Characterization of the Contributions a Detoxified Stx-Prophage Makes to the Fitness of Its Bacterial Host

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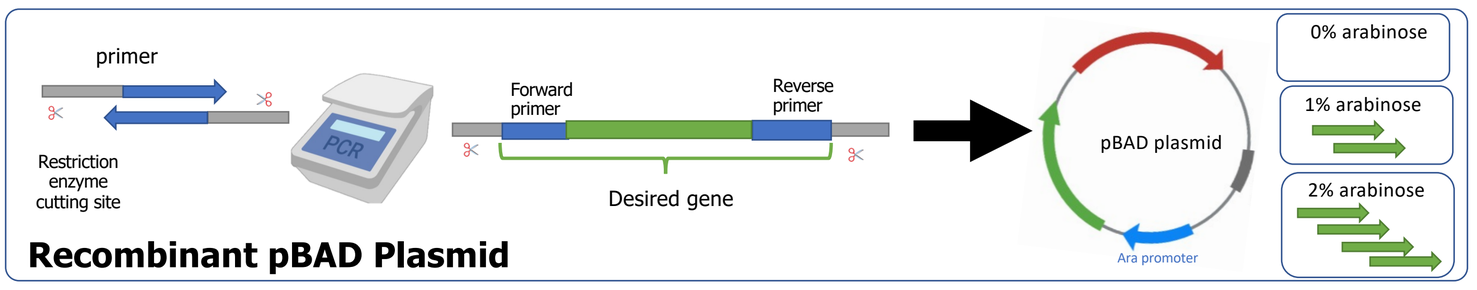
1. Introduction

- Stx-phages are responsible for the horizontal transmission of Shiga toxin-encoding genes between bacteria.
- Due to the ability of prophages to carry and introduce additional genetic material to the host cell, Stx-phages can confer traits on their bacterial lysogen.
- It was found ***vb_24B*_13c**, *res* and *stk* are under the control of same promoter, *pRM*, which drives *cI* expression.

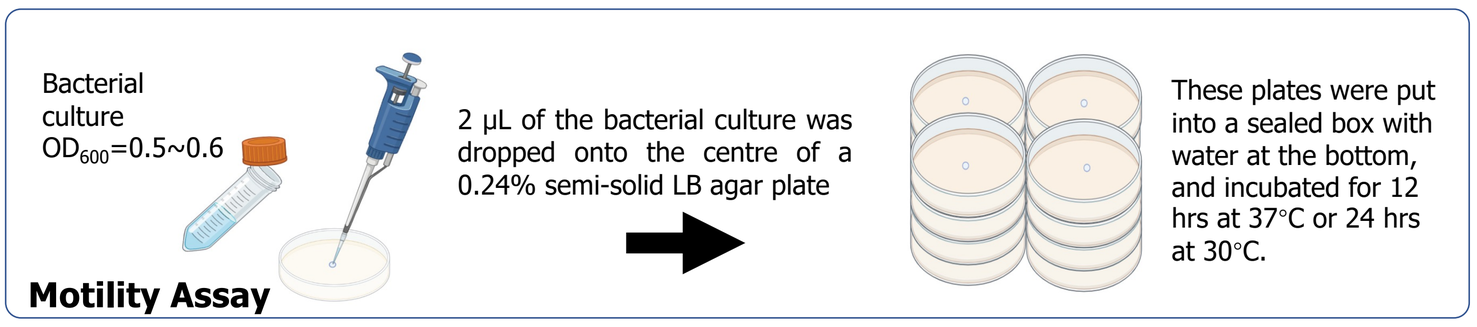
2. Aim

- Identify the function of a prophage gene, *vb_24B_13c* with regards to the motility of its bacterial host *Escherichia coli.*
- Analyse the effects of Stx-phage-mediated α-amylase upregulation in phage lysogens, which results in a shift in metabolic pathways towards preferential utilisation of maltose as a carbon source in comparison to naïve *E. coli* strains.
- Demonstrate that *cI* expression from the Stx prophage prophage has a significant role in host reprogramming of metabolic function.

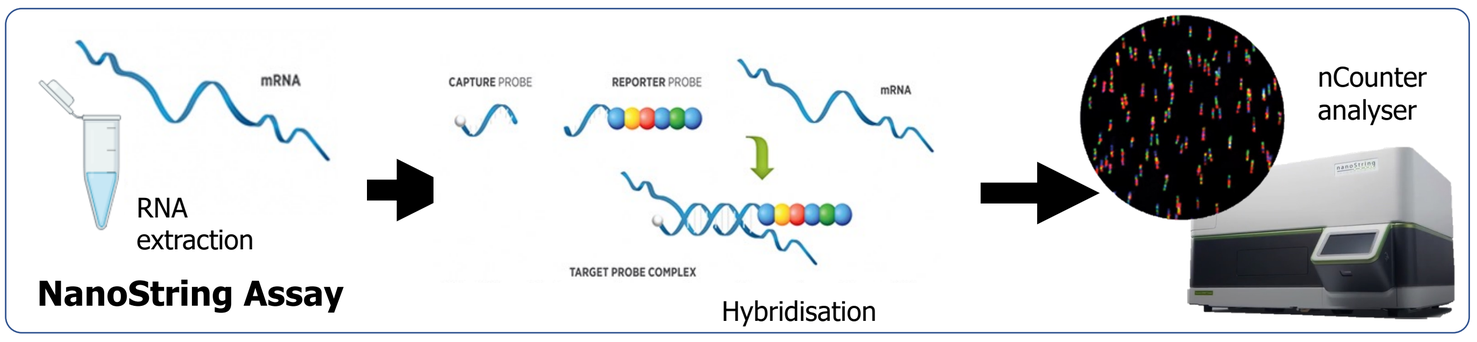
3. Methodology



Recombinant pBAD Plasmid



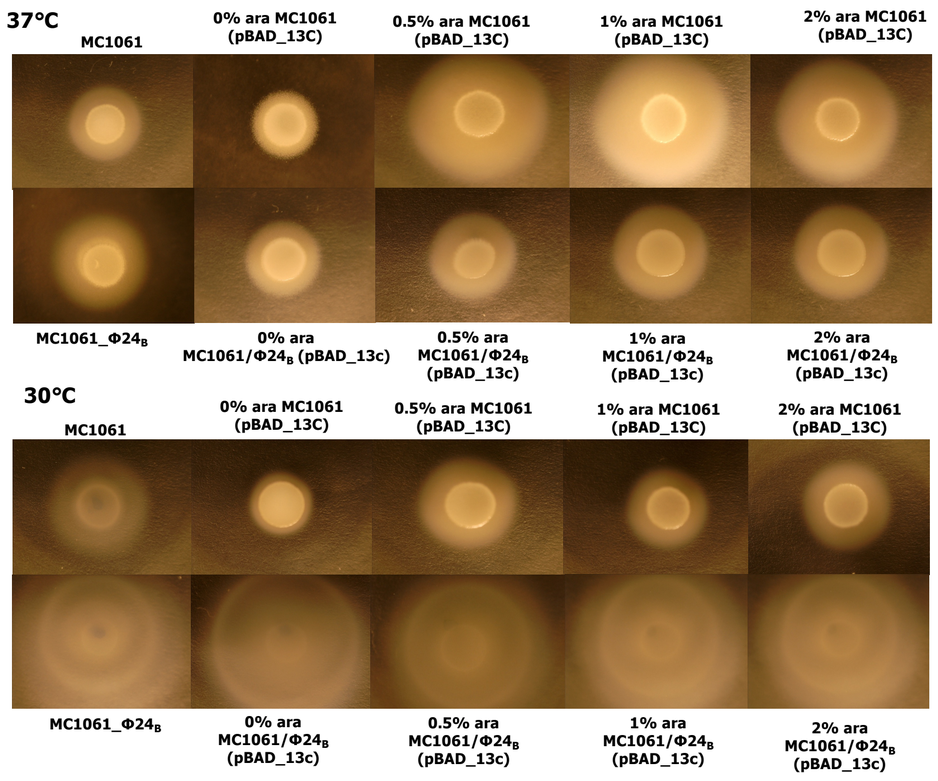
Motility Assay



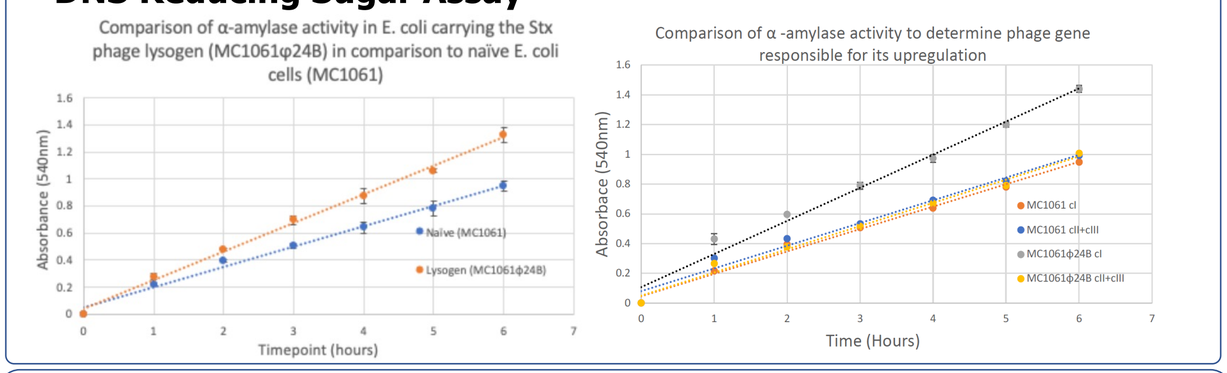
NanoString Assay

4. Results

Motility Assay



DNS Reducing Sugar Assay



Lysogen vs Naïve

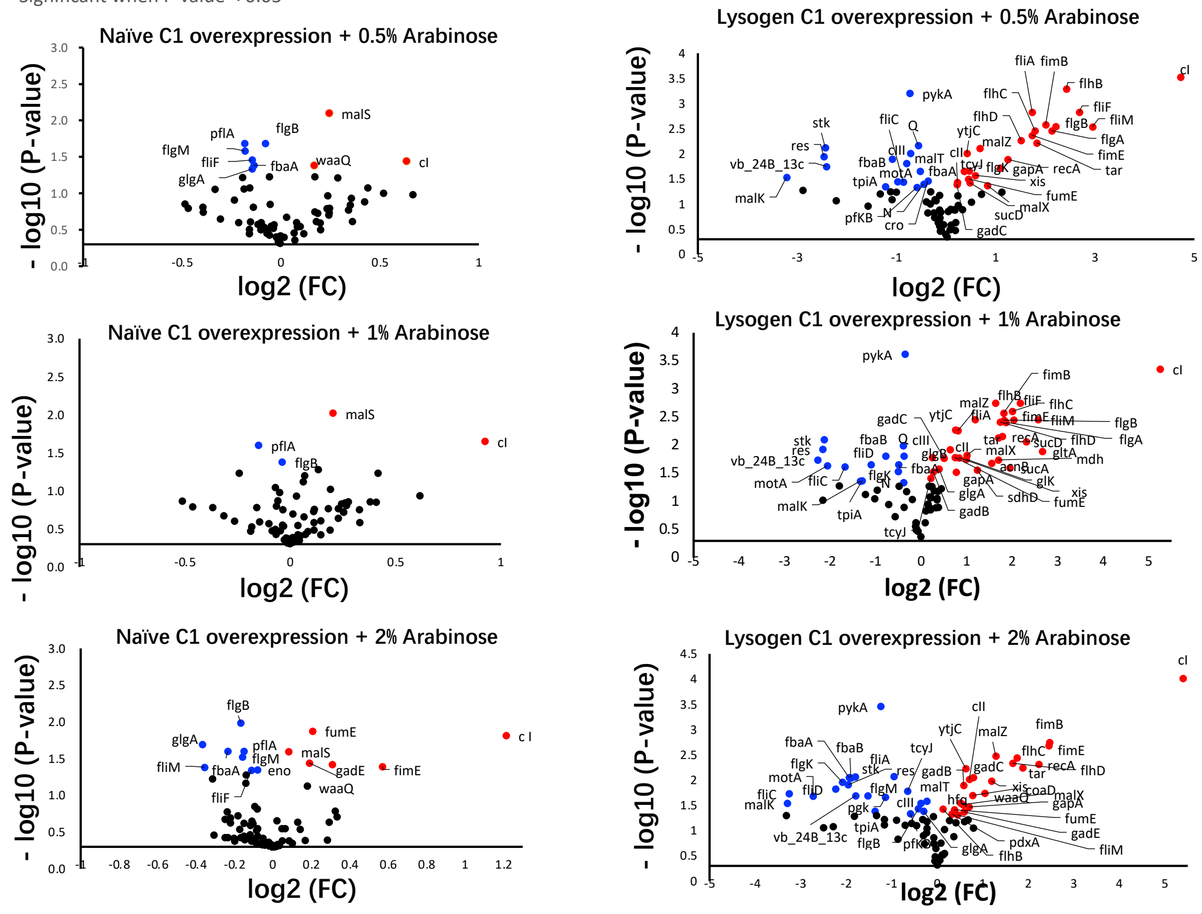
Up or Down regulation log2(Fold Change)

acnA	1.16767674	glgB	0.72442583	amyA	-1.3285116
acnB	0.42636722	gltA	0.72142024	eno	-0.1040197
coaD	0.09154691	hfq	0.39930364	fbaA	-0.9874869
crp	0.30746717	malK	0.60845577	fbaB	-1.4864365
cyaA	0.53724202	malS	0.87540022	fimA	-0.4780976
fimB	0.29140787	malT	0.25027272	flgA	-0.8180972
fimE	2.01182111	malX	0.86834864	flgB	-0.3674319
flhC	0.03895141	malY	0.23397118	flgK	-0.0436084
flhD	0.49512134	malZ	0.52155886	flgM	-0.5273083
fliD	0.22794733	motA	1.20905445	flhB	-0.6937654
fumE	0.38023628	recA	0.3231064	fliA	-1.2434516
gadB	0.58546596	sdhD	1.70820377	fliC	-0.5866648
gadC	1.08340694	sucA	0.48174909	fliF	-0.3258271
gadE	1.26321434	tar	0.74485723	fliM	-1.0183686
glgA	0.5433285	waaQ	1.54260398	gadW	-0.5720736
		pdxA	0.13166842	gadX	-0.2412738
				gapA	-0.6971507
				glK	-0.130381

gpmA	-0.4095611
gpmM	-0.8600698
icd	-0.087575
malE	-1.3230424
malF	-0.5587599
malP	-0.3313379
malQ	-0.220825
mdh	-0.2021172
pfkB	-0.6658566
pfkA	-0.2619042
pflA	-0.4928836
pflB	-0.8795375
pgi	-0.8417096
pgk	-0.6012007
pykA	-0.6710289
pykF	-0.8709161
sucD	-0.3720578
tcyJ	-1.0807951
tpiA	-1.1869355

Lysogen vs Naïve with *cI* overexpressed in different levels

*Significant when P-value < 0.05



5. Discussion & Conclusion

- The motility of MC1061/$\Phi24_B$::Cat was enhanced compared to naïve cell, at both 37°C and 30°C, indicating **prophage $\Phi24_B$ contributes to the motility of its host.**
- The motility of MC1061 (pBAD_13c) was enhanced compared to naïve cell at 37°C with 0.5%, 1.0% or 2.0% arabinose, indicating **prophage gene *vb_24B_13c* can contribute to the motility of naïve cells** even without the presence of other prophage genes**.**
- The motility of MC1061 (pBAD_13c) was NOT enhanced compared to naïve cell at 30°C with 0.5%, 1.0% or 2.0% arabinose, indicating **temperature can be one of the factors contributing to its host's motility.**
- The results from an amylase assay as measured using DNS Reducing Sugar Reagent validated our previous RNASeq data which showed that **α-amylase activity is upregulated in the lysogen.**
- *NanoString* data indicates that **the overexpresion of prophage repressor gene, *cI,* may be able to inhibit the expression of some other prophage genes** (*e.g. vb_24B_13c, res* and *stk*), which also shows **the lysogen's expression of *cI* is not high enough to repress some prophage genes.**
- In summary, **prophage having a significant role in host reprogramming of metabolic function.**

6. Acknowledgements

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