Transcriptional analysis of heterologous gene expression using the endogenous σ^D promoter from *Bacillus halodurans*

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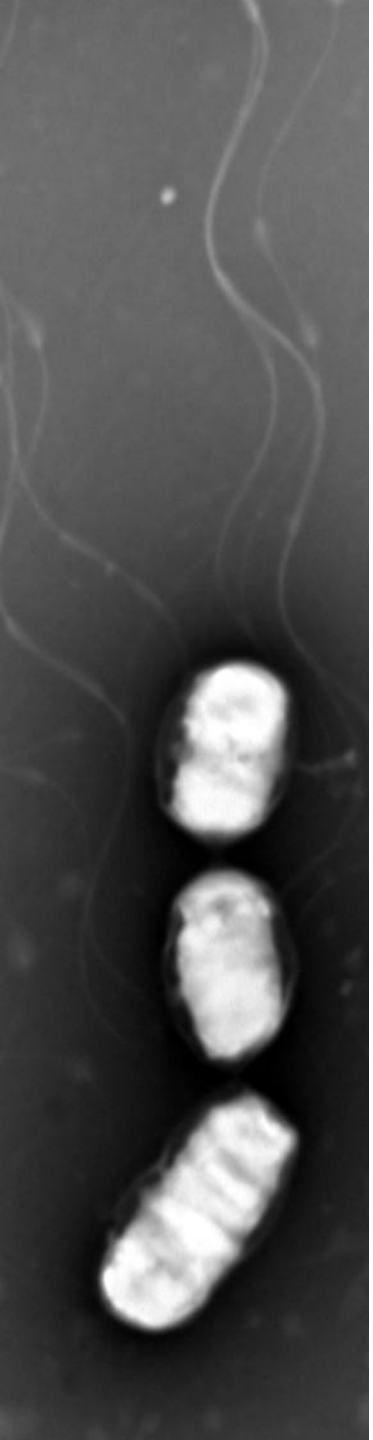
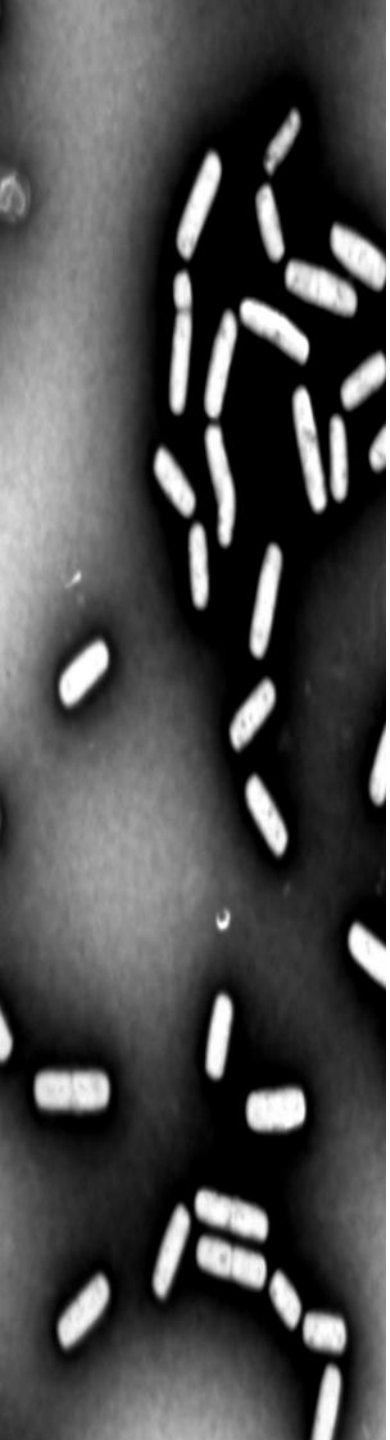
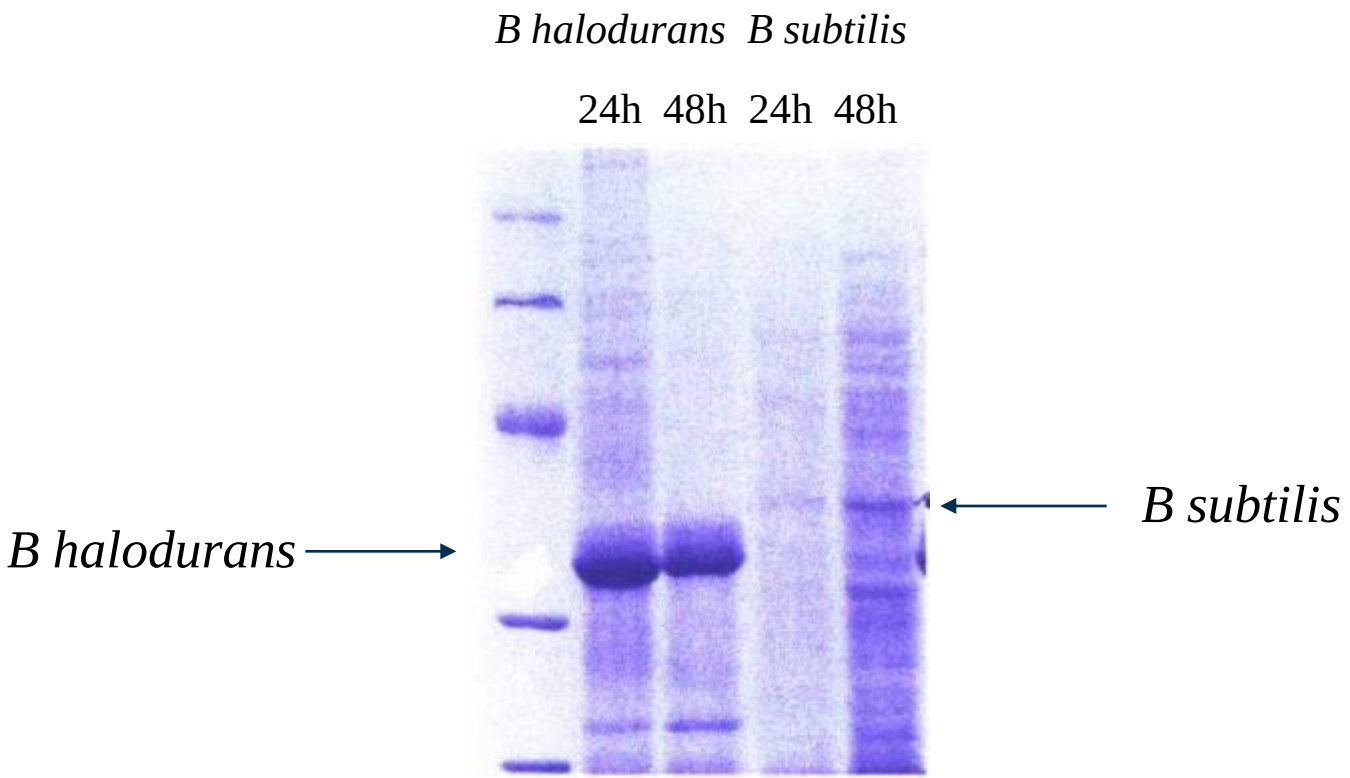


Bacillus halodurans Alk36

- Isolate identified by 16S rRNA homology study- found to have 99.9% sequence identity to the published genome of *B halodurans* C125.
- Ability to over-produce cell surface protein
- Ability to grow over a wide pH range: pH 7.5 to 10.5
- Ability to grow over a wide temperature range: 30°C - 60°C.



PAGE showing over-production of cell surface protein by *B halodurans* Alk36



Protein identified as flagellin, product of the *hag* gene.

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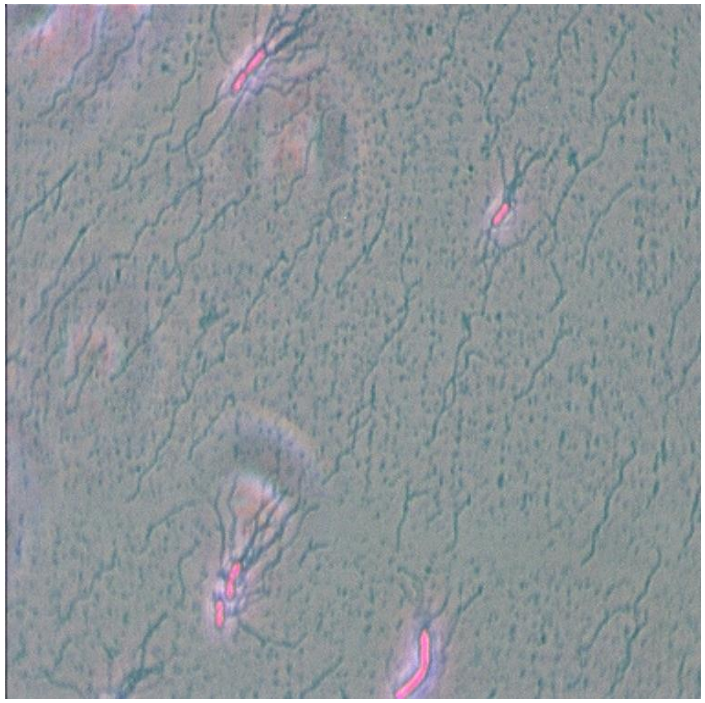
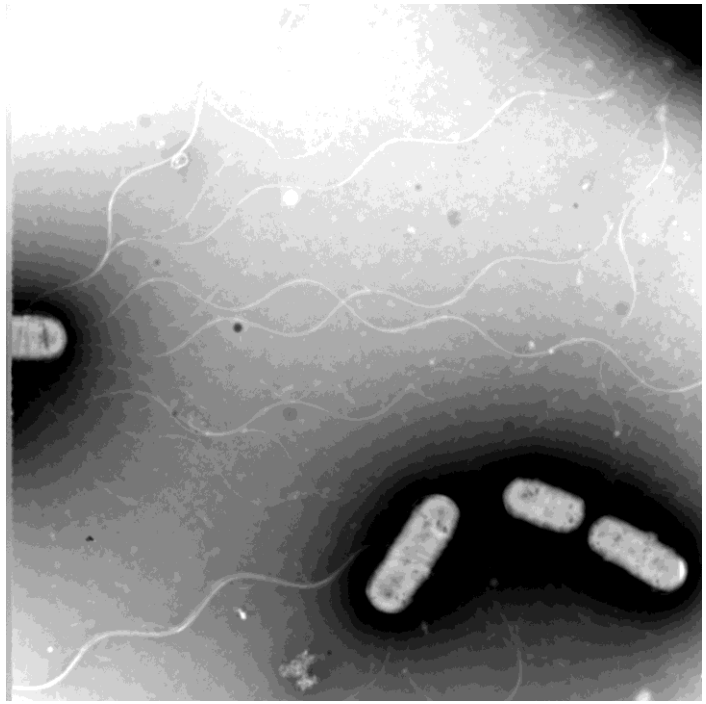
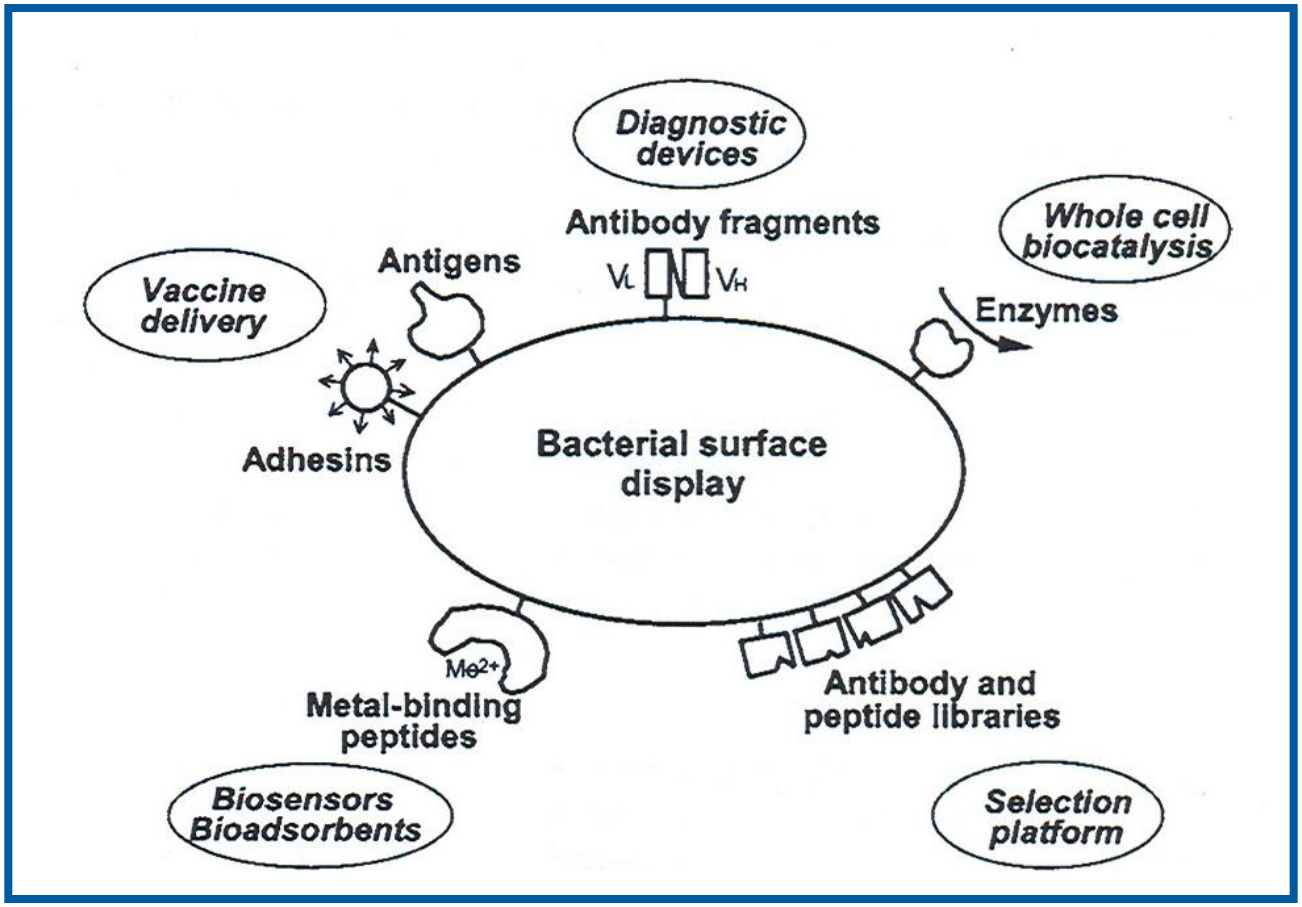
Micro-photograph and TEM showing *B halodurans* flagella





Applications of Surface display



Wernerus and Stahl (2004) Biotechnol. Appl. Biochem. **40** : 209-228

Crampton et al (2007) Applied Microbiology and Biotechnology 75: 599-607

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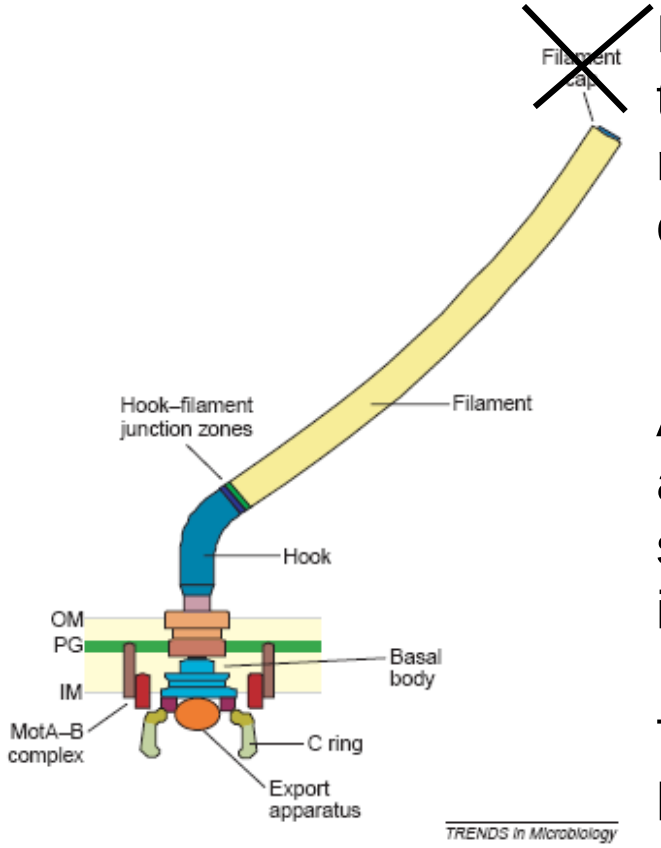
Secretion of peptides using a modified type III secretion apparatus.



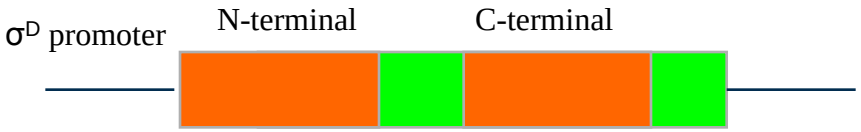
Flagella contain at their distal end the capping protein FliD which is responsible for the polymerization of flagellin (FliC) monomers

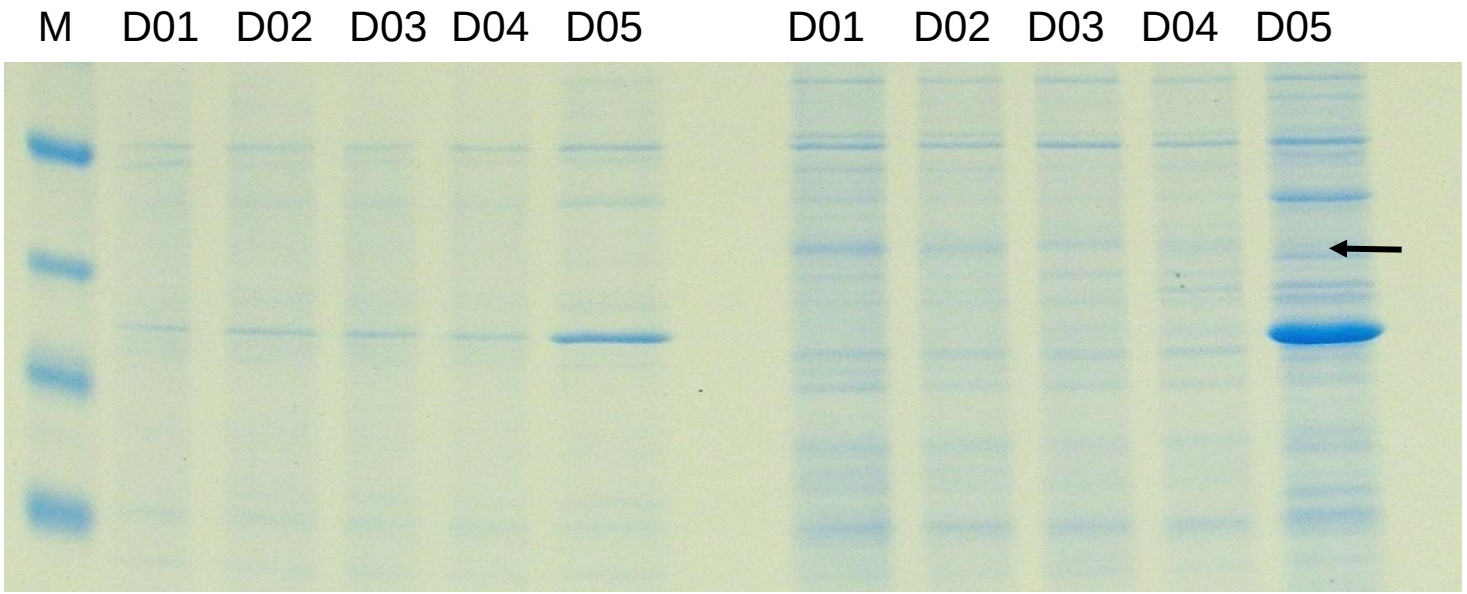
A non-functional FliD fails to assemble flagella and leads to secretion of flagellin monomers into the culture medium.

These monomers can contain a heterologous peptide.

Beatson et al (2006). Trends in Microbiology 14: 151-155.

Evaluation of different genetic backgrounds on peptide secretion during log and stationary phase

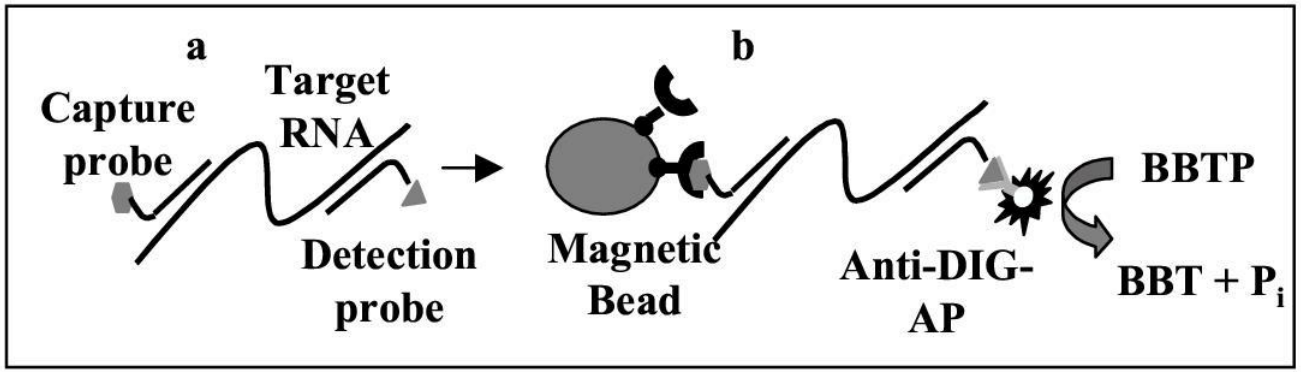




B. halodurans BhFD05 (Δ*hag,* Δ*fliD* Δ*wprA,* Δ*alp,* Δ*apr,* Δ*vpr,* Δ*asp)*

Berger et al (2009) Applied and Environmental Microbiology 75: 271-274.

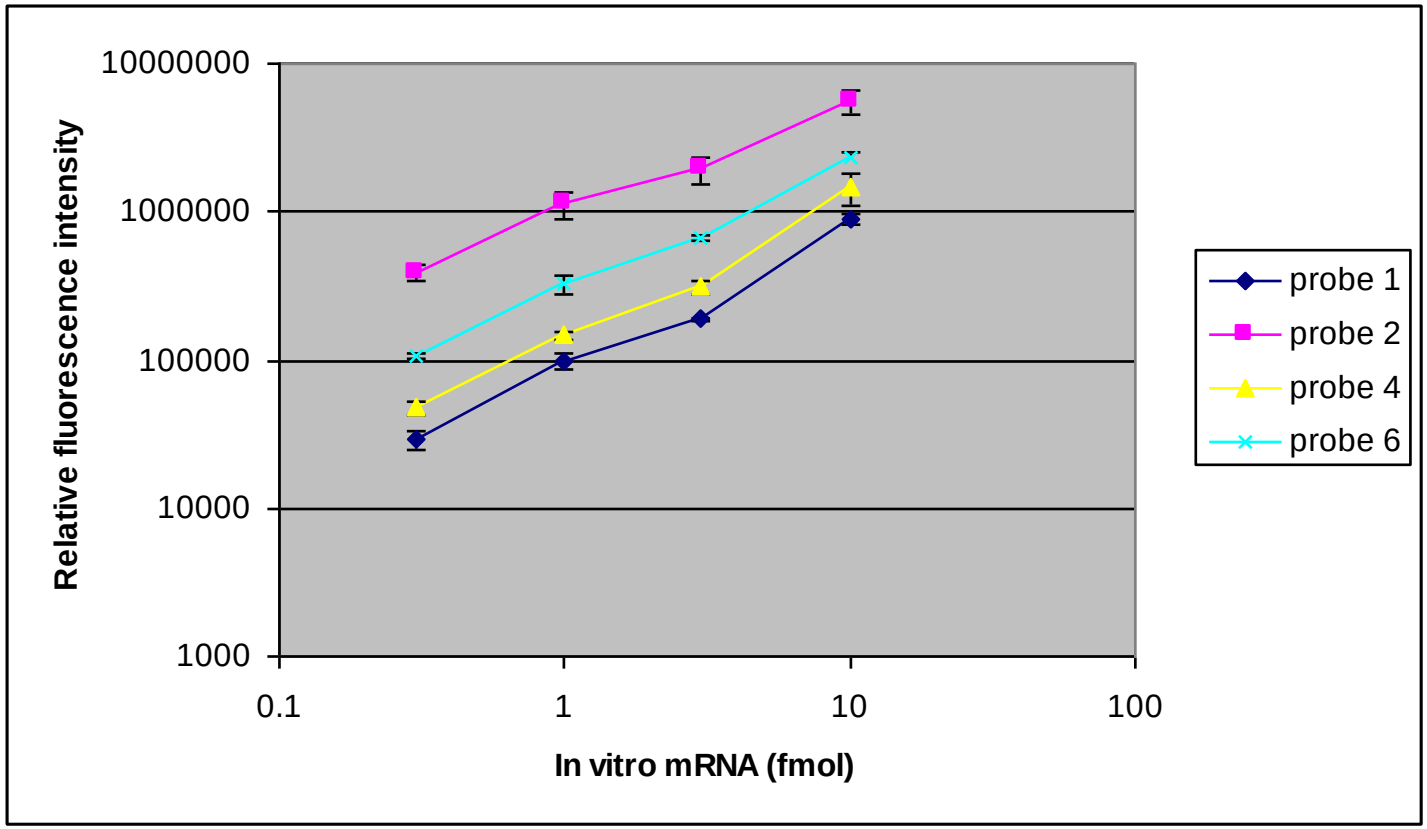
Transcript Analysis using Sandwich Hybridisation Assay

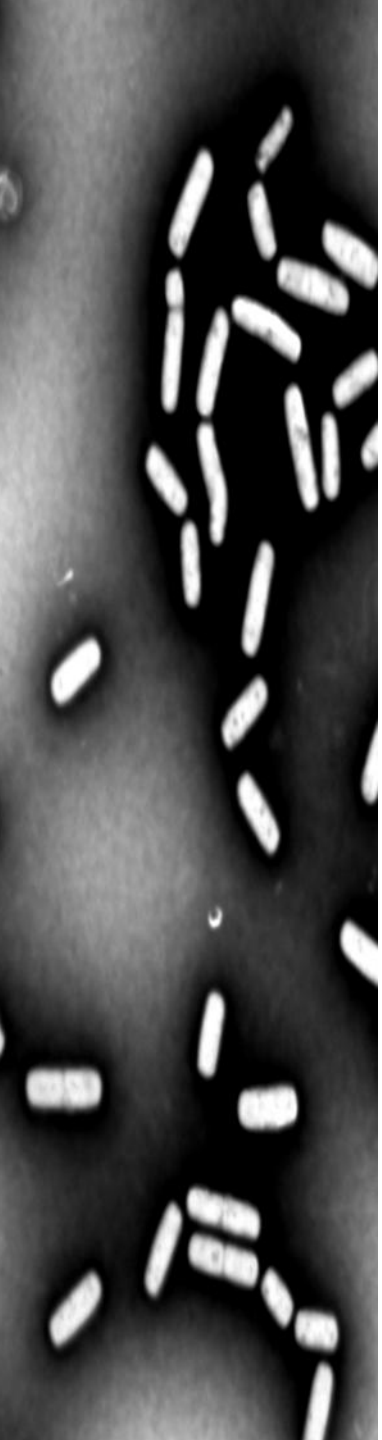


Rautio et al. (2003) Microb. Cell Fact. **2:** 1-9

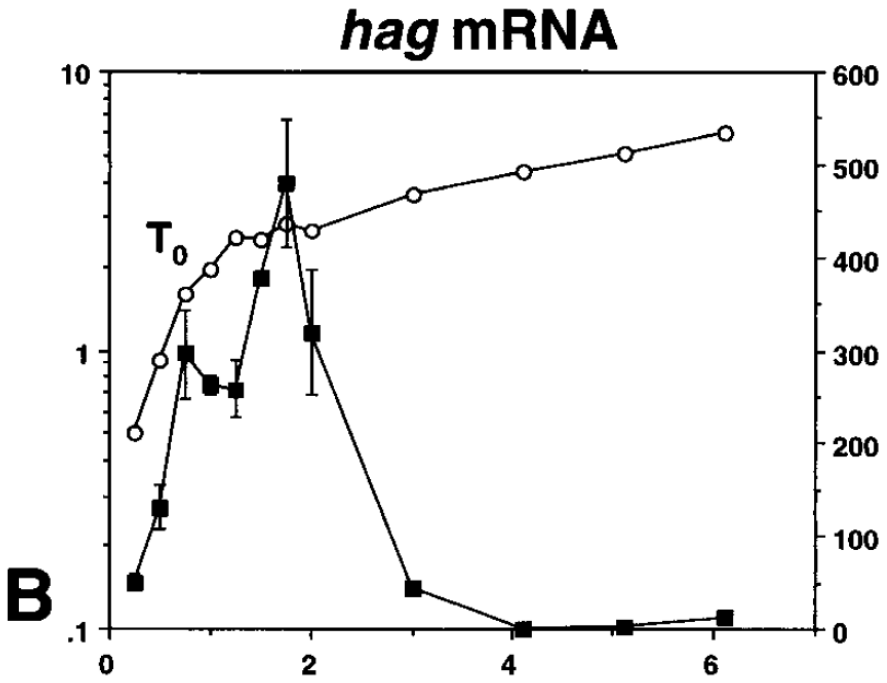
probe	tm (salt adjusted)	nt	position	label	sequence 5'to3'
1Detection127	60	20	127		CATCGTCTCCTGCACGGTTA
1Capture147	60	20	147	5' biotin	AGAGATGGCAAGACCTGCAG
1Helper107	60	21	107		ATGCGAAGACCTGAAGAAAGC
1Helper169	60	22	169		GGATTTGCGCACGCATTTTTTC
2Detection209	60	22	209		ATACCGTCTTGTGAGTTACGAG
2Helper233	60	24	233		CCTTCAGCTGTTTGAATTAACGAA
2Capture257	60	24	257	5' biotin	ATAGAATGTACTTCATCAAGCGCA
2Capture257M	60	24		5' biotin	ATAGAATTGCATCTTACAAGCGCA
2Helper278	60	21	278		AGCTCACGCATACGTTGAAGA
2Helper187	60	20	187		ACGCTTGATCCAAACCACGG
4Capture333	60	22	333	5' biotin	GTTAAGAGCTGCTTGATCTTGC
4Helper311	60	24	311		TCAACATTCGTTTCGTTCGAAGAT
4Detection287	60	20	287		TGAACCGCTAGCTCACGCAT
4Helper267	58	24	267		ACGTTGAAGAATAGAATGTACTTC
4Helper357	58	24	357		CTCAACTAATTGTTGGAATTCATC
6Capture642	60	21	642	5' biotin	GATTGTATGCTCTAGGCGGTT
6Capture642M	60	21		5' biotin	GATTGTTACGTTCGAGCGGTT
6Detection621	60	22	621		TTGCATAGCTCCTAGGTAAGAG
6Helper599	59	23	599		CGACCTTCTGAAACAGCTTTTAA
6Helper669	60	26	669		GTTTTCAGAAGCATTATCAAGGTTTT

Probe Set Validation



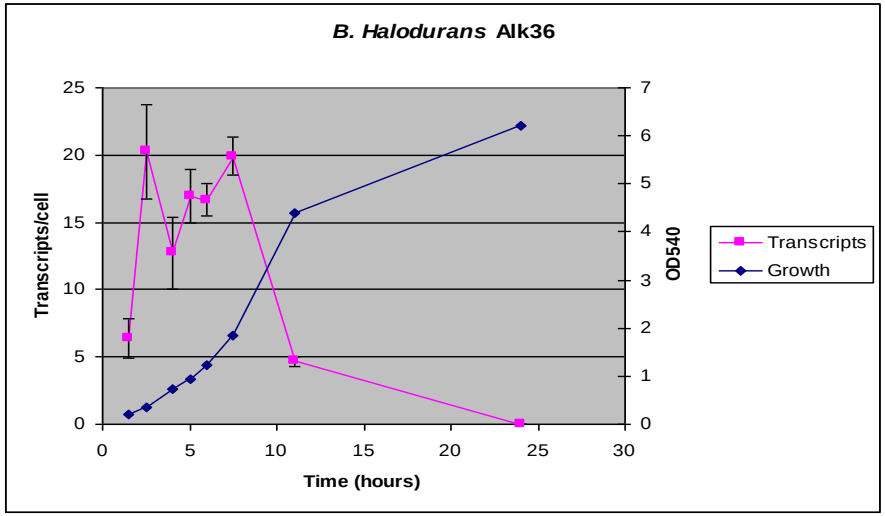


hag mRNA Transcript Levels During Growth in Complex Media



B. subtilis

Mirel et al. (2000) J. Bact. **182:** 3055-3062



B. halodurans

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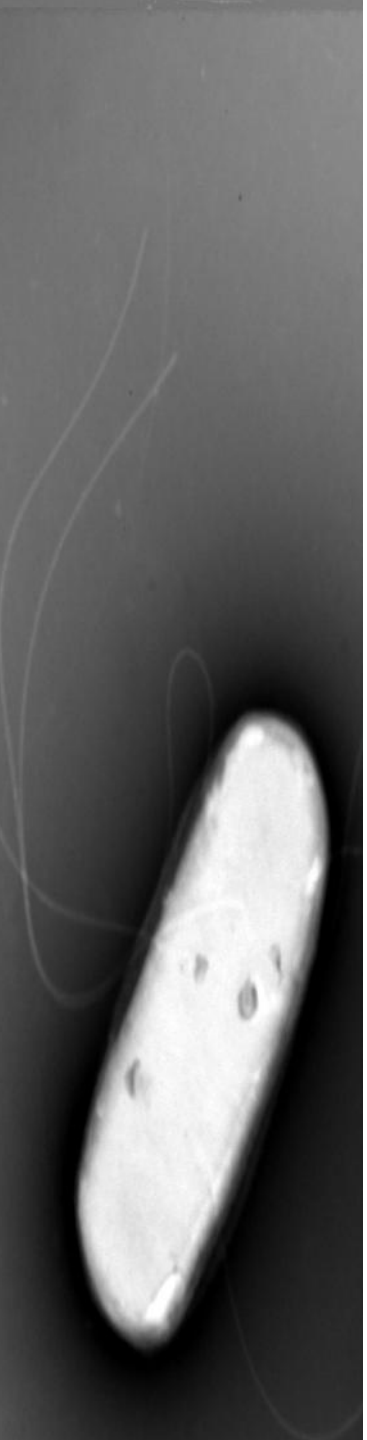
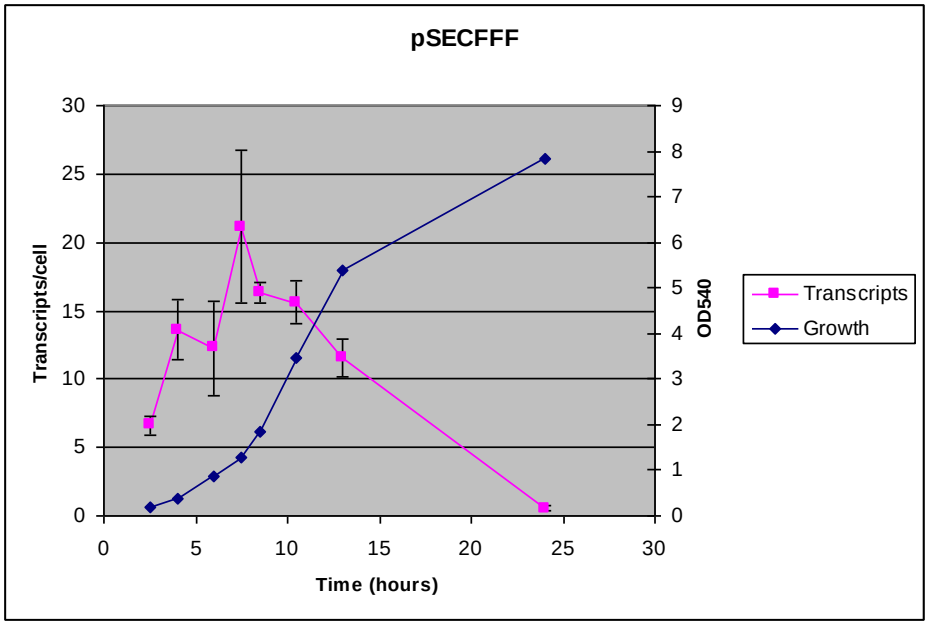
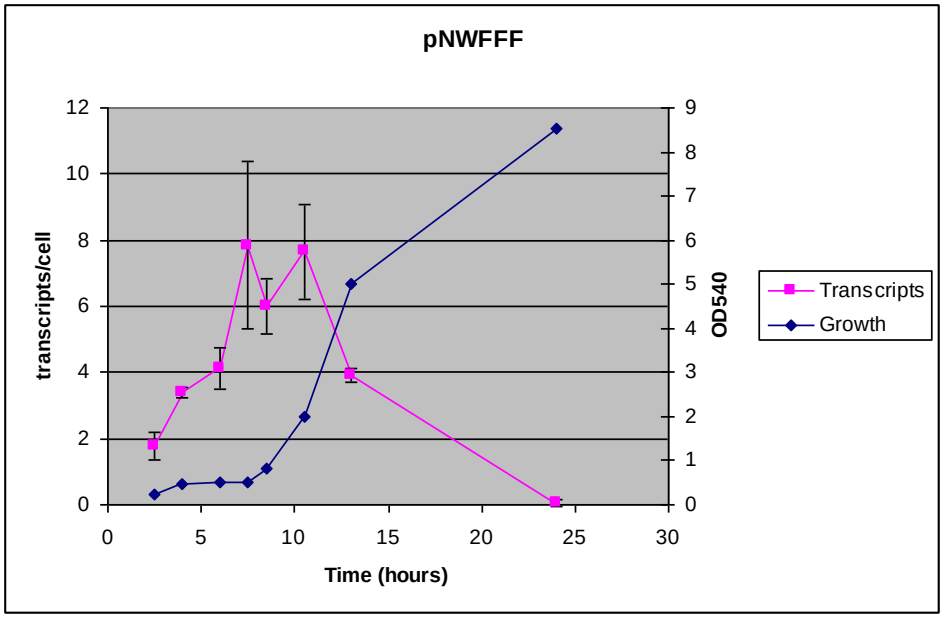


Vector Effect on *hag* Gene Expression





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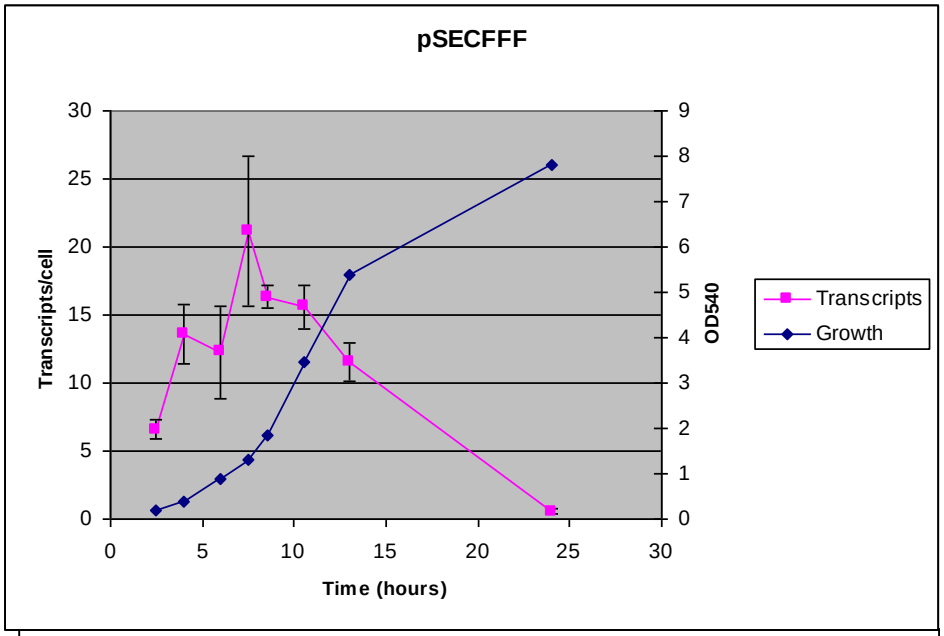
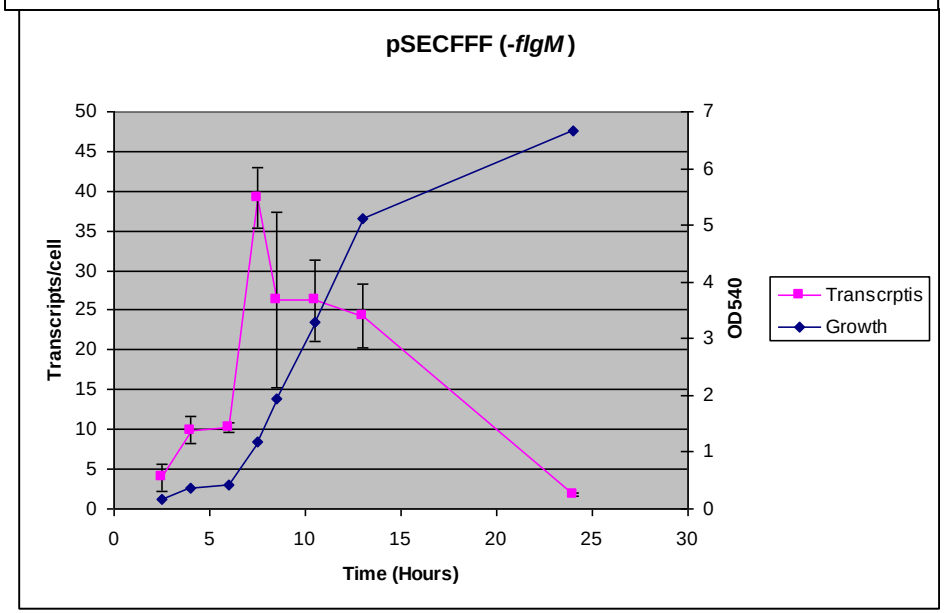
hag mRNA Transcript Levels in a *flgM*-null (anti-sigma factor) Mutant





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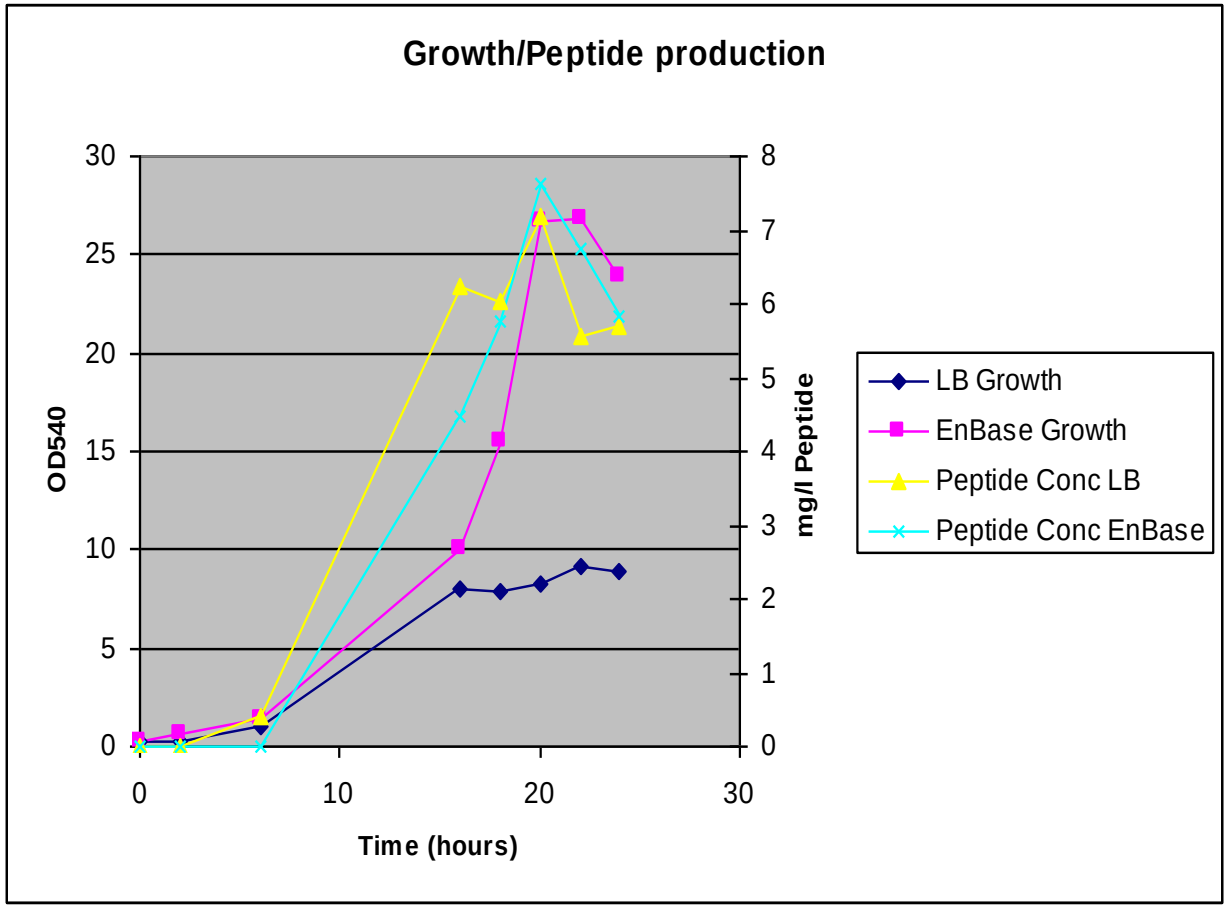
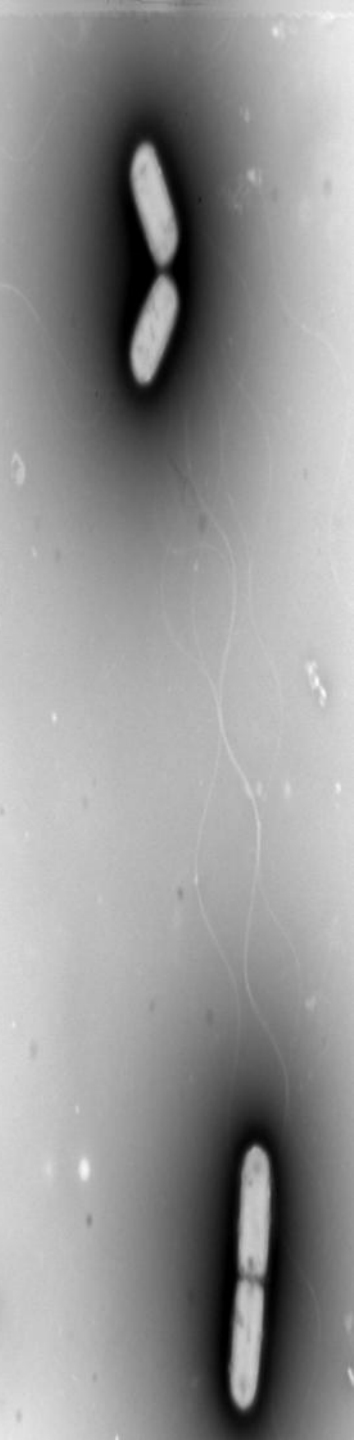
Peptide Production vs Growth





Conclusion

• Successful implementation of a high throughput mRNA sandwich hybridisation quantification assay.

• *B. halodurans* σ^D promoter regulated in a similar fashion in complex media as *B. subtilis*.

• Vector choice influences transcript levels.

• *flgM*-null mutant improves transcript levels but not protein levels.

• Peptide production follows transcript profile for LB.

Future Work

- Validation using RT PCR
- Transcriptional analysis Fermentation
- Micro-array analysis of global gene expression during transition between exponential and stationary phase
- Directed evolution of σ^D promoter

Acknowledgements

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END