Glucose sensing in the solventogenic clostridia

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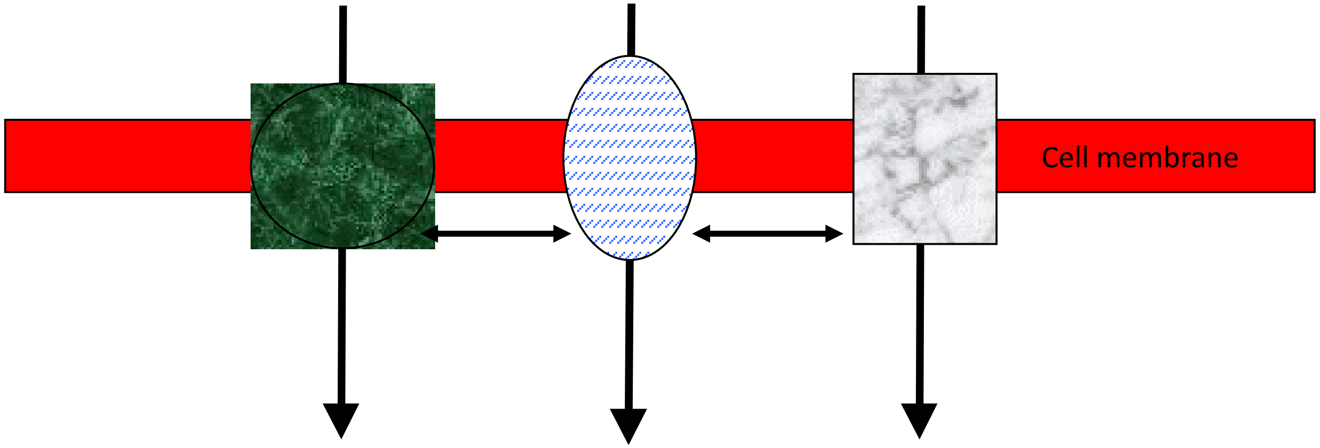
Metabolisable carbohydrates



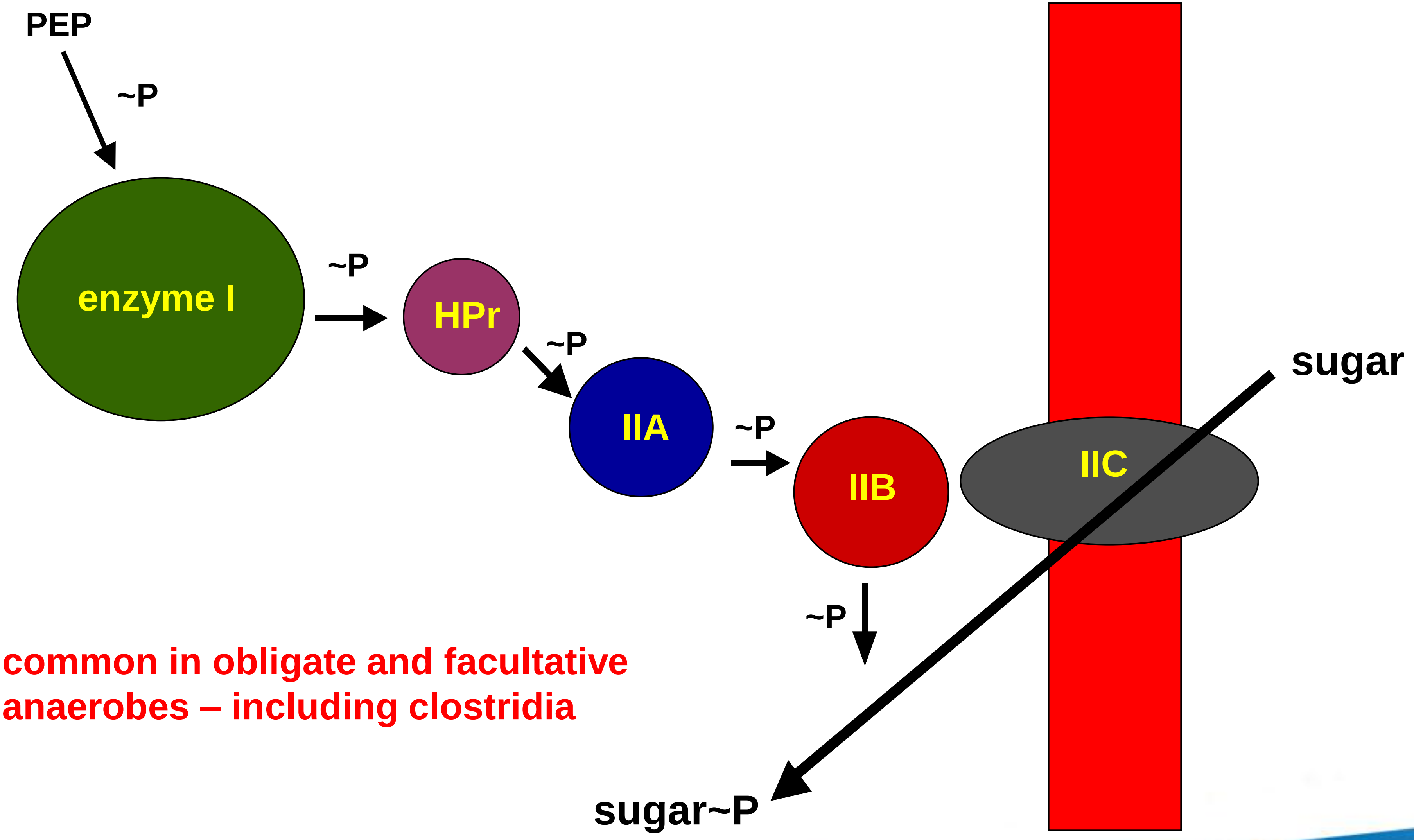
"The ideal microorganism for biofuel production will possess high substrate utilization and processing capacities, fast and deregulated pathways for sugar transport, good tolerance to inhibitors and product, and high metabolic fluxes and will produce a single fermentation product"

Alper & Stephanopoulos

Nature Reviews Microbiology 7, 715-723 (2009)

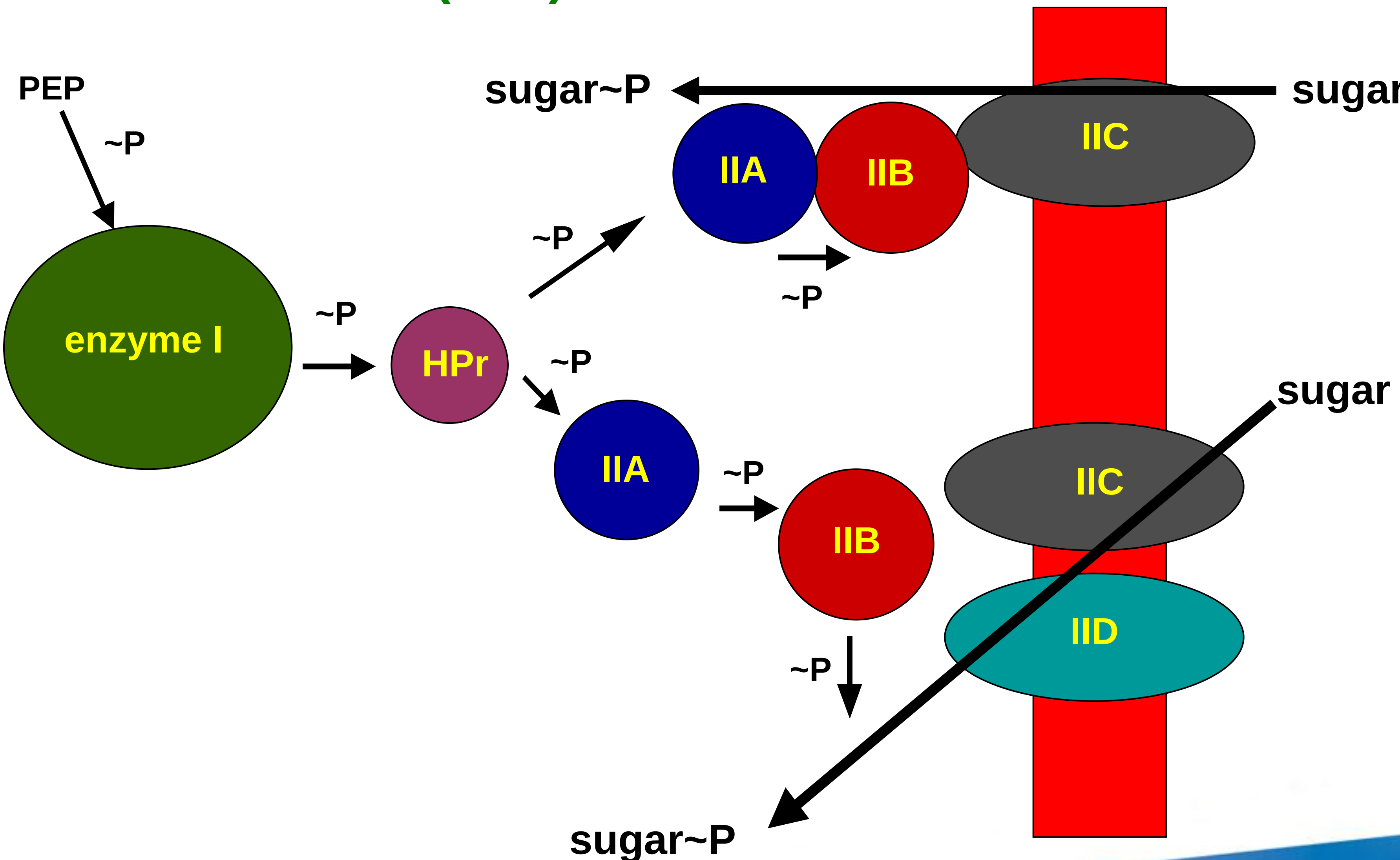
Bacteria control metabolic activities in response to the nutrient status of the environment - not according to the requirements of a biotechnologist

The bacterial phosphotransferase system (PTS)

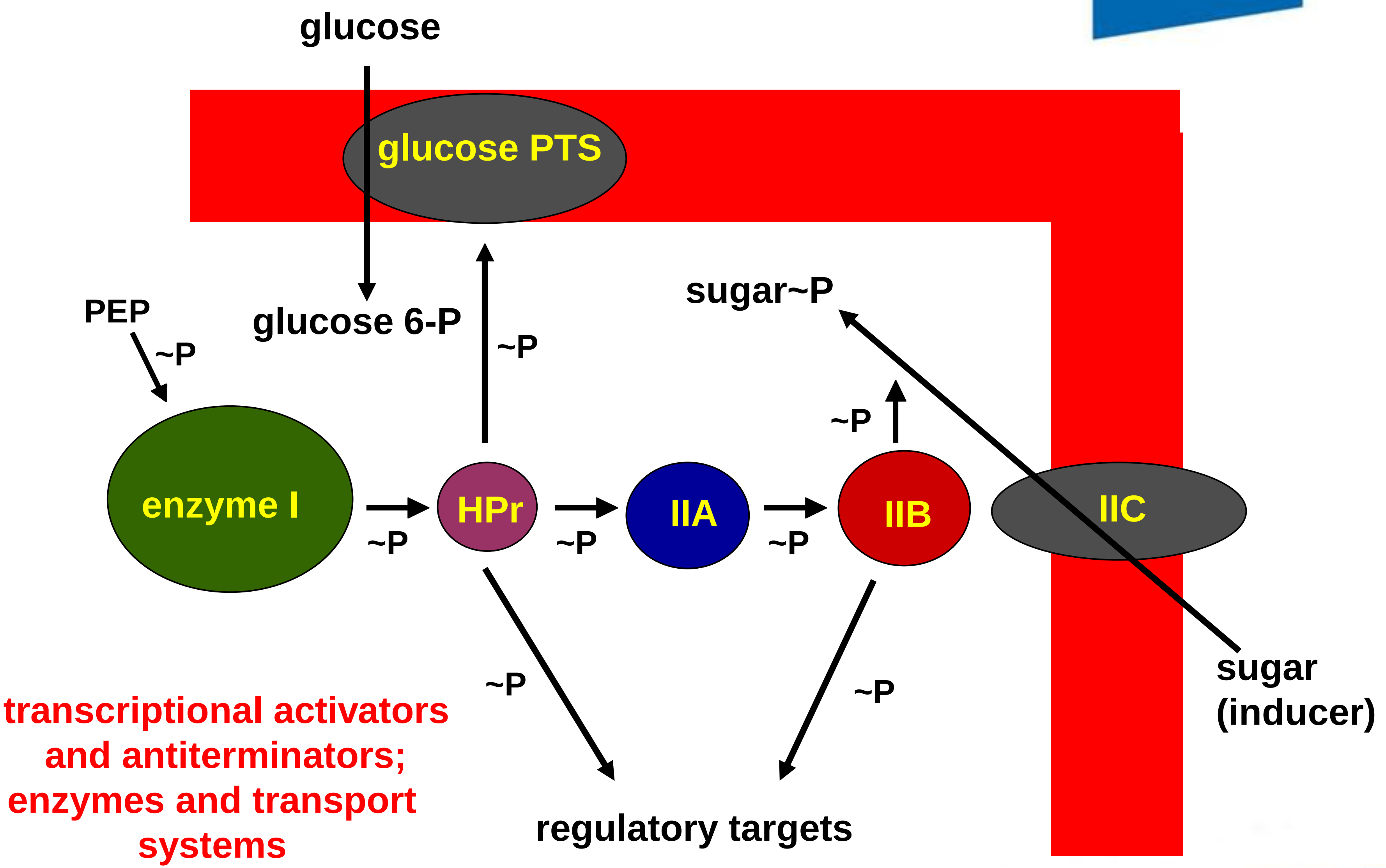


common in obligate and facultative anaerobes – including clostridia

The bacterial phosphotransferase system (PTS)

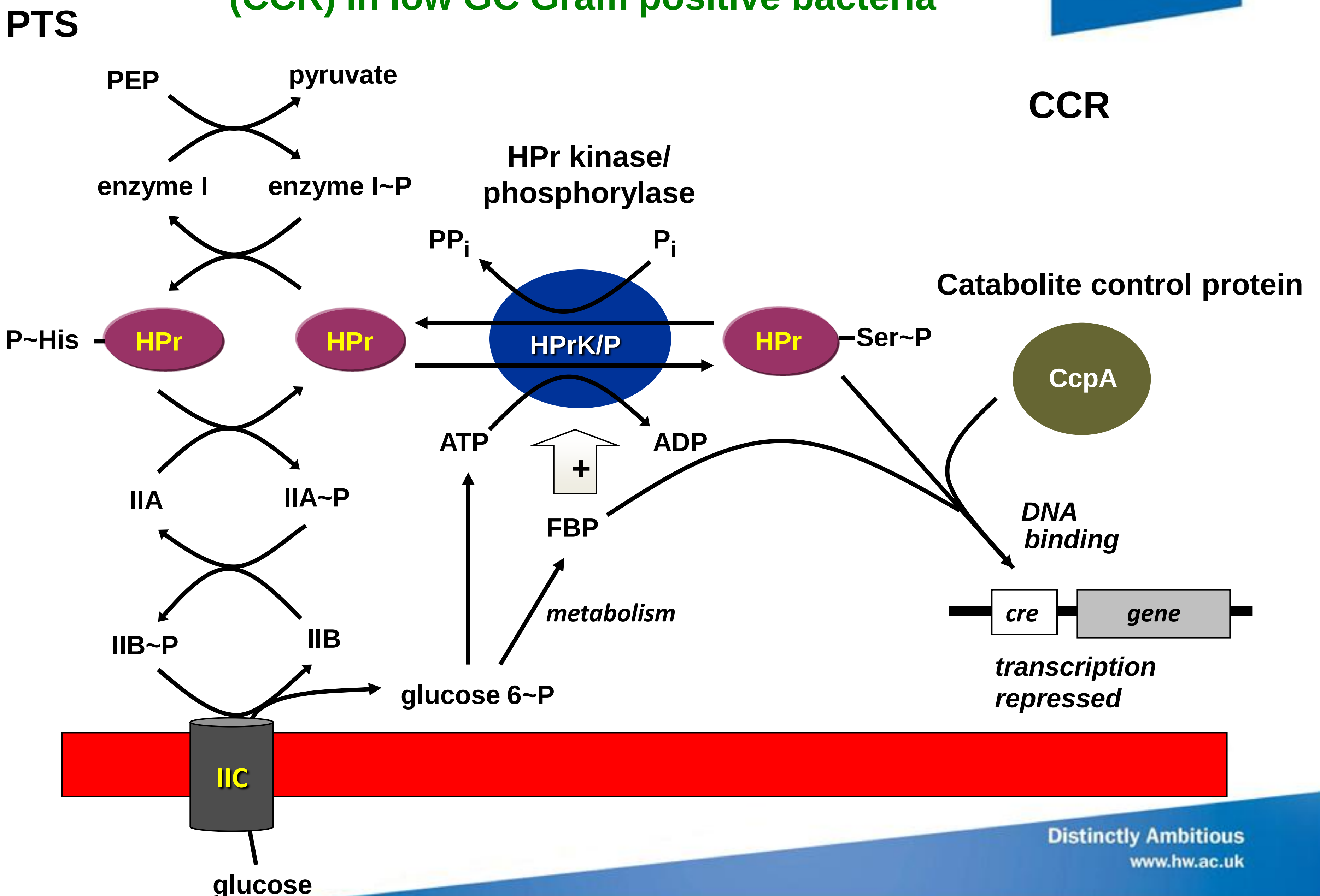


The PTS as an environmental sensor

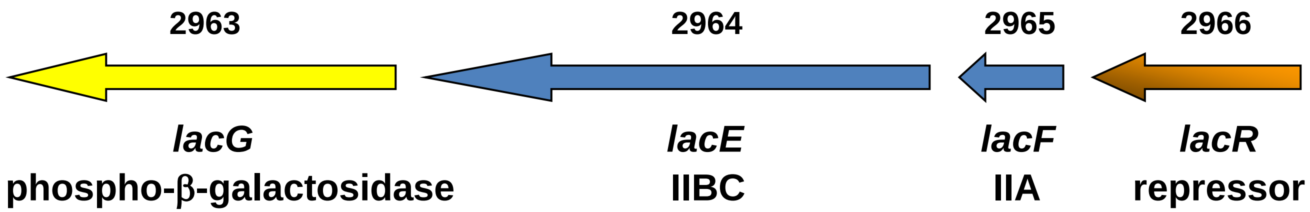


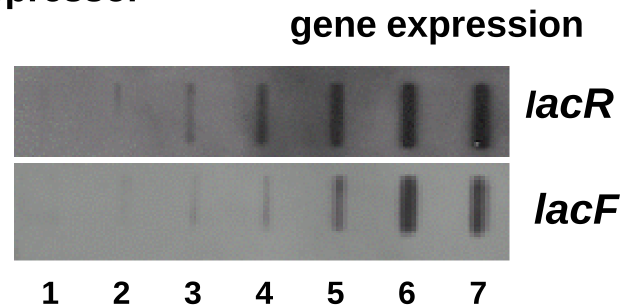
allosteric and covalent interactions

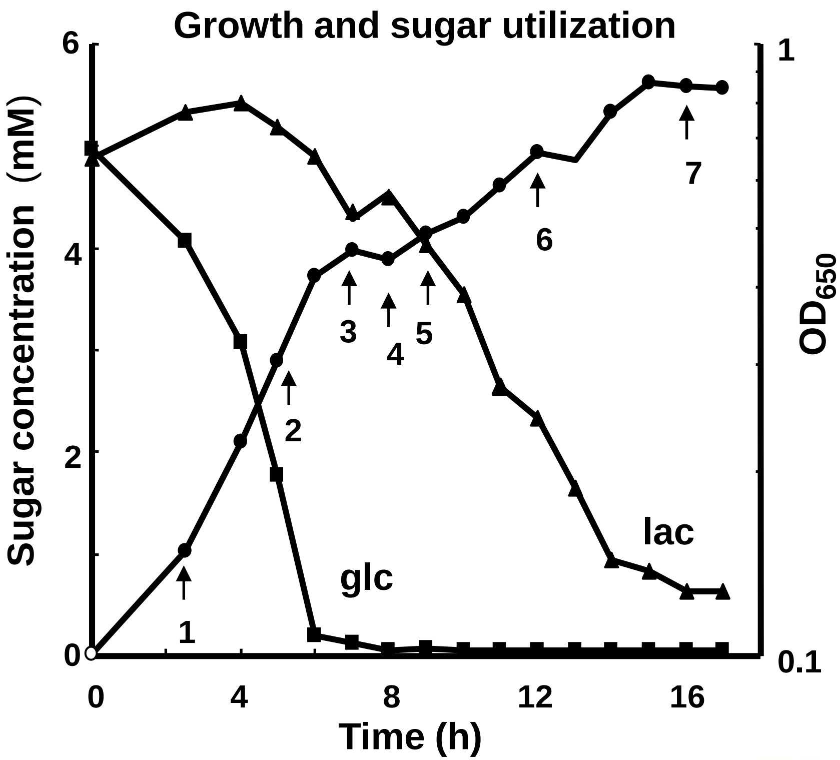
Global mechanism of carbon catabolite repression (CCR) in low GC Gram positive bacteria

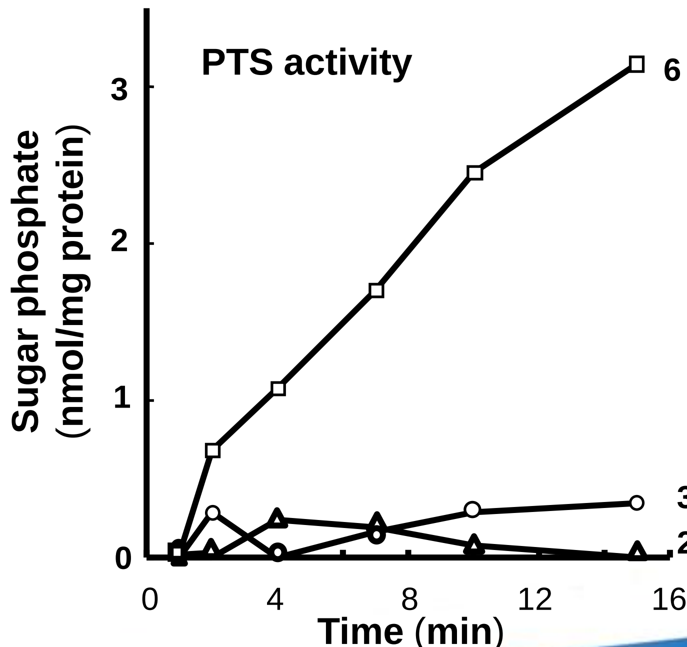


Diauxic growth of *C. acetobutylicum* on glucose and lactose









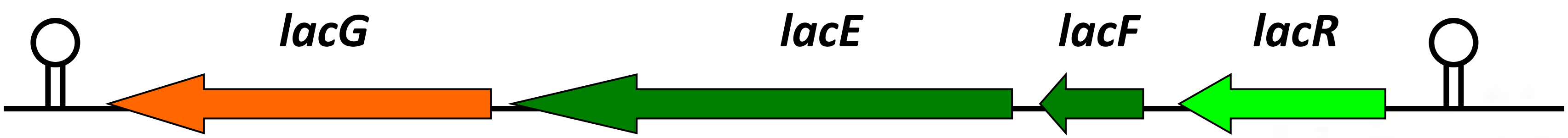
Putative regulatory region of the *C. acetobutylicum lac* operon

TAAAGTTGAGAAATAAGAGTATCCCTAGAAGTGGTTTCTAGGGATATTTTCATATACTAAGGTTTTTA

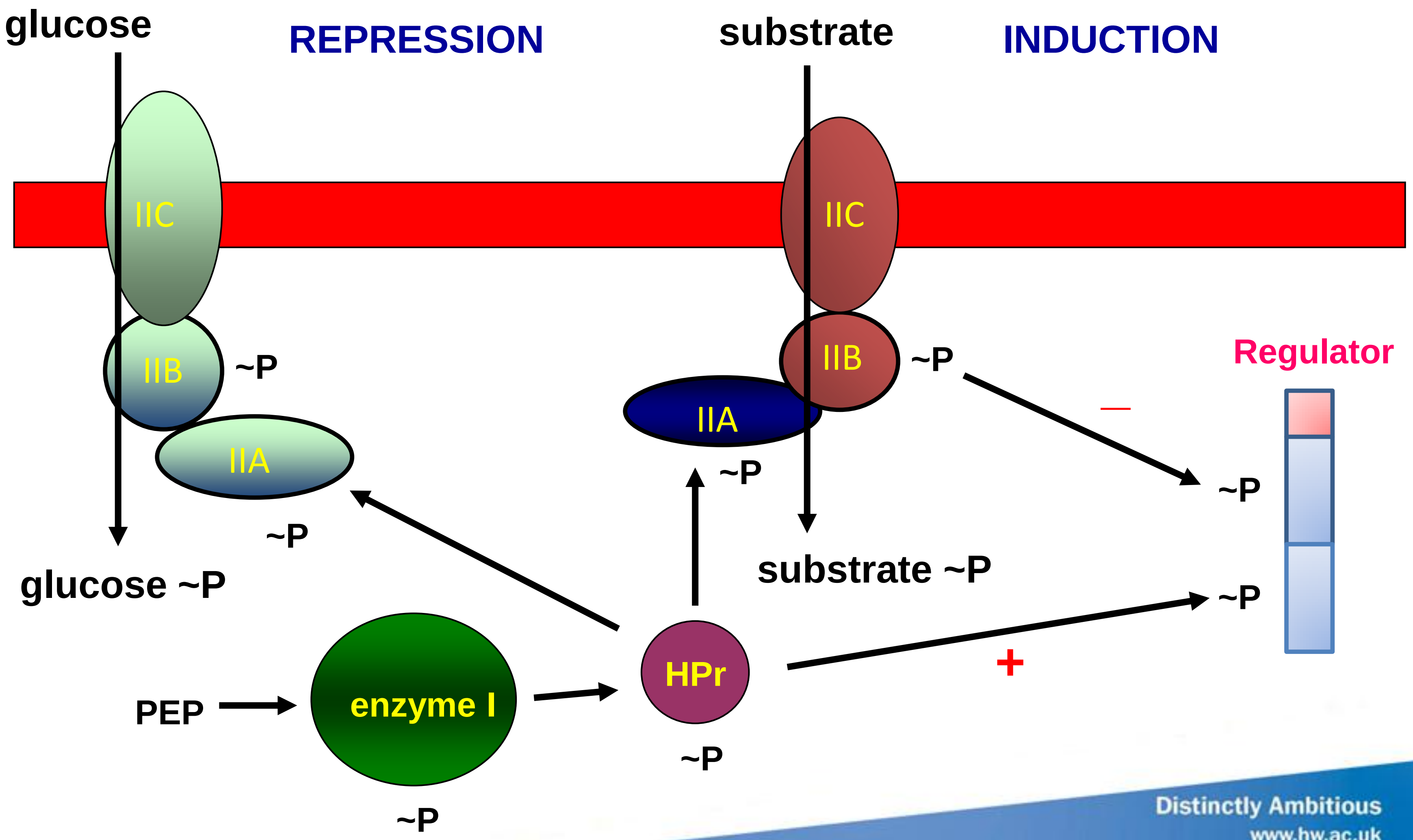
TAGTTTTAGTGAATCTTAAAACTTTATCTAATAGAAATCGCTTTCTATAAAGTATTATGTTAAATATTAAA
AGTAATCAACGGTTATTTTGTGTTAGAAAAAATCAAATTACTGTCATAGTATTCTTTAACAAAATAGTAAC
AGTAAAAA**AGAGCAGTGTAAACGGTTTG**ATTTTGAATAATGTAATATTTTTATTGTTTTATG*TTAAAAAA*
*CAAA*AAAA*TTTGTATTTTAA*CAAACAAAAACAGAACATAGATAAAATAAAAATATCACAACAA

TATTA**TTGACA**AACCTTATTTAAAGATATA**TTATAT**AAA**TGTAAACGAAAACA**AGTACATAAAAGAACA
-35 **-10** **CRE**
AAATAATTCATAAAAAAGCGCACTTATAGAAAACGATAACTAACCATGAAATGGTGAATCTGTGGAAA

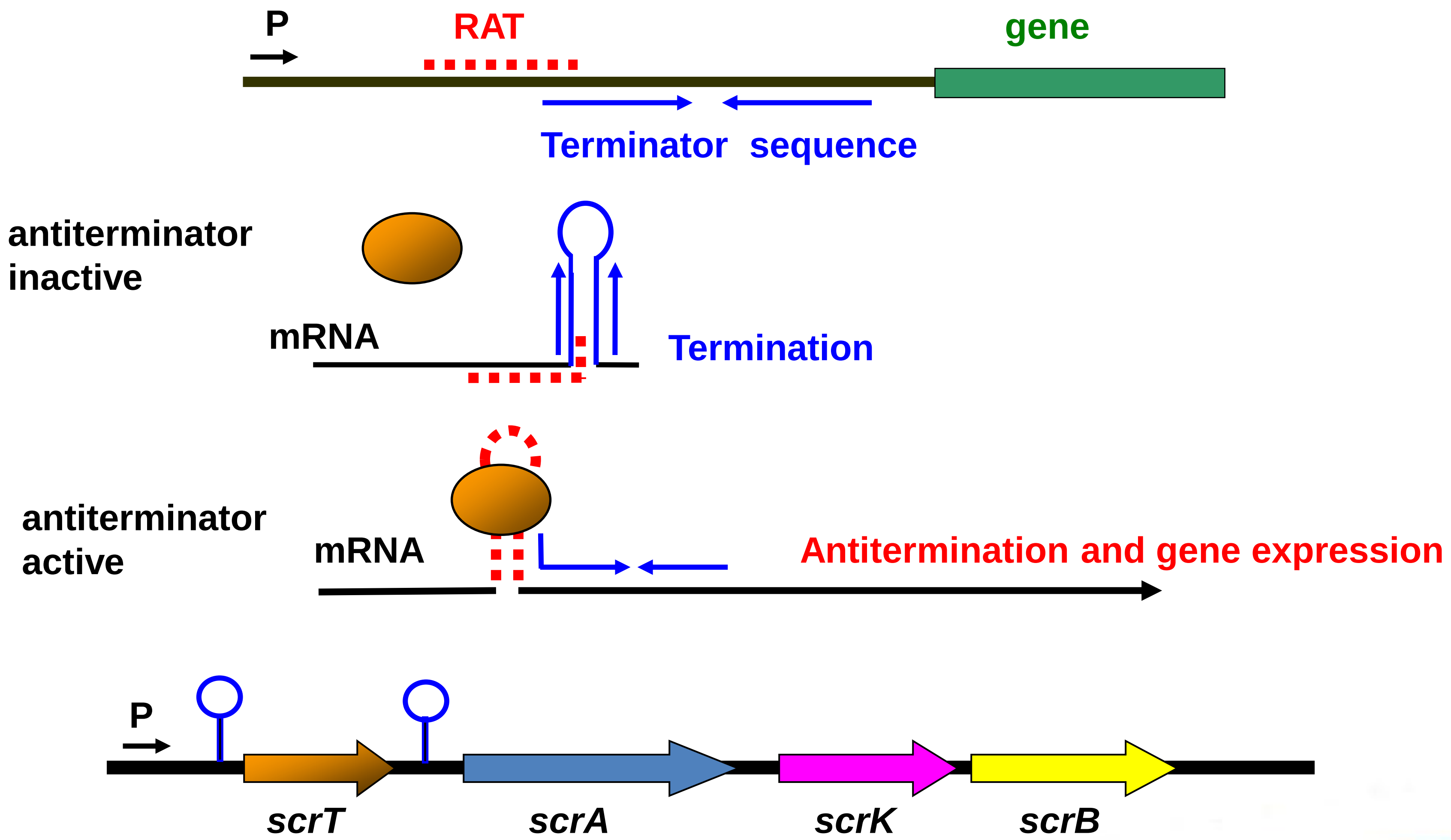
ATTACAATTAAATTTACTT**CGATATGAGGTGATGATGAA**TGTGTTAAAAGAACAAAGACATGAAATGAT
RBS



PTS-dependent induction and repression of individual operons



Antitermination in the *C. acetobutylicum* sucrose operon



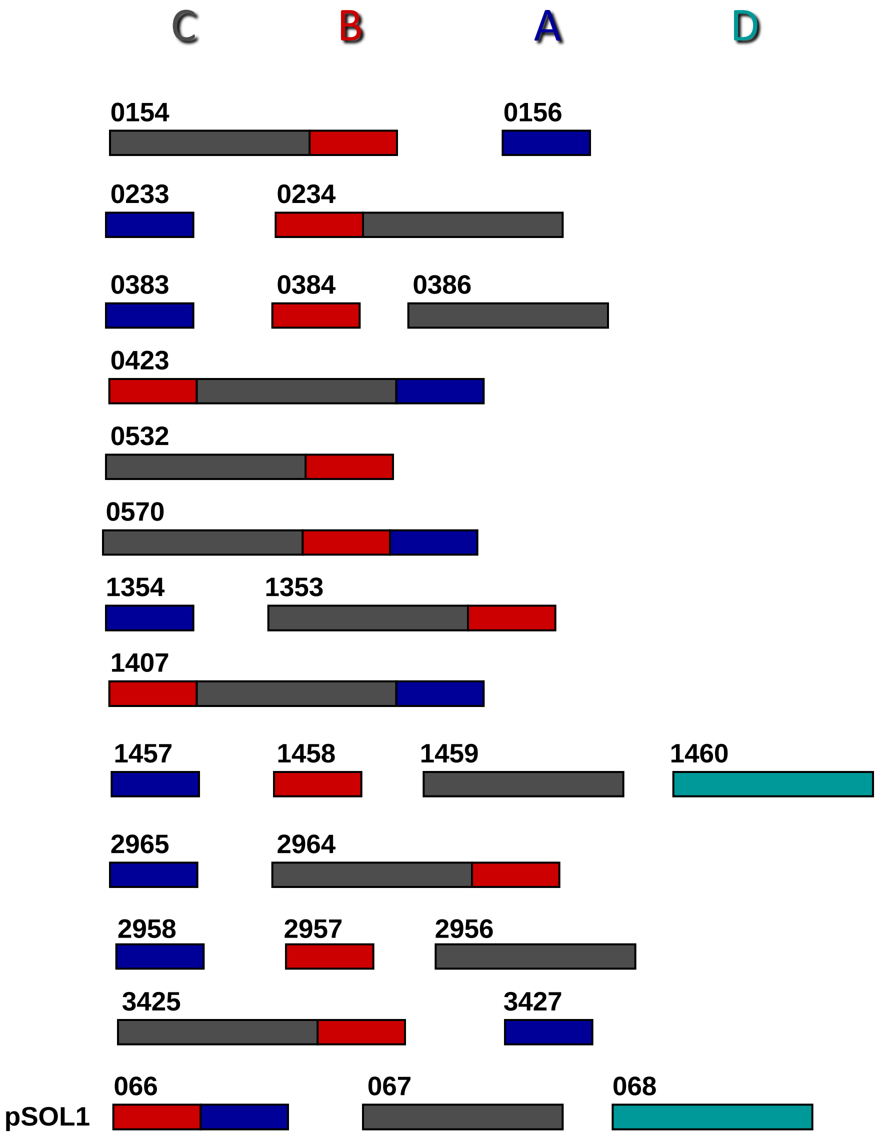
Understanding CCR in clostridia

CCR by glucose is dependent on uptake and phosphorylation by the PTS

Manipulating CCR is dependent on understanding:

- **the role of the PTS in sensing and uptake of glucose**
- **the signal transduction pathway**

Phosphotransferases of *C. acetobutylicum* ATCC 824



Substrate

mannitol

fructose

ß-glucoside (cellobiose)

sucrose

maltose

glucose GlcG

N-acetylglucosamine

ß-glucoside

fructose/mannose?

lactose

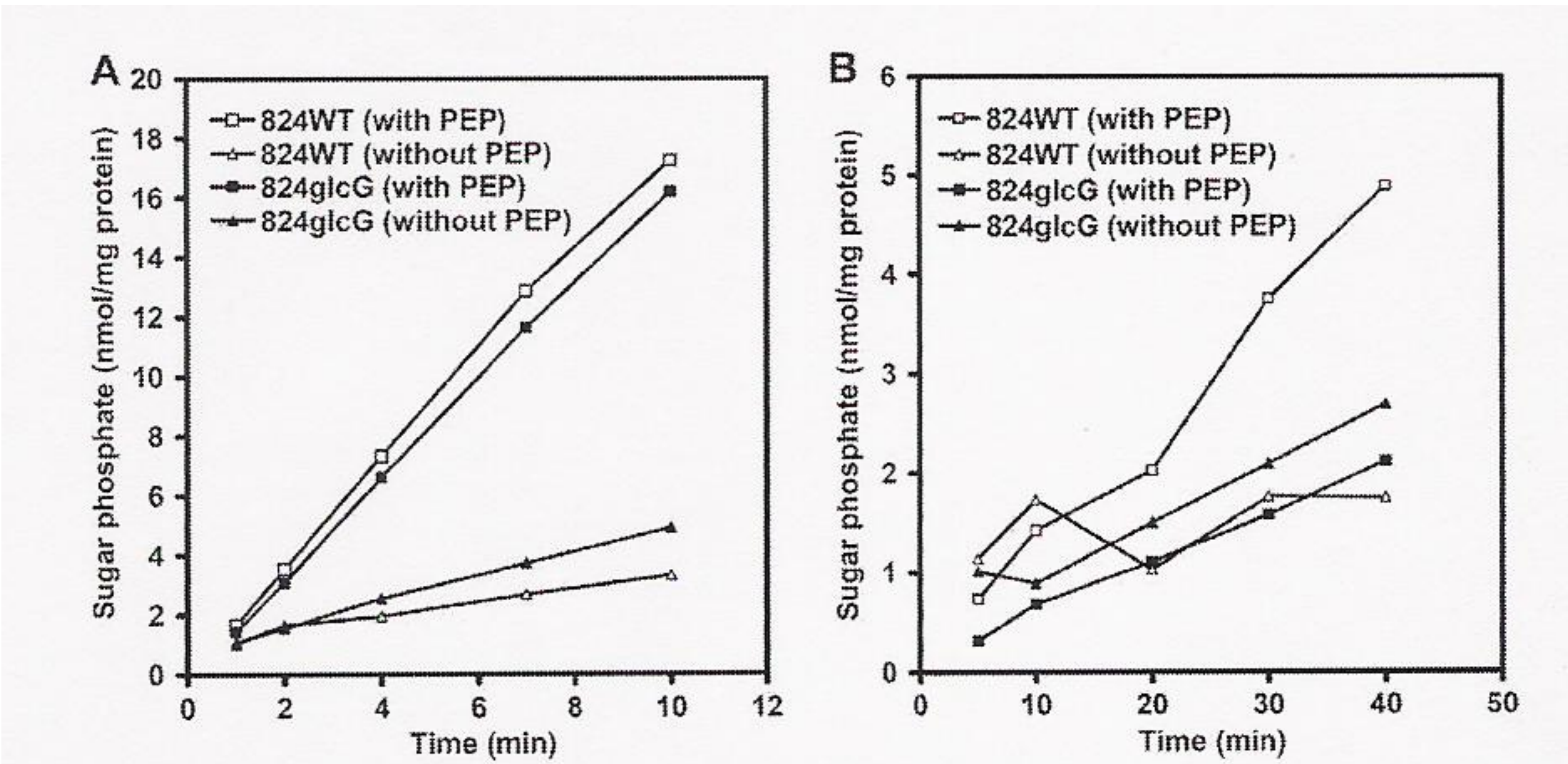
galactose

α-glucoside

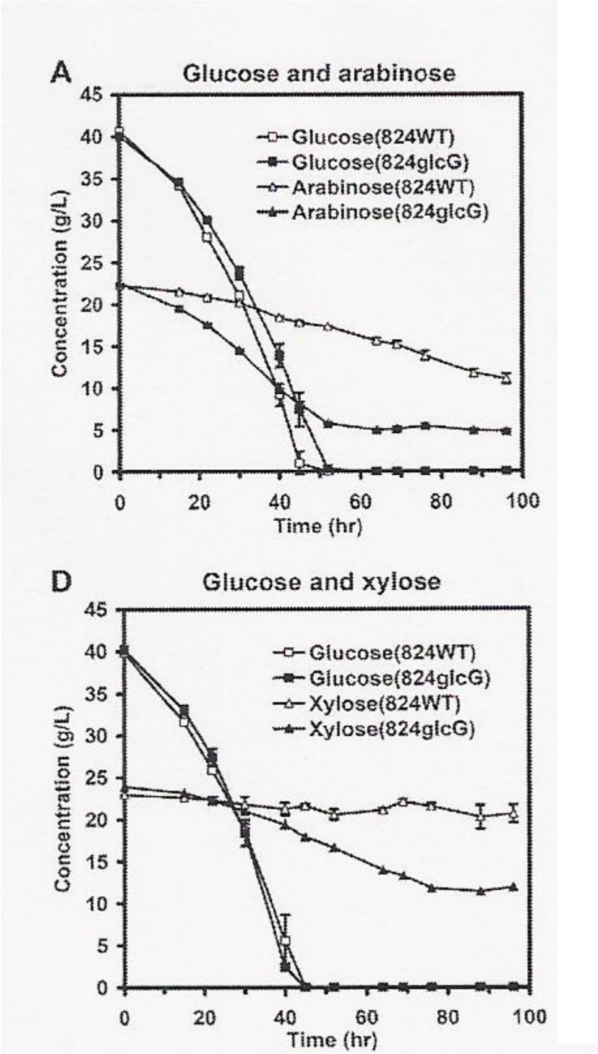
??

PTS activity in wild-type and *glcG* mutant

glucose phosphorylation **methyl-α-glucoside phosphorylation**

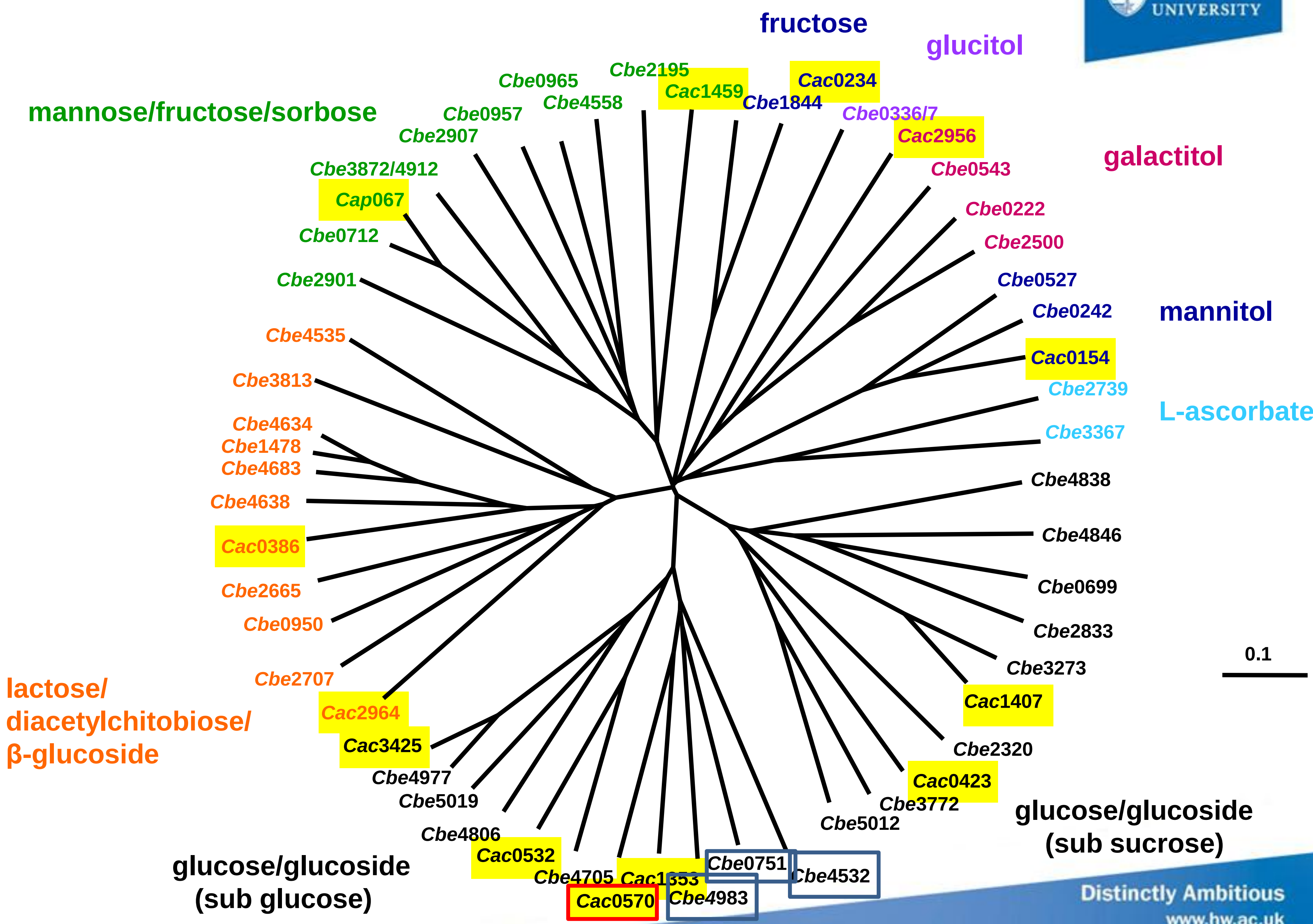


Fermentation profile of wild-type vs *glcG* mutant

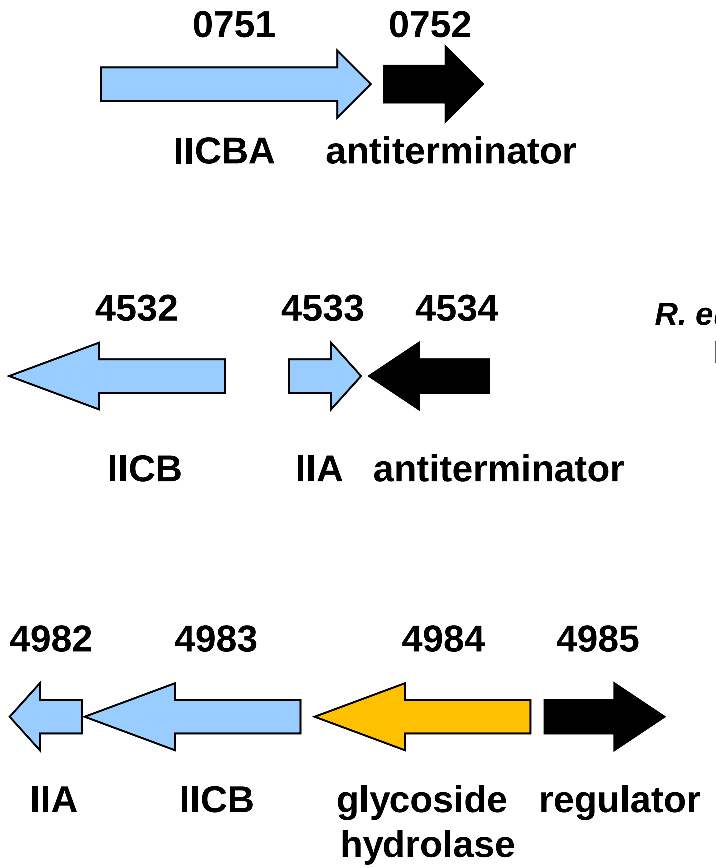


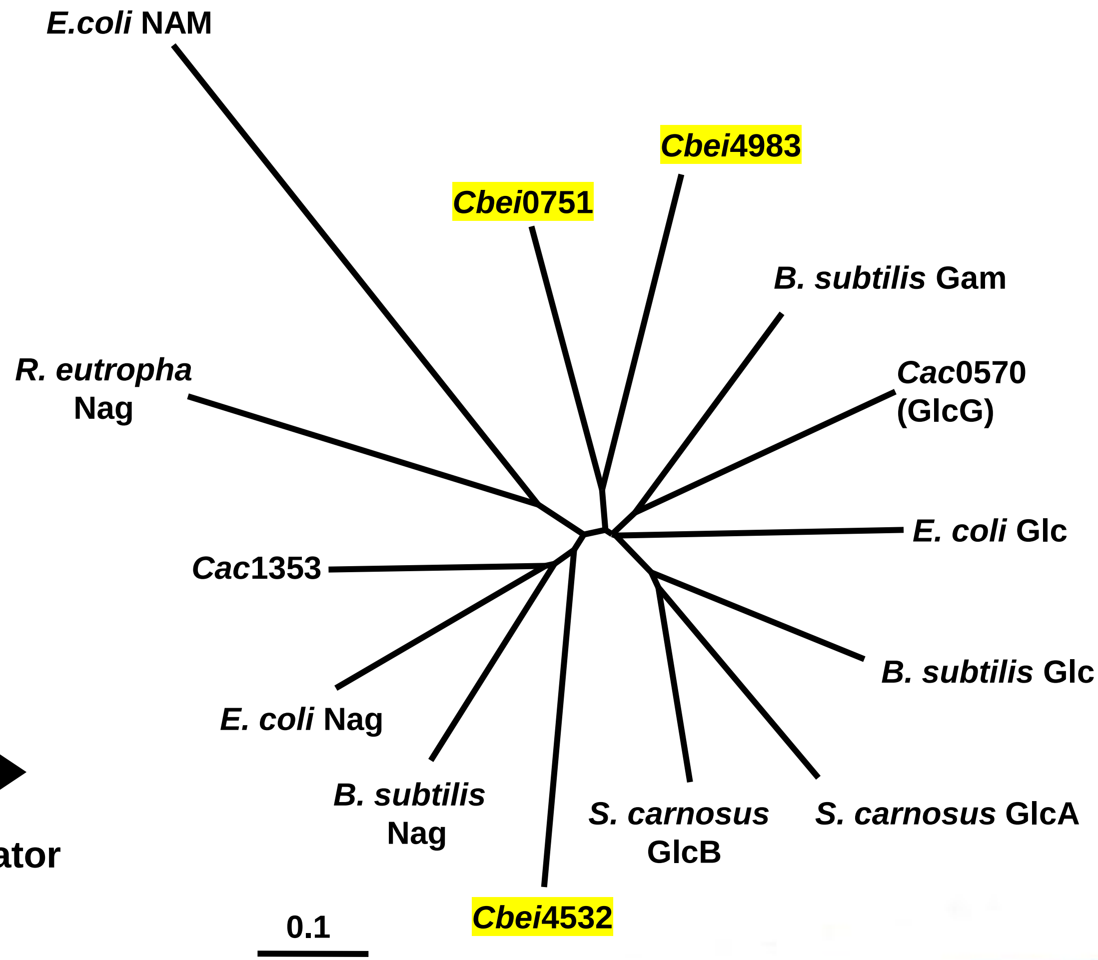
- **glucose fermented at a similar rate in both strains**
- **arabinose and xylose fermented more efficiently in the *glcG* mutant compared to wild-type**
- **considerable amount of xylose remaining at the end of the fermentation**

Phylogenetic tree of clostridial IIC domains

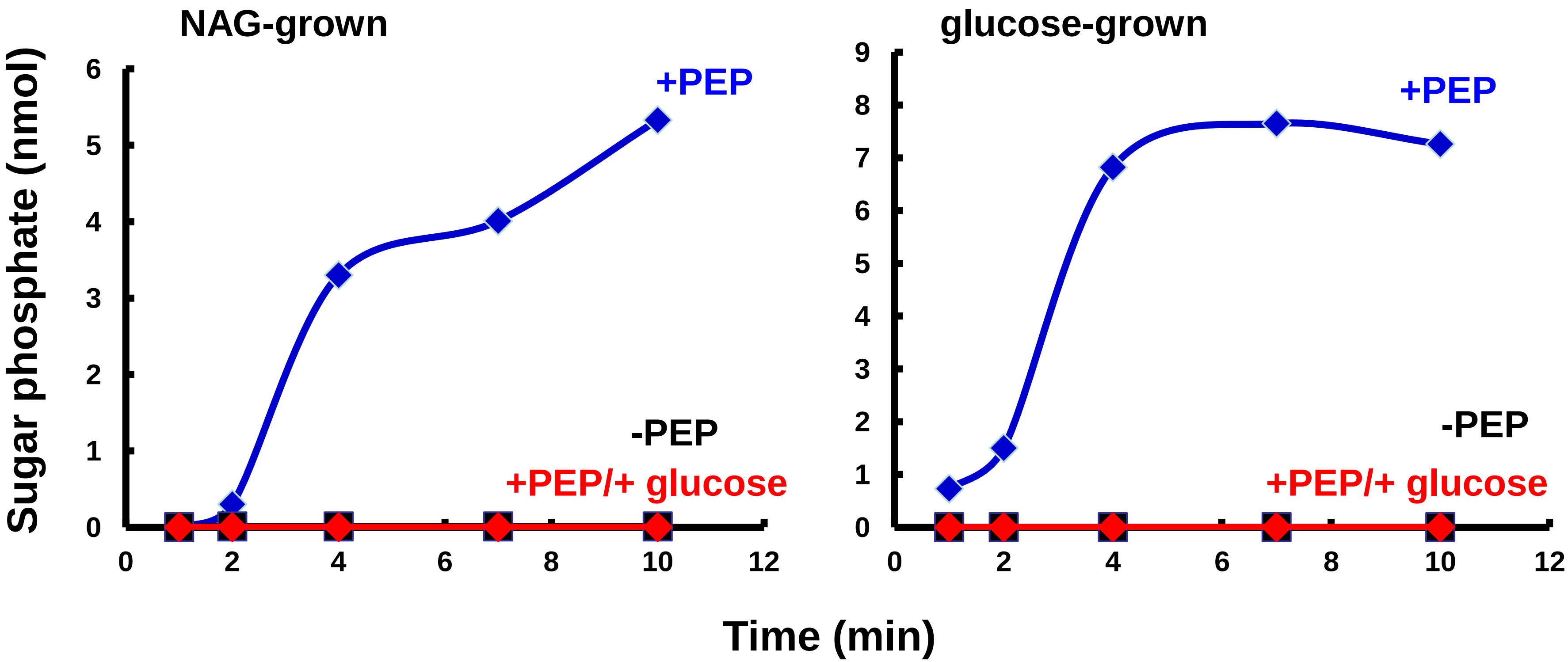


C. beijerinckii glucose family phosphotransferases





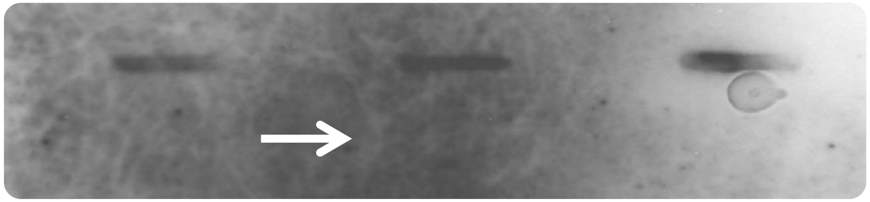
NAG PTS activity in *C. beijerinckii* grown on NAG and glucose



Expression of putative *nag pts* genes in *C. beijerinckii* grown on NAG and glucose

cbei4532

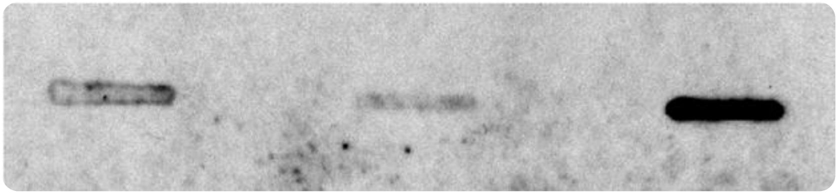
glucose NAG glucose + NAG



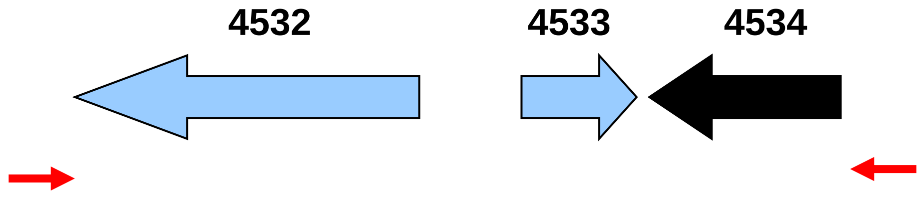
glucitol

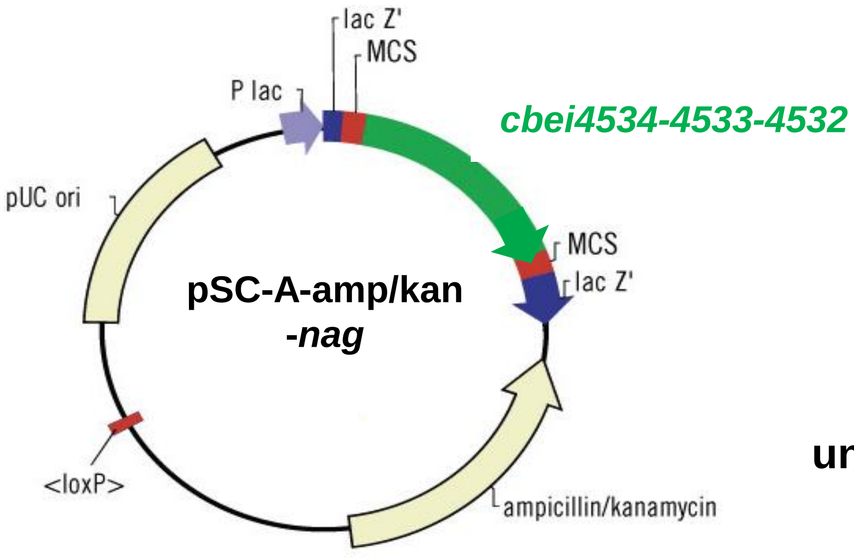
cbei4533

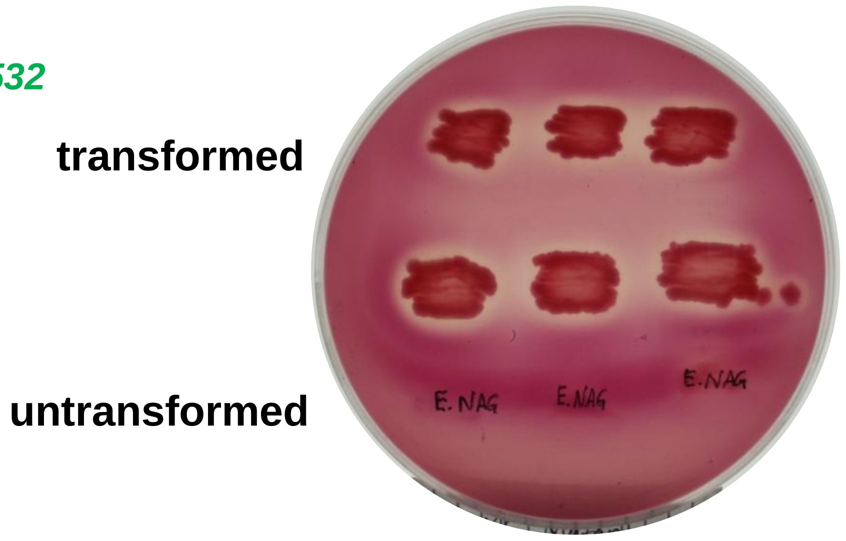
glucose NAG glucose + NAG



Cloning of *C. beijerinckii* nag genes

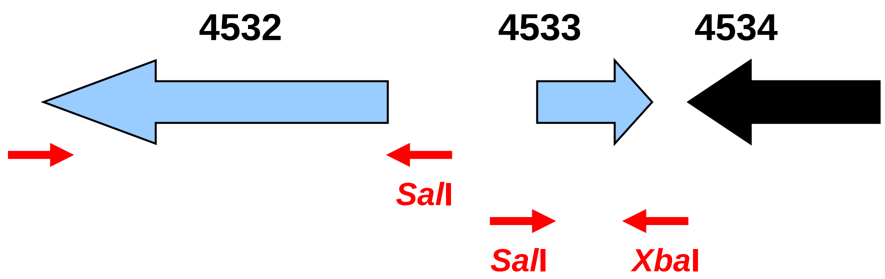


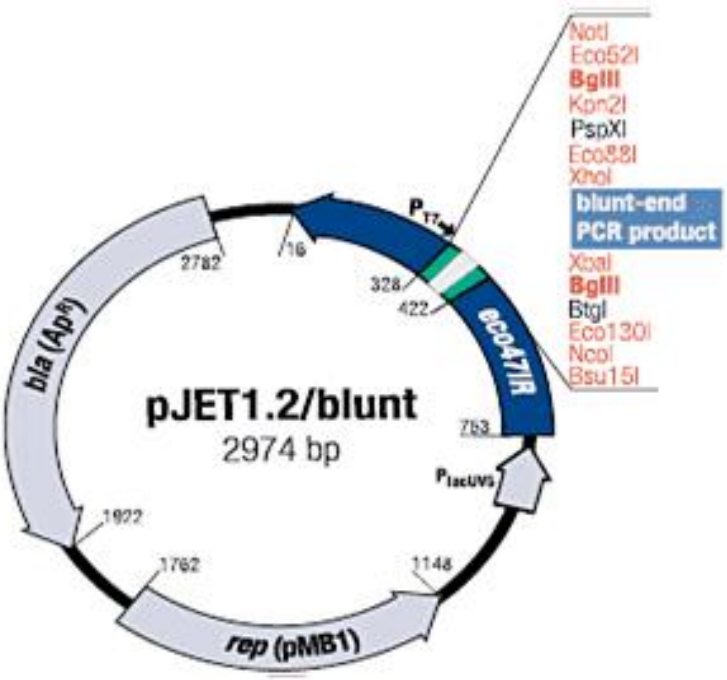


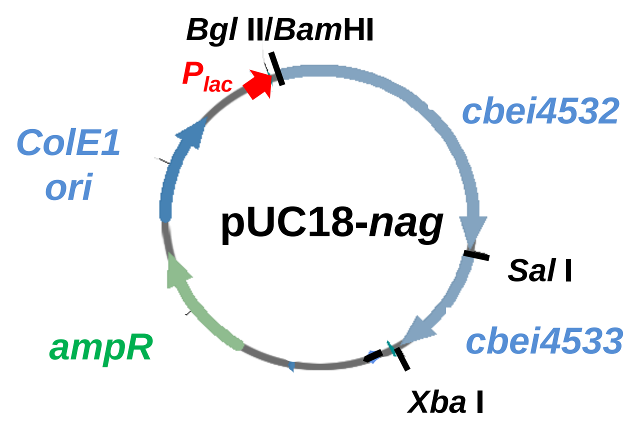


MacConkey + NAG

Construction of an artifical *nag* operon



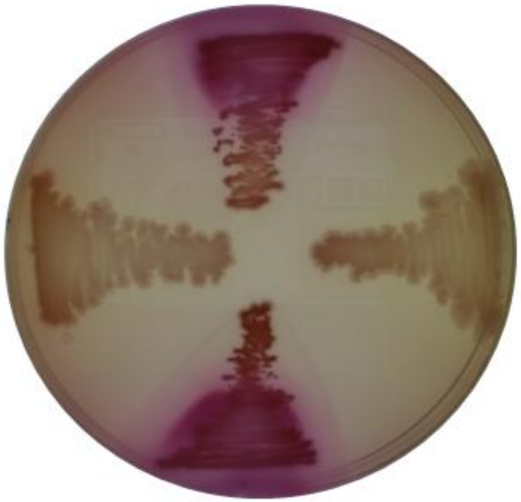




Complementation of *E. coli nagE* mutant by pUC18-*nag*

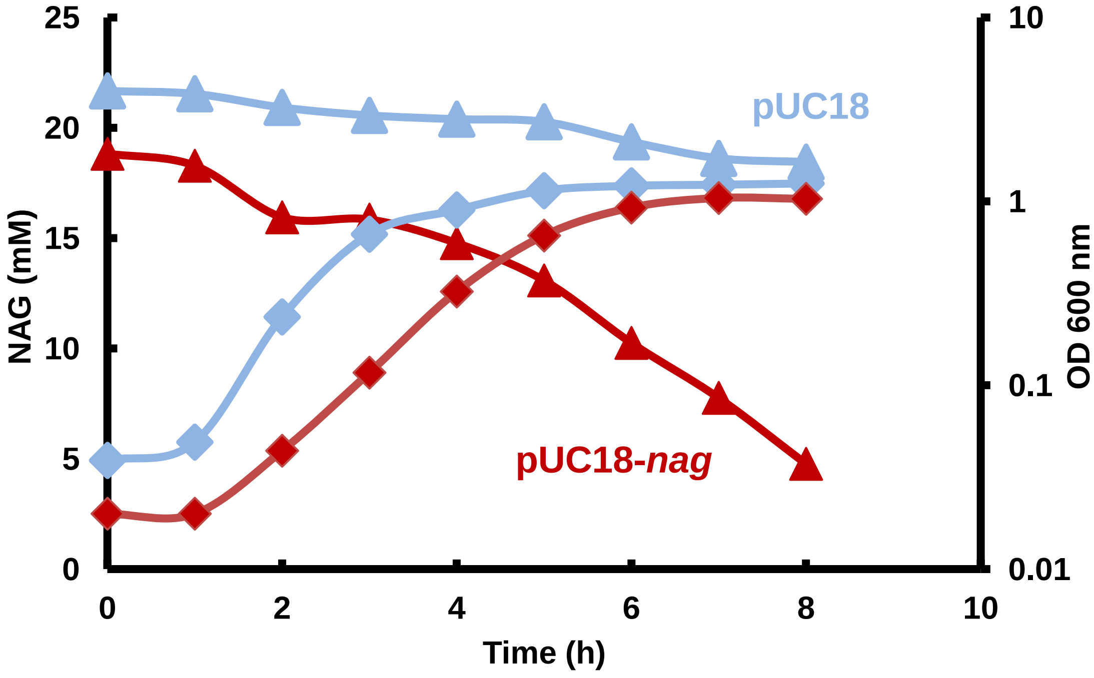
MacConkey agar + NAG

pUC18



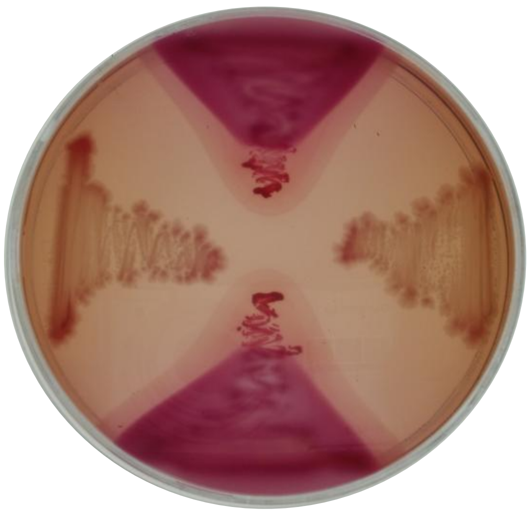
pUC18-*nag*

LB + NAG



Complementation of *E. coli* ZSC113 by pUC18-*nag*

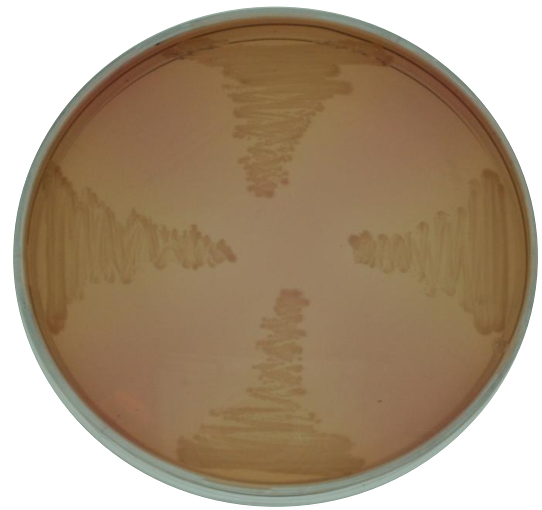
MacConkey agar + glucose



pUC18

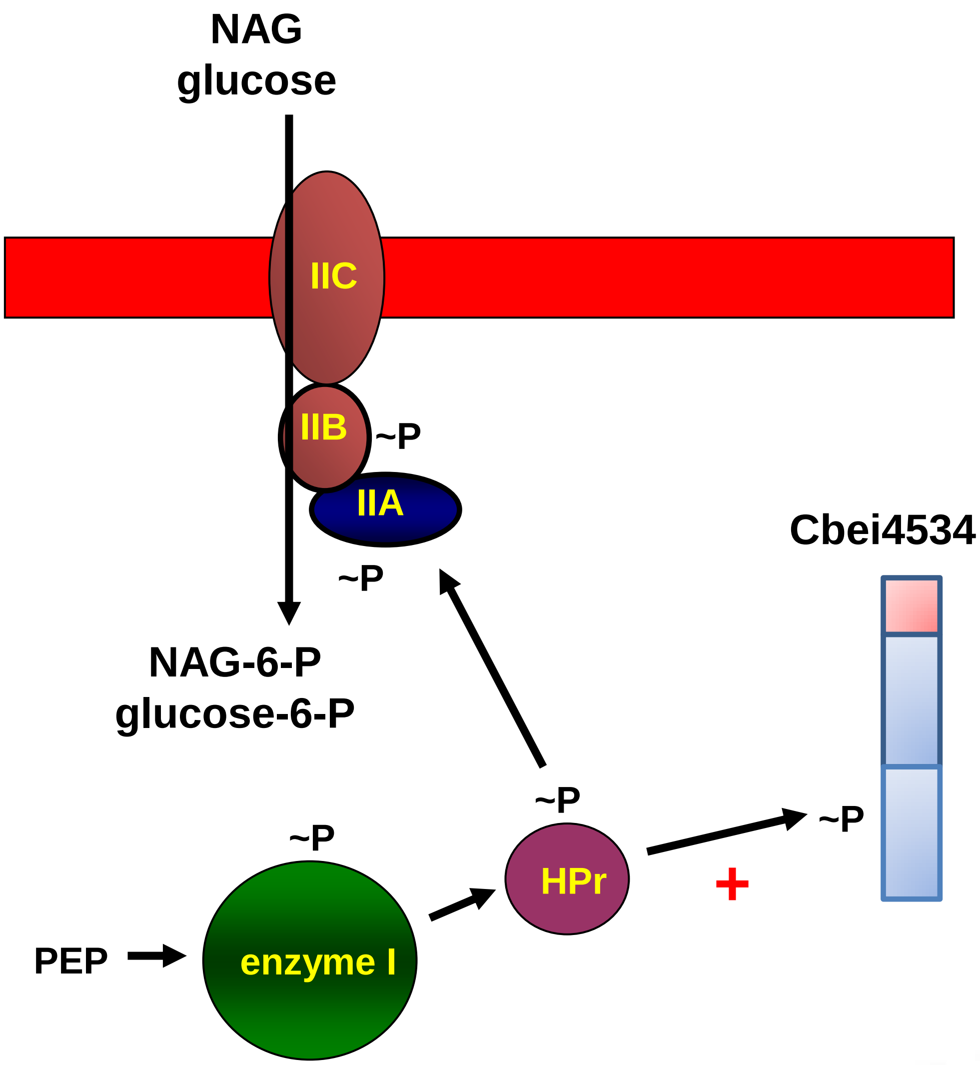
pUC18-*nag*

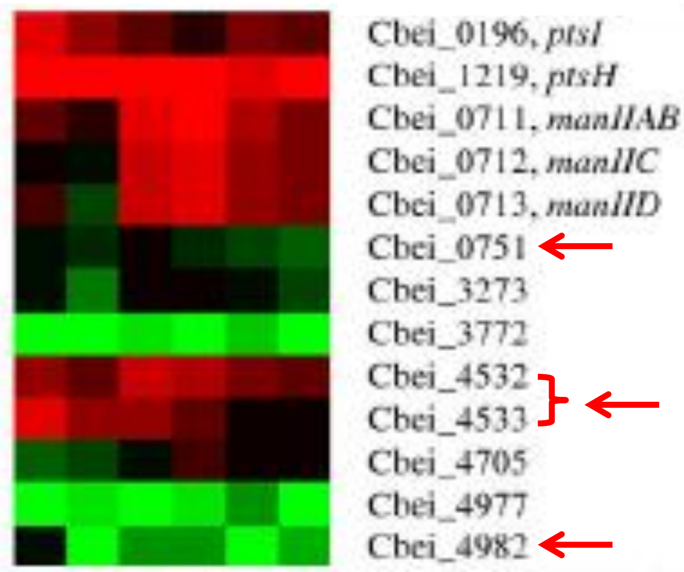
MacConkey agar plus mannose



pUC18-*nag*

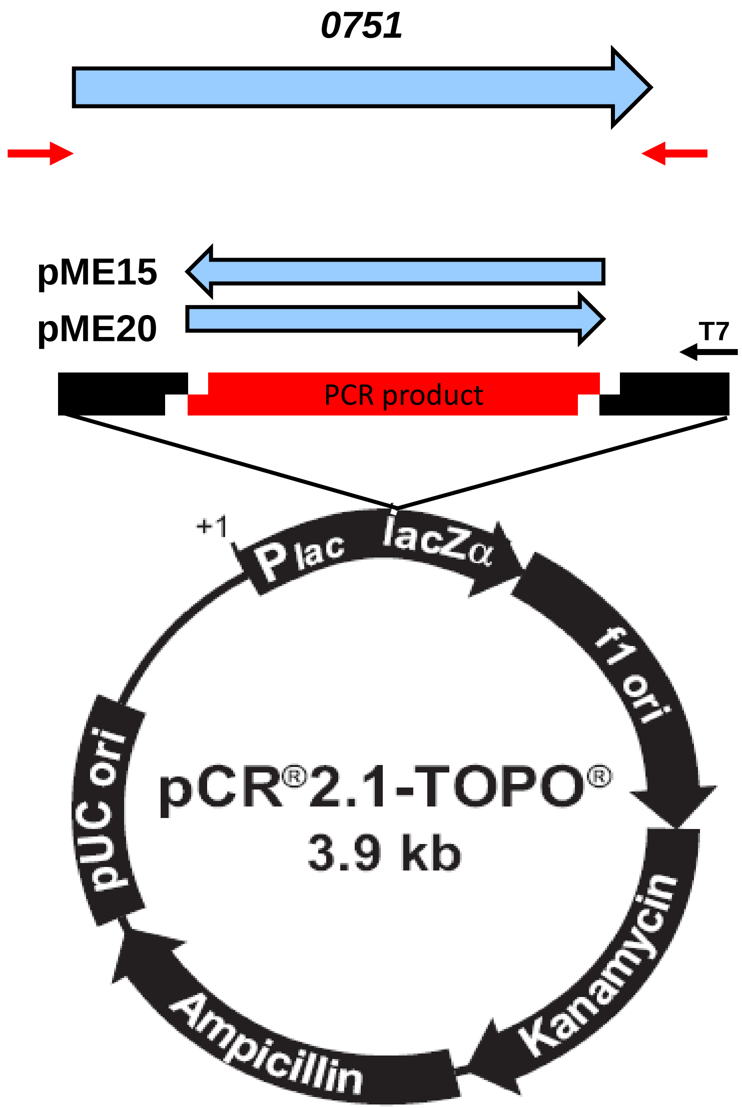
Induction of *nag* gene expression in *C. beijerinckii*



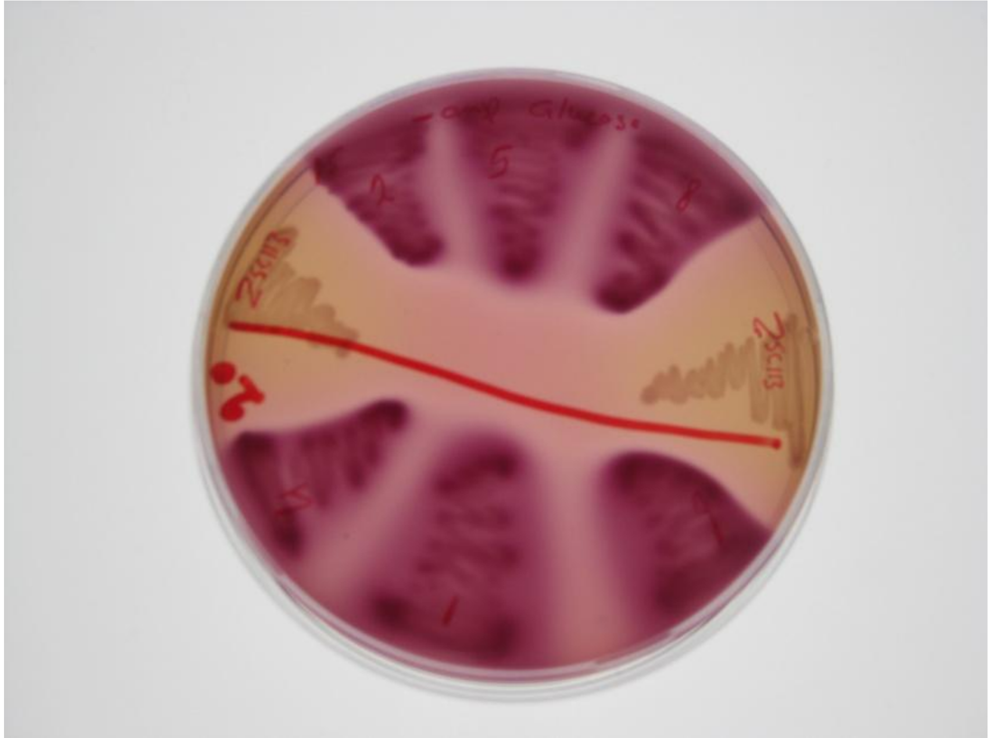


Wang *et al* (2012) *BMC Genomics* **13:**102

Cloning of *Cbei 0751*



Complementation of *E. coli* ZSC113 for glucose fermentation



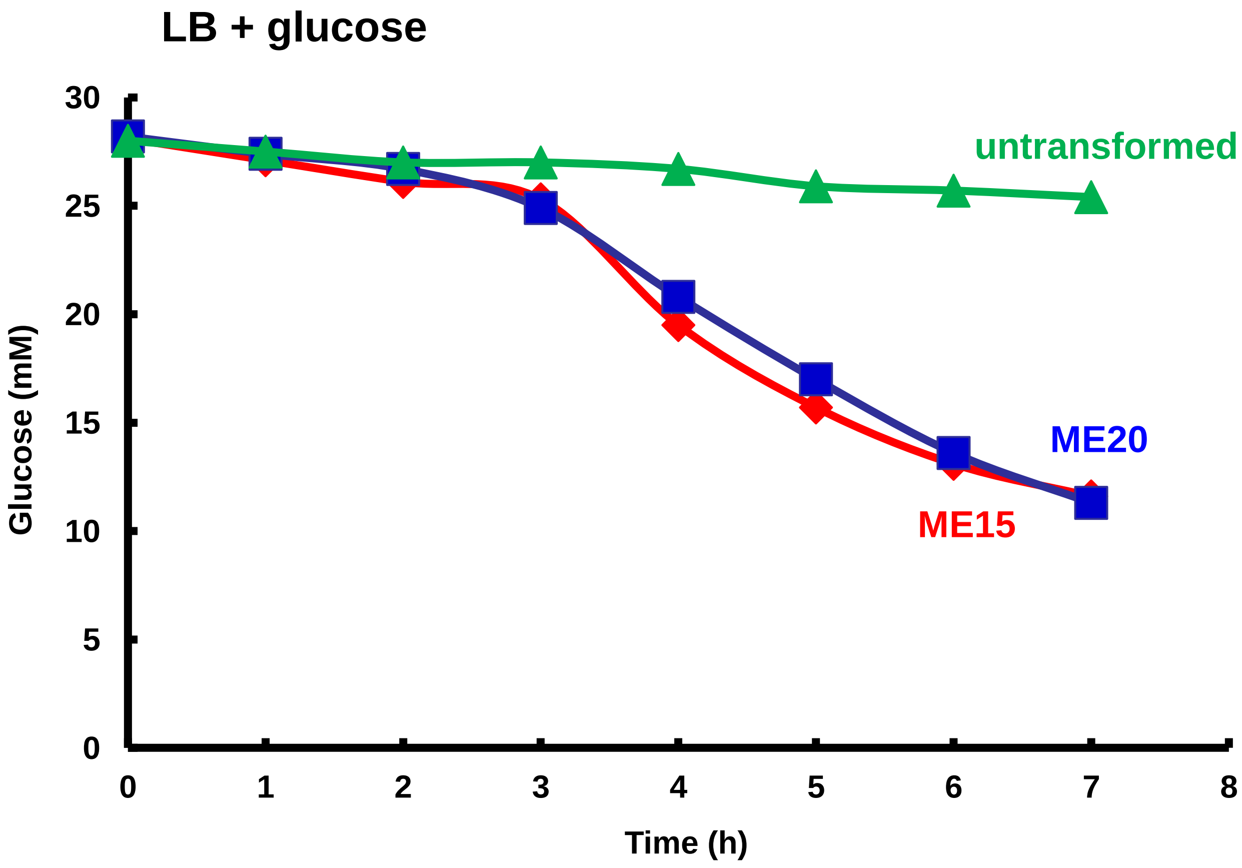
ME15

untransformed cells

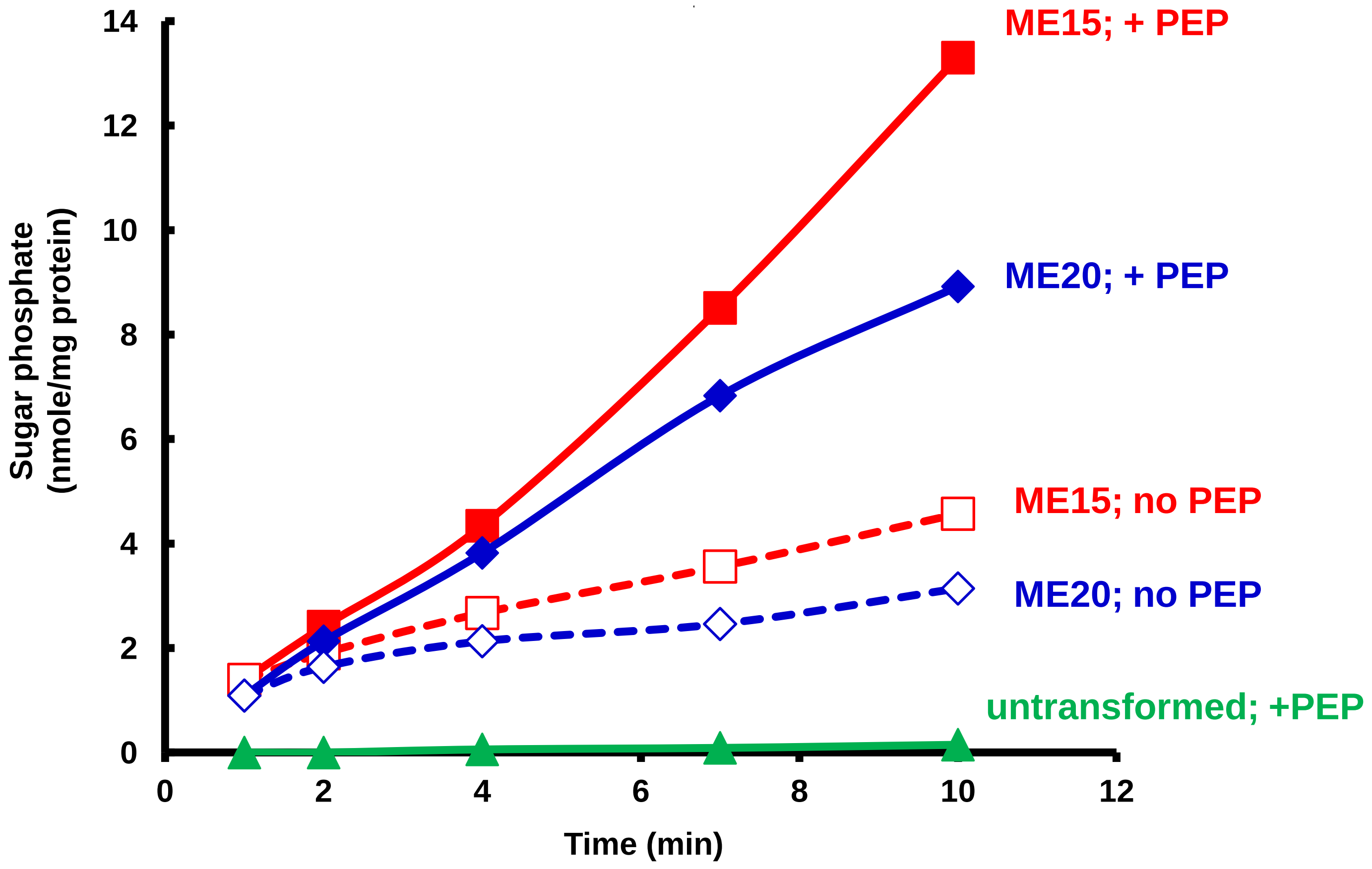
ME20

MacConkey agar + glucose

Glucose utilization by ZSC113 transformants

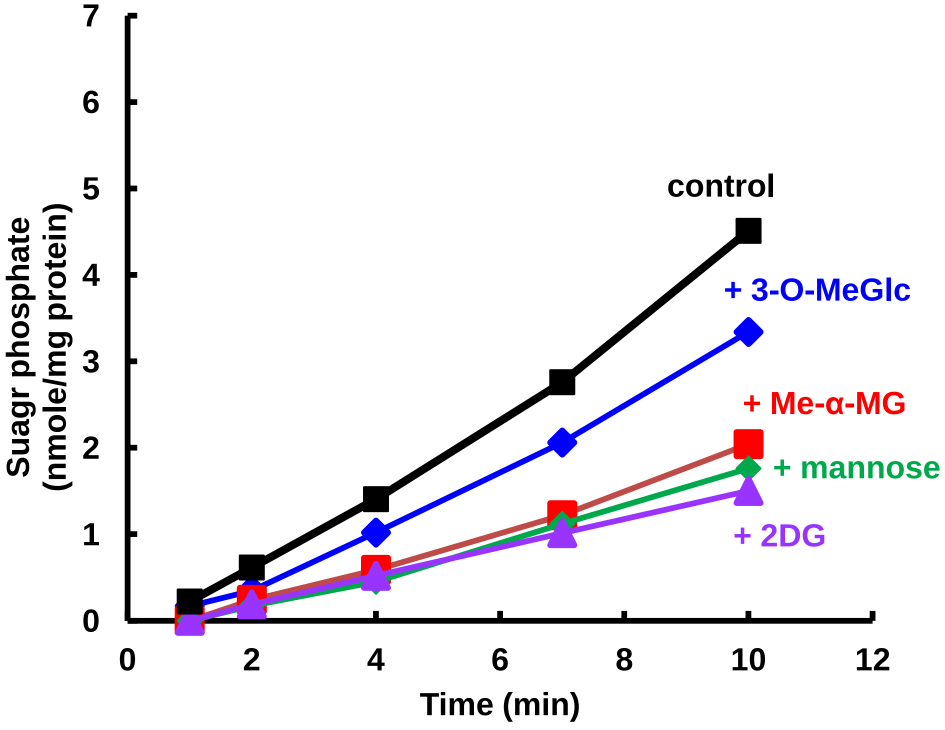


Glucose PTS activity in cell extracts

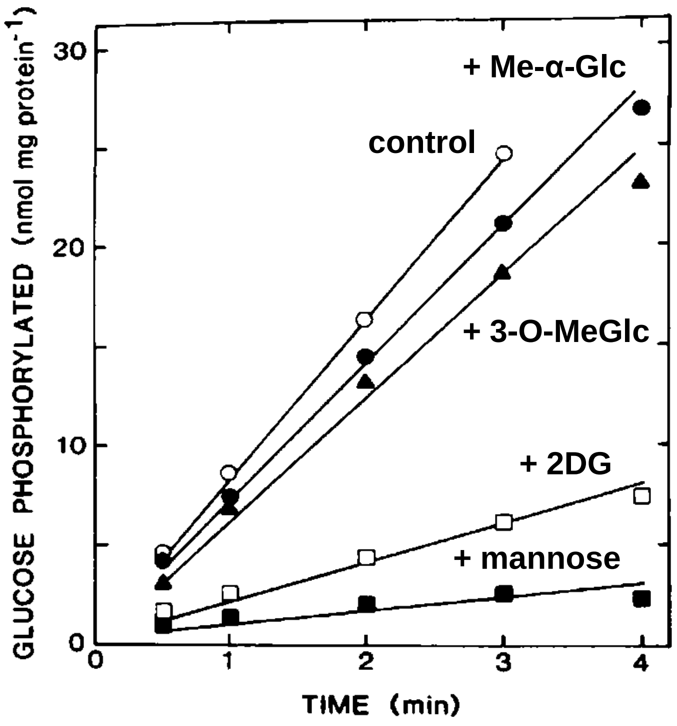


Inhibition of glucose phosphorylation by cell extracts

E. coli ME20



C. beijerinckii NCIMB 8052



Mitchell *et al* (1991) *Appl. Environ. Microbiol.* **57,** 2534-2539.

Conclusions:

- *cbei4532/4533* **encode a PTS that translocates both N-acetylglucosamine and glucose**
- *cbei0571* **encodes a PTS that translocates both glucose and mannose**
- **both phosphotransferase systems can contribute to glucose sensing and catabolite repression**

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