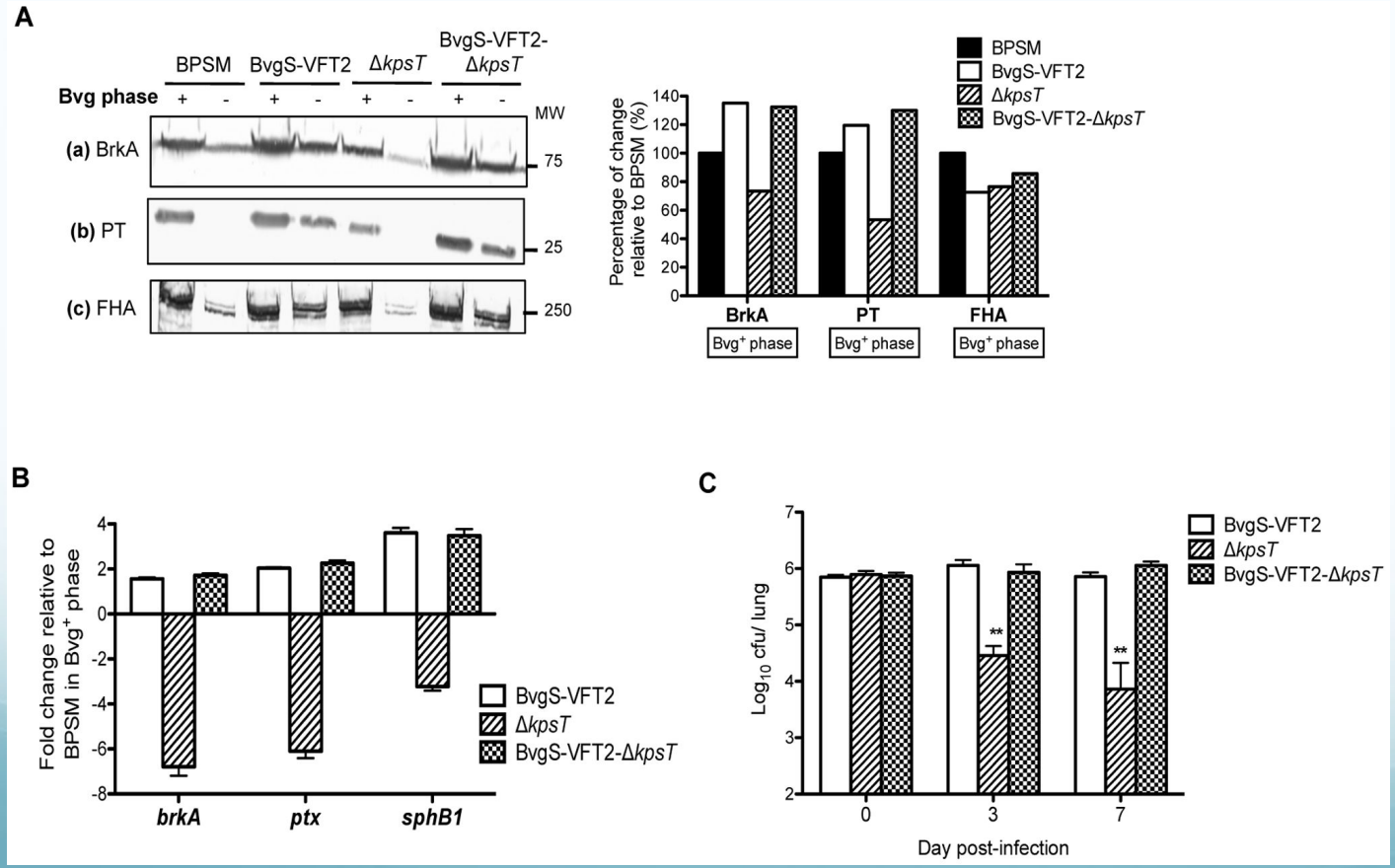
Production of *bvg*-regulated virulence proteins in Δ*kpsT* mutant.

Figure 5: Absence of KpsT alters the global gene expression pattern in *B. pertussis*



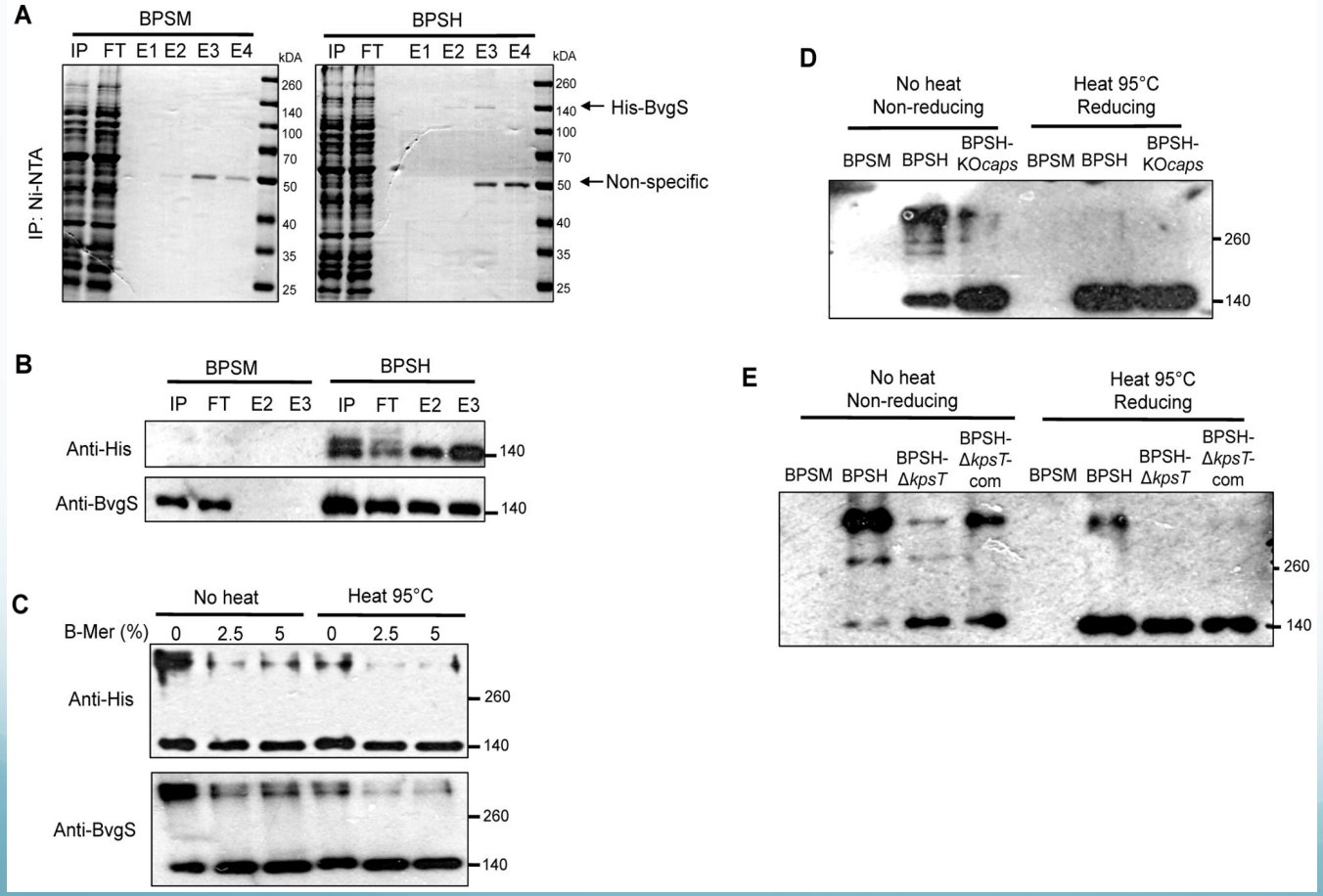
Transcriptional activity in Δ*kpsT* mutant.(A) Relative transcriptional activity of *vags*.

Figure 6:Functional link between KpsT and the BvgA/S signaling pathway



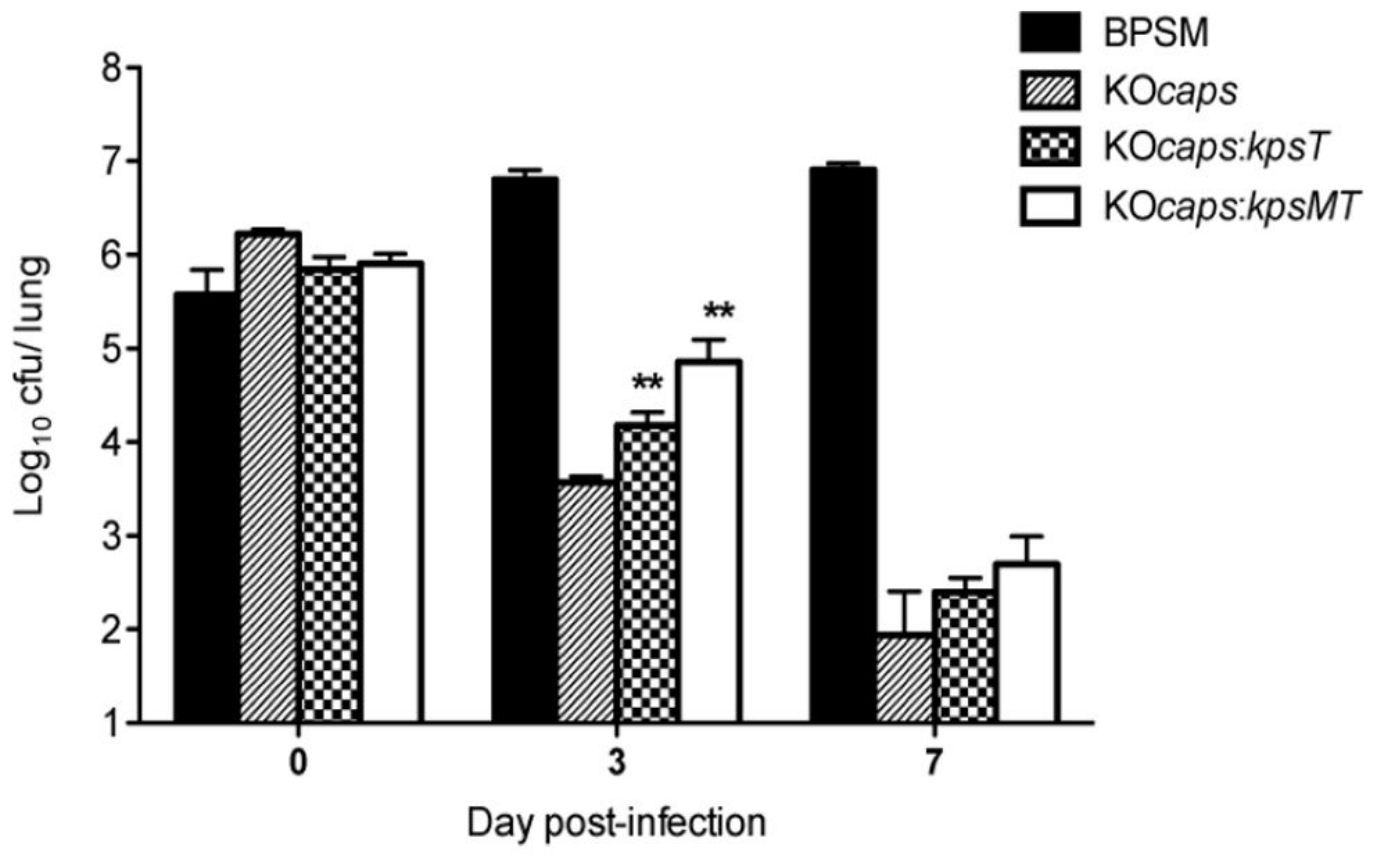
Characterization of the BvgS-VFT2-Δ*kpsT* mutant. (A) Production of *bvg*-regulated virulence proteins.

Figure 7: Expression and purification of His-BvgS from *B. pertussis* strains.



Coomassie blue analysis, Western blot analysis, Detections of BvgS associated oligomers and BvgS monomer

Figure 8: Mice were infected intranasally with *B. pertussis* BPSM, *kpsT, kpsMT* strains



Lung colonization profile of *B. pertussis* KO*caps*:*kpsT* and KO*caps*: *kpsMT* strains.

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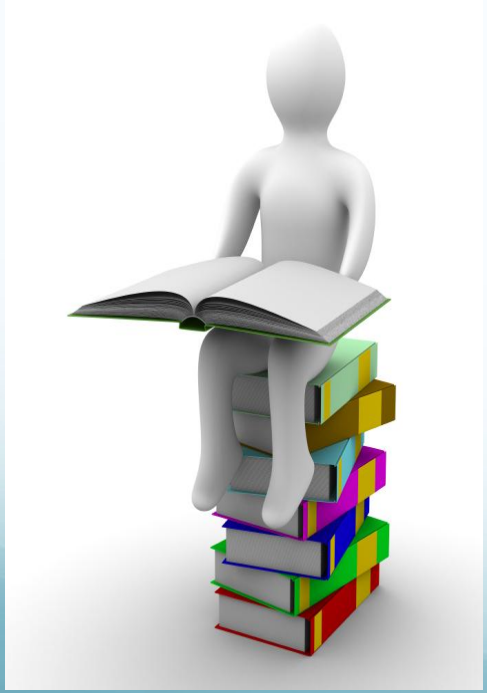
Discussion



- The results of this experiment were compared to findings in previous studies.
- In conclusion, this research portrays that the *B. pertussis* PS capsule transporter-export machinery and in particular KpsT are necessary for optimal expression of virulence genes and therefore play an important role in pertussis pathogenesis.

Acknowledgements

- Dr. Dahlquist
- Dr. Dionisio
- Authors of research article: Evidence for a Role of the Polysaccharide Capsule Transport Proteins in Pertussis Pathogenesis.
- Biological Databases Students
  - Thank you for listening!

References

Hoo, R., Lam, J.H., Huot, L., Pant, A., Li, R., Hot, D., & Alonso, S. (2014). Evidence for a Role of the Polysaccharide Capsule Transport Proteins in Pertussis Pathogenesis. PLoS ONE, 9(12):e115243. doi: 10.1371/journal.pone.0115243

Pictures:

http://media.historyofvaccines.org/images/000744_540.jpg

http://mediad.publicbroadcasting.net/p/shared/npr/styles/x_large/nprshared/201404/306870910.jpg


Questions