Evolution of new metabolic functions in bacteria

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Survival in the natural environment

The extraordinary versatility and adaptability exhibited by microorganisms are primarily responsible for their success in occupying a broad spectrum of niches.

Growth conditions of microorganisms in their natural habitats are often dramatically different from the nutritionally rich and stable environments they encounter in the laboratory.

In spite of facing a variety of stress in their natural environments, bacteria are very resilient and have evolved several strategies to compete and survive.

Bacterial models of evolution

The classic experiment by Salvadore Luria and Max Delbruck in 1943 demonstrated for the first time the power of microbial systems to study evolutionary processes, eighty five years after Darwin and Wallace proposed the idea of evolution by natural selection.

Luria S. E and M. Delbruck *Genetics* **28**: 491-511, 1943

Different strategies of using bacterial cultures to study evolution in the laboratory

Subsequent evolutionary studies using bacteria made use of three different ways of culturing:

Continuous culture – Hall and coworkers

Batch Cultures (Re-seeded) - Lenski & coworkers

(Long Term) – Kolter & coworkers

Silent or “Cryptic” Genetic Systems

In spite of the evolutionary pressure to maintain a compact genome, many bacteria carry genes that are silent, but are capable of being activated by a single genetic event. The presence of these “cryptic” genes is felt only upon acquisition of the new phenotype as a result of their activation.

Cryptic genes **=/=** Pseudogenes

Pseudogenes cannot be activated as they have accumulated several mutations whereas cryptic genes can be activated most often by a single mutational event.

Cryptic genes constitute an evolutionary puzzle

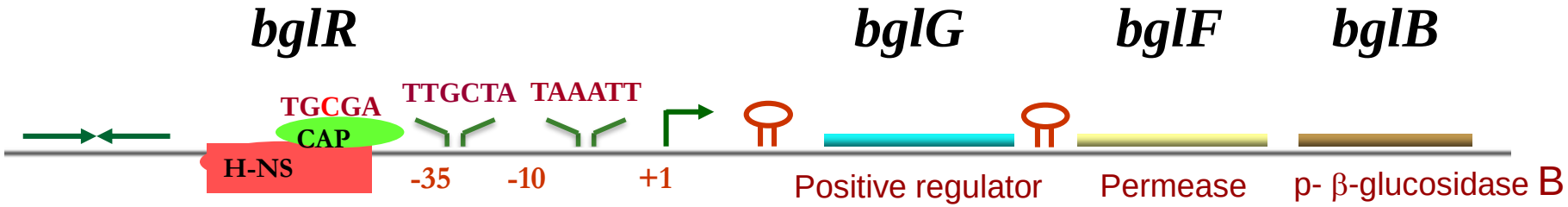
If there is no periodic selection for the function of a gene, it is likely to accumulate mutations and be lost from the genome

Since silent genes are not expected to contribute to the fitness of an organism, their presence in the genome without accumulating inactivating mutations is an enigma

How does one solve this puzzle?

Look for a paradigm

The *bgl* operon of *Escherichia coli*



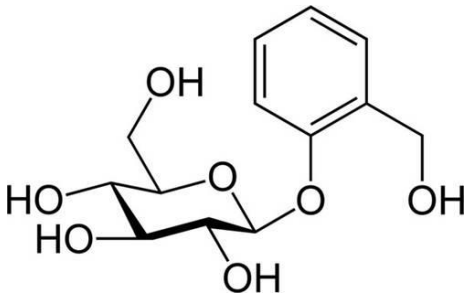
Inverted repeat

Rho independent terminators

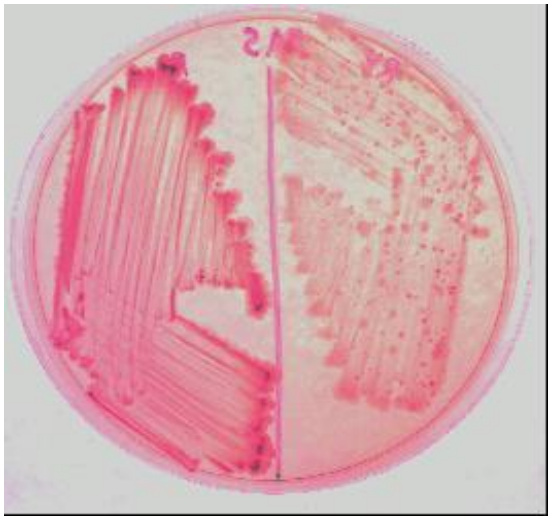
CAP CAP-cAMP binding site

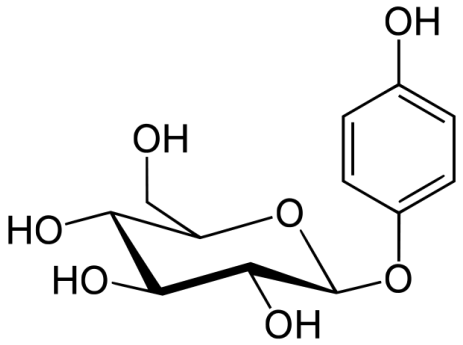
H-NS H-NS binding site

Transcription start site



salicin

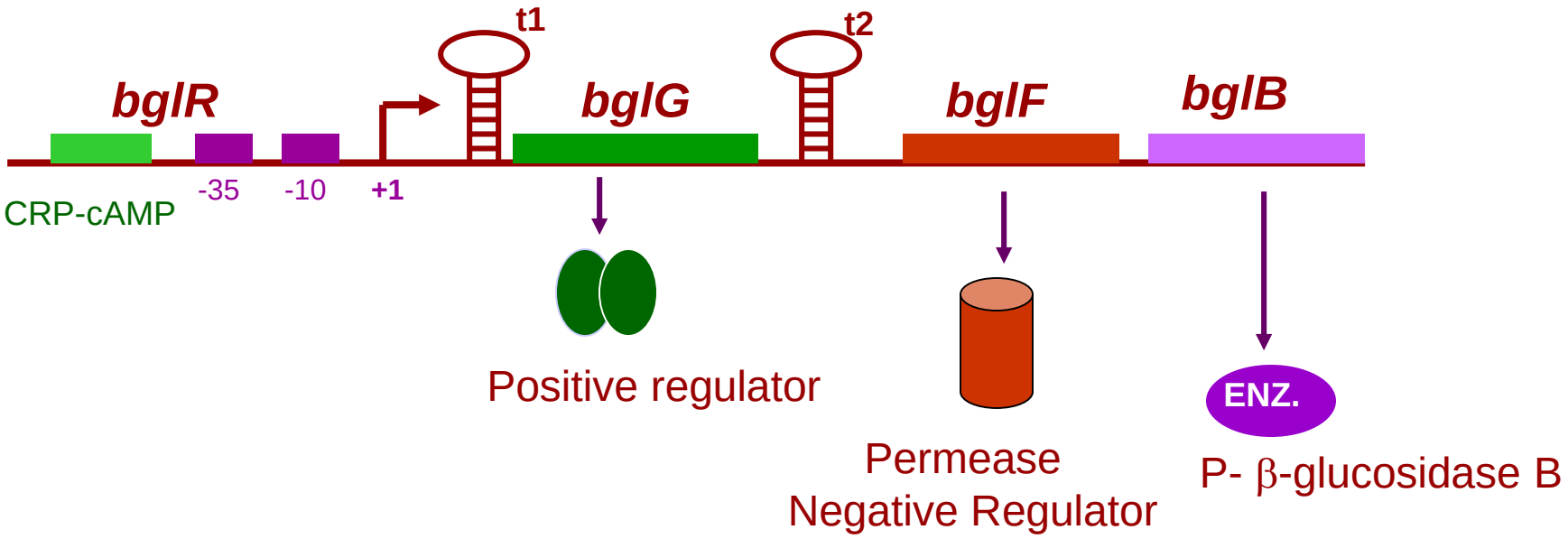




arbutin

Activation of the operon allows growth on salicin and arbutin

Regulation of the *bgl* operon



Induction by β-glucosides

- β-glucosides

+ β-glucosides

Does the *bgl* operon have a role in stationary phase?

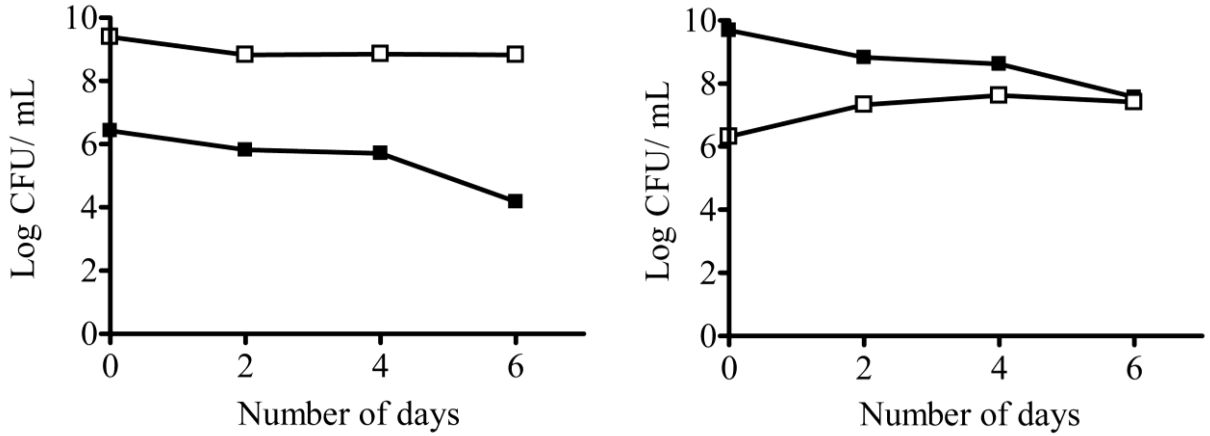
Bgl^{+} mutations present in survivors of prolonged stationary phase even when grown in the absence of β-glucosides.

Expression of the wild type *bgl* operon is enhanced in stationary phase even in the absence of an activating mutation.

Madan *et al* 2005, 2008

Activation of the *bgl* operon confers a growth advantage in stationary phase

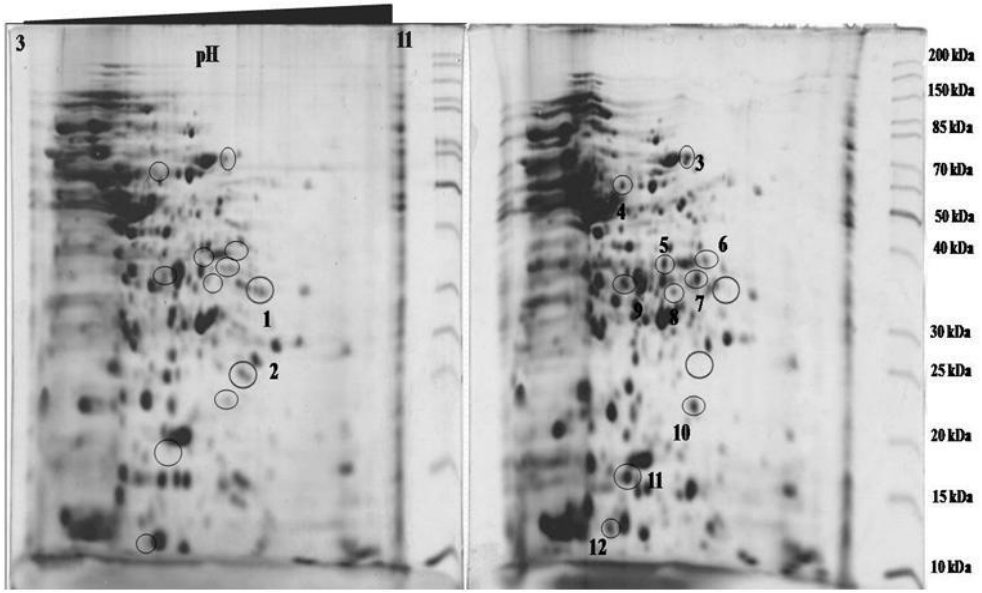
Strains carrying an activated *bgl* operon (Bgl^+) out-compete the parent (Bgl^-), thereby exhibiting a GASP phenotype when grown in L broth

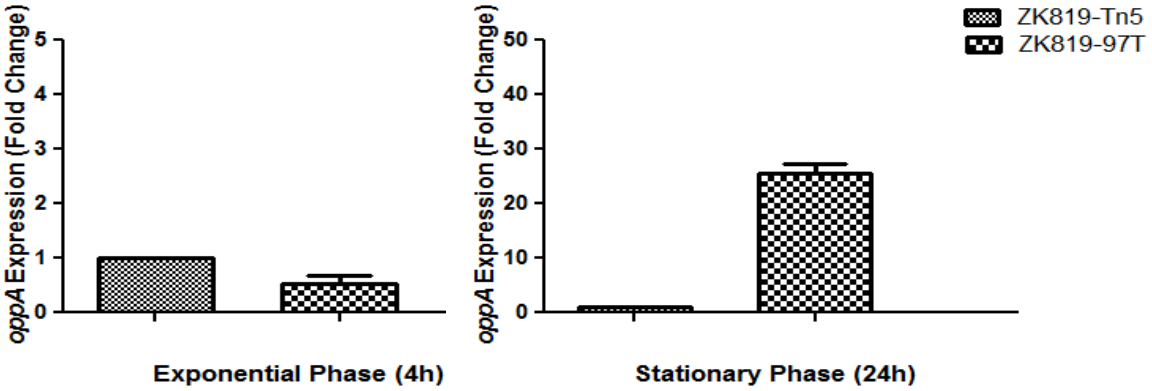


(Madan *et al* J. Bacteriol 2005)

How does activation of the *bgl* operon confer a GASP phenotype?

Proteome profiles of Bgl^{+} and Bgl^{-} strains show differences in stationary phase

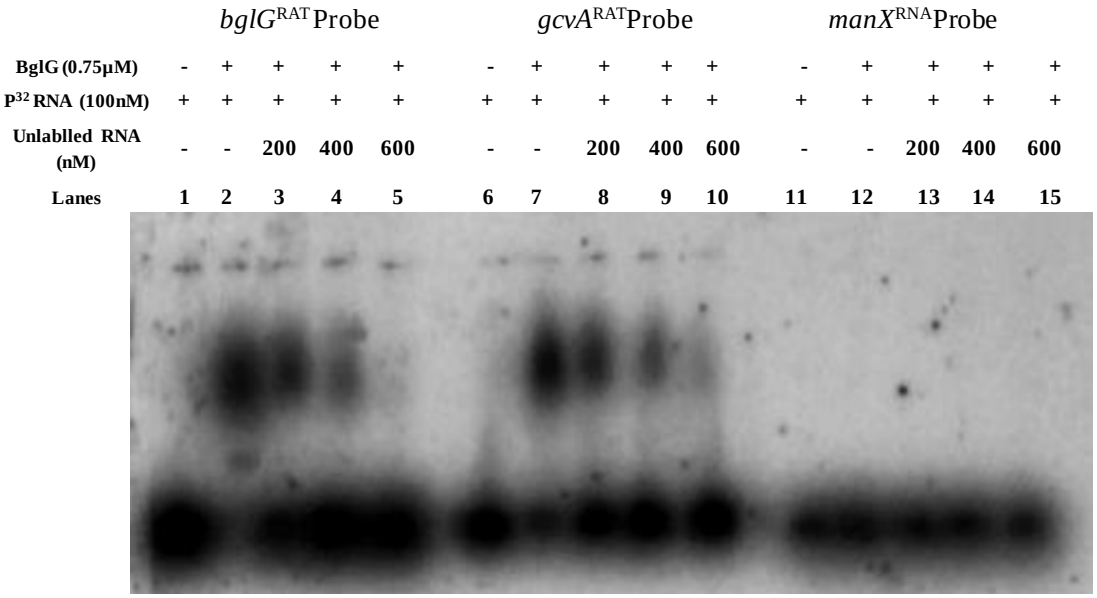




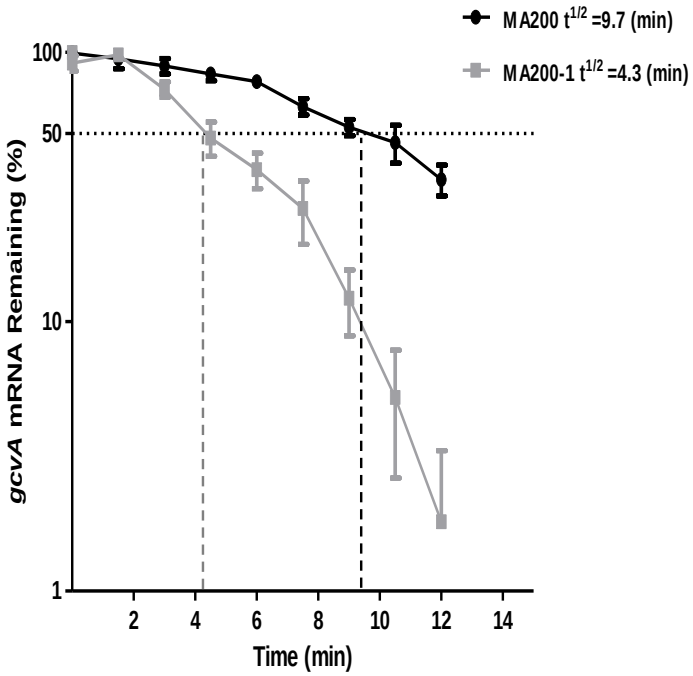
Expression of *oppA* encoding an oligo-peptide transporter is up-regulated in Bgl^{+} cells during stationary phase

Harwani *et al* J Bact. 2012

BglG binds to *gcvA* transcript and destabilises it



BglG binds to *gcvA* mRNA



Half-life of *gcv*A mRNA is reduced in the presence of higher levels of BglG

Reduced levels of GcvA leads to the down-regulation of the regulatory RNA *gcvB* that is a negative regulator of *oppA* resulting in higher levels of OppA enabling the import of oligo-peptides released by dying siblings.

Harwani *et al* J Bact. 2012

Ability to hydrolize aromatc β-glucosides enables defense against predators

Members of *Enterobacteriaceae* that actively hydrolyze aromatic β-glucosides such as salicin, arbutin, and esculin are able to avoid predation by the bacteriovorous amoeba *Dictyostelium discoideum*, nematode *Caenorhabditis elegans* and predatory *Streptomyces* sp.

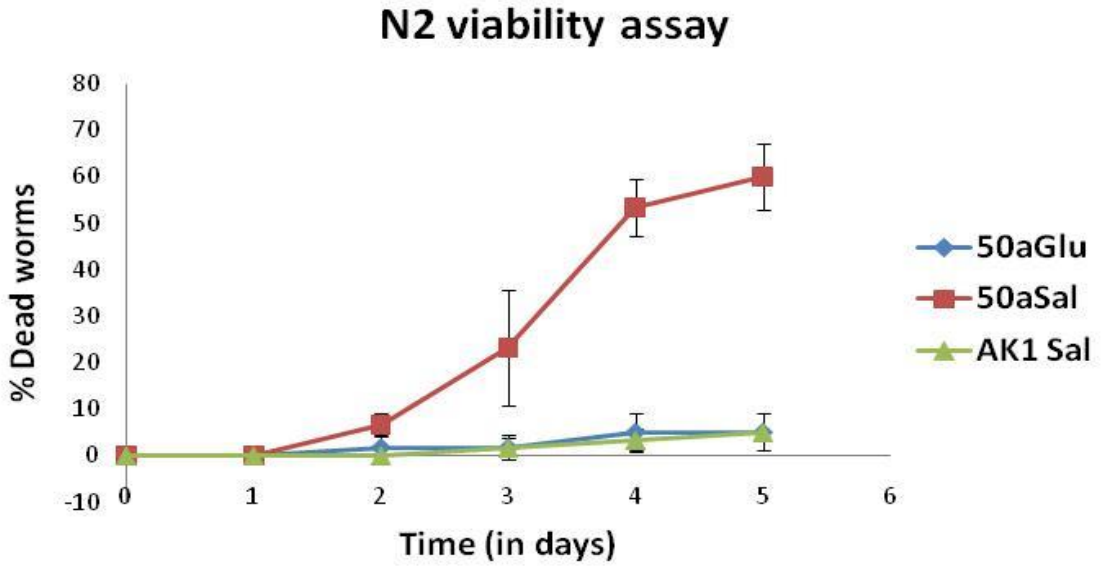
How does it work?

Presence of both Bgl^+ bacteria and aromatc β-glucosides is necessary for growth inhibition of *D. discoidium*

Experimental Conditions	Plaque formation
AK1 (Sal^-) + AX2	+
AK1 + AX2 + 35mM Salicin	+
AK102 (Sal^+) + AX2	+
AK102 + AX2 + 35mM Salicin	–
Klebsiella (Sal^+) + AX2	+
Klebsiella + AX2 + 35mM Salicin	–
MS201 (Sal^+) + AX2	+
MS201+ AX2 + 35mM Salicin	–
50a (Sal^+) + AX2	+
50a + AX2 + 35mM Salicin	–

Sonowal *et al* Proc. Royal Soc. 2013

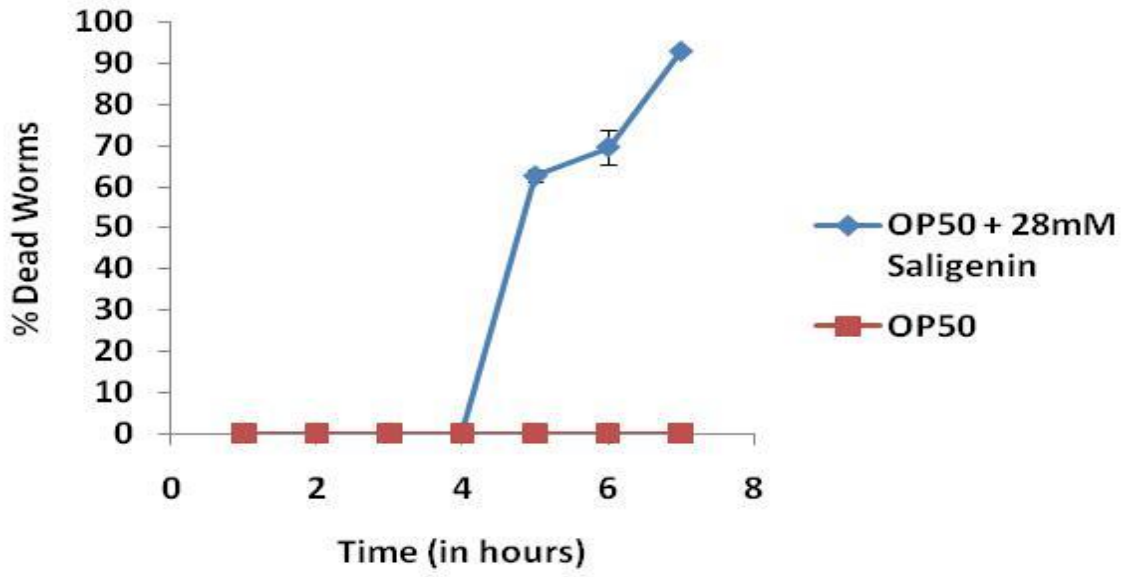
Salicin metabolism by bacterial prey is fatal to *C. elegans*



Survival of the N2 strain of *C. elegans* (plotted as percentage dead worms versus time) was monitored on NGM plates containing 35mM salicin and the Sal^+ bacterial strain 50a (red), 55mM glucose and 50a (blue) or 35mM Salicin and the Sal^- bacterial strain AK1 (green). Bacteria were grown on the respective medium for 36 hrs at 37^{o}C before addition of ~20 one day old adults to each plate. N=3. Error bars indicate SD.

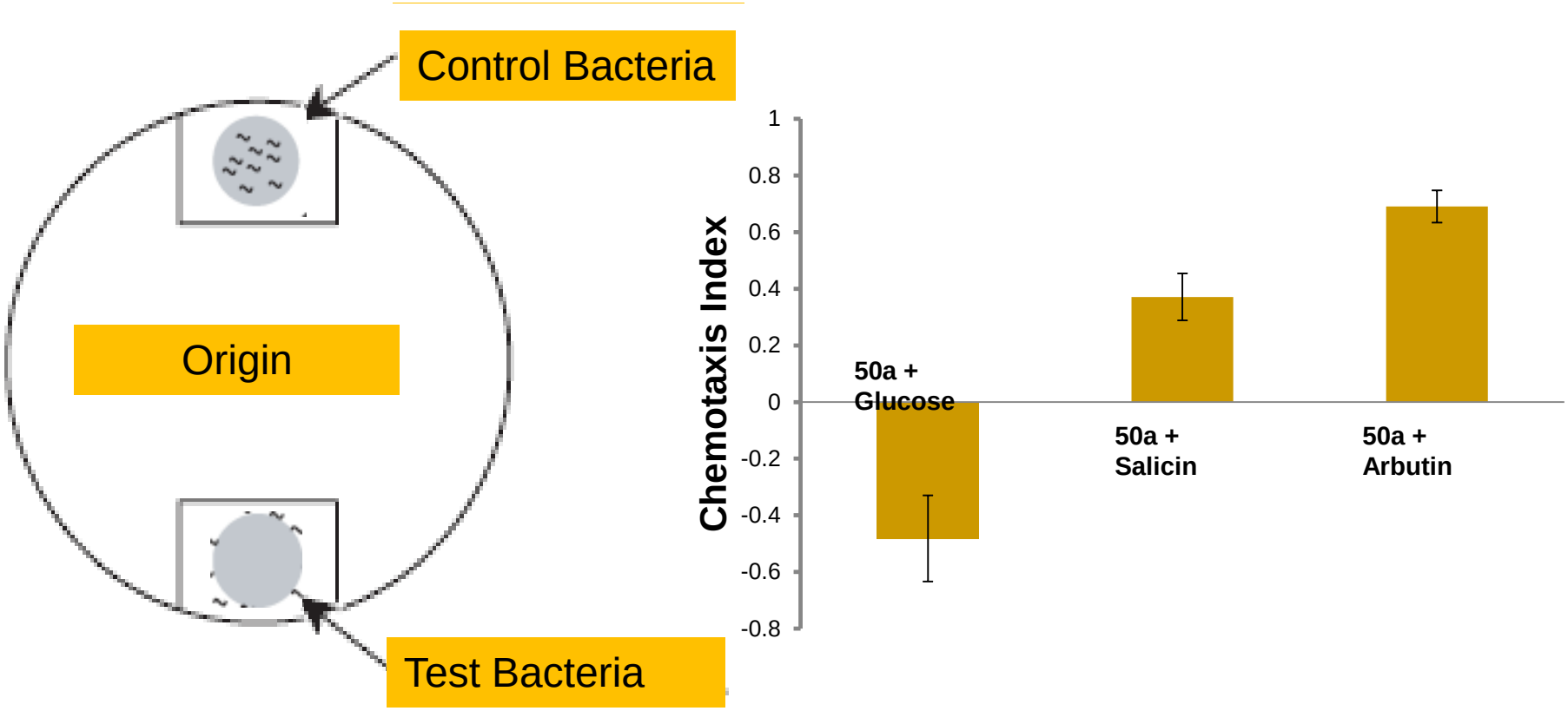
Sonowal *et al* Proc. Royal Soc. 2013

Effect of saligenin on predator viability



Effect of saligenin (28mM) on N2 young adults when present in their growth medium of NGM + OP50 lawn (blue line). The control plates had N2 worms on NGM medium + OP50 without saligenin (red line). N=2

C. elegans is attracted by bacteria degrading salicin



Chemotaxis Index = $\frac{\text{Number of worms in test spot} - \text{Number of worms in control spot}}{\text{Total number of worms}}$

Summary so far

- The activated *bgl* operon confers a substantial fitness advantage by providing a new metabolic capability both in growth phase as well as stationary phase and in addition protection from predation in the soil environment, thereby constituting a major selective force for its retention in the genome.

- The activated form of the operon could confer a fitness disadvantage in the exponential phase in the gut environment (so far no experimental support for this possibility).

- The transition between the silent and active states is likely to be dictated by the oscillations between the soil and gut environments, regulated at the population level.

Two “silent” operons can collaborate to acquire new metabolic functions

Mutants of *E. coli* that have lost *bglB* function can accumulate mutations in the silent *asc* genes to gain the ability to utilize salicin, with BglF providing the transport function and AscB providing the phospho-beta-glucosidase activity.

Shigella sonnei strains that carry a defective *bglB* gene recruit the silent gene *SSO1595* encoding a phospho-beta-glucosidase, and gain the ability to utilize salicin. SSO1595 is activated by genome rearrangements that introduce a new promoter upstream of the gene.

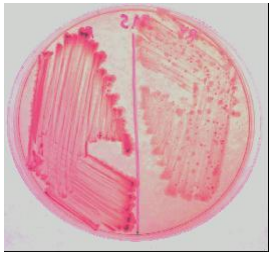
Desai and Mahadevan 2010

Evolution of new metabolic functions by mutating pre-existing genes

What happens when *bglB* and *ascB* are non-functional?

The Δ*bglB* Δ*ascB* strain can utilise arbutin with the orphan gene *bglA* providing the hydrolytic function. However, BglA *cannot* mediate hydrolysis of salicin and esculin.

When forced to grow on esculin/salicin as the sole carbon source, the Δ*bglB* Δ*ascB* strain (Esc^- Sal^-) evolves in the following trajectory:



Arbutin$^+$ → Esculin$^+$ → Salicin$^+$

(Mutants can be isolated as papillae on MacConkey medium)

Over-expression of *bglA* is sufficient to confer the ability to grow on esculin

Deletion of *bglA* in the Esc^+ mutant results in an Arb^- Esc^- phenotype, indicating the Involvement of *bglA* in the Arb^+ and Esc^+ phenotypes.

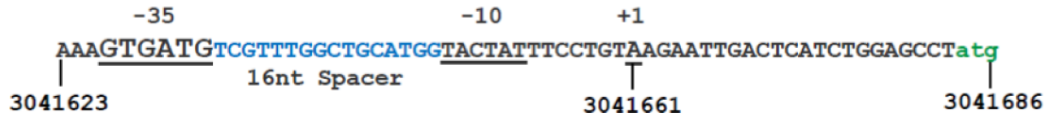
Over-expression of wild type *bglA* in the Esc^- parent conferred an Esc^+ phenotype, indicating that BglA has low level activity against esculin, which is augmented when over-expressed

Plasmid present	Arbutin	Esculin	Salicin
pACDH	**+**	**-**	**-**
pACDH-*bglA*^{WT}	**+**	**+**	**-**
pACDH-*bglA*$^{Esc+}$	**+**	**+**	**-**
pACDH-*bglA*$^{Sal+}$	**+**	**+**	**+**
pACDH-*bglA*^{T583G}	**+**	**+**	**+**

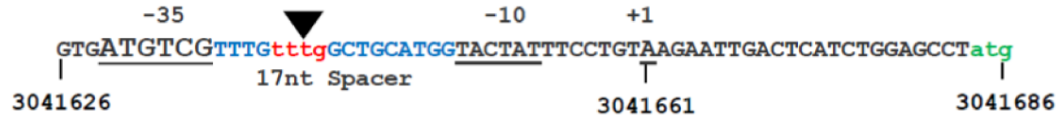
What happens in the Esc$^+$ mutant?

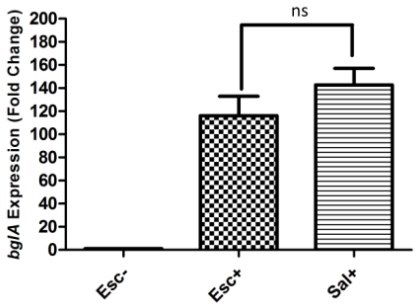
Nucleotide sequencing of the *bglA* locus in the mutant revealed a 4 nt. insertion within the *bglA* promoter with the coding sequences intact. The mutation results in a significant enhancement of transcription of *bglA* and more than doubles its stability

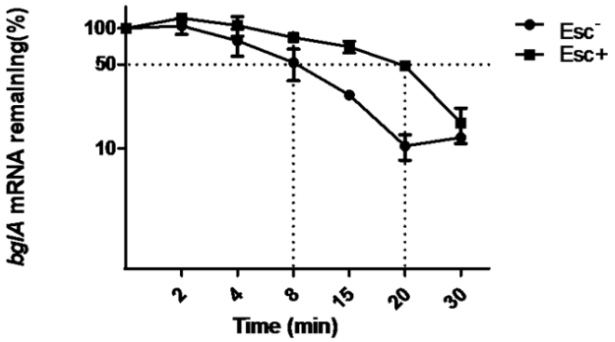
***bglA* promoter in wild-type:**



***bglA* promoter in mutant:**

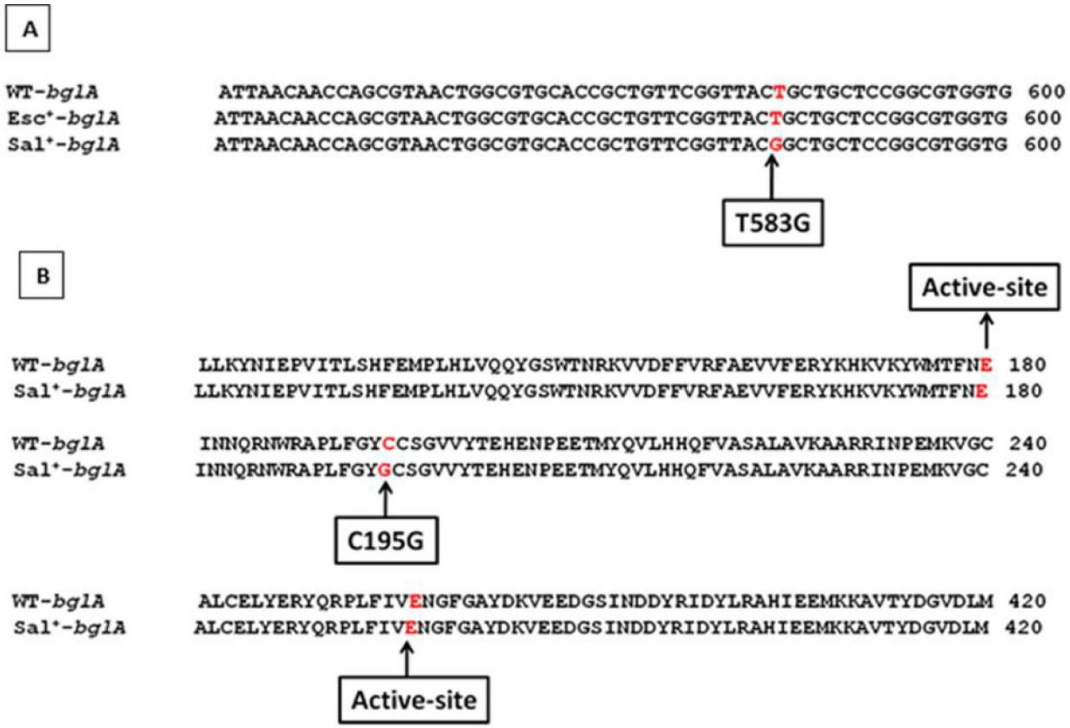






The transition to the Sal$^+$ state

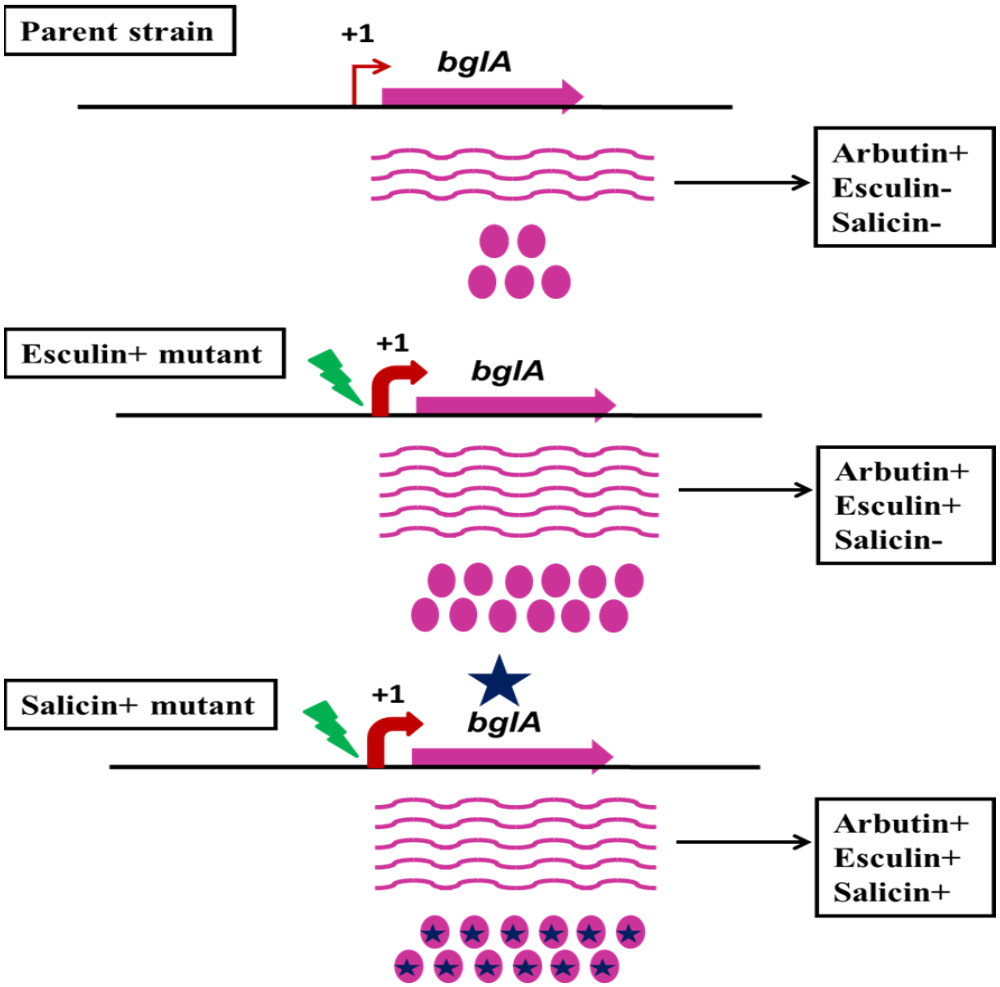
The Esc$^+$ to Sal$^+$ state involves an additional mutation



T to G transversion in the *bglA* coding sequence results in a C195G substitution close to the active site of the BglA glycosyl hydrolase.

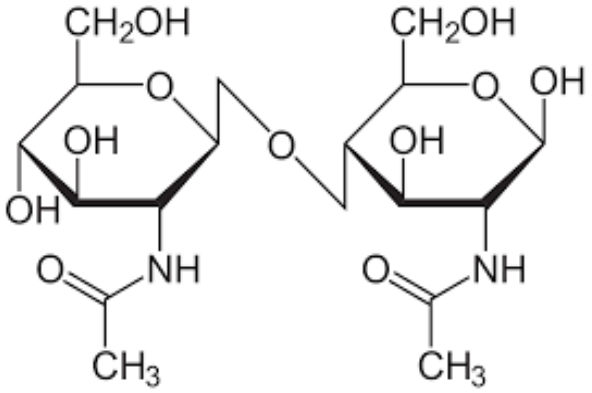
Interestingly BglA^{C195G} retains its activity against arbutin and esculin.

Summary

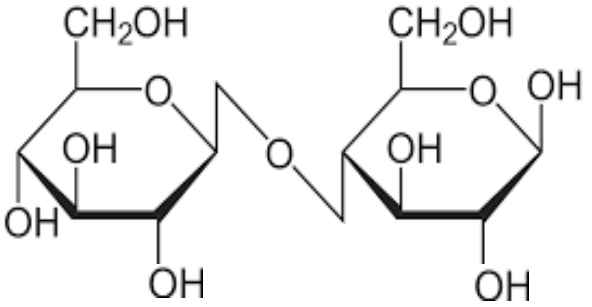


Zangoui et al *J. Bacteriol* (2015)

Many wild type *E. coli* strains can utilize chitobiose, but not cellobiose



Chitobiose
(Derivative
of chitin)

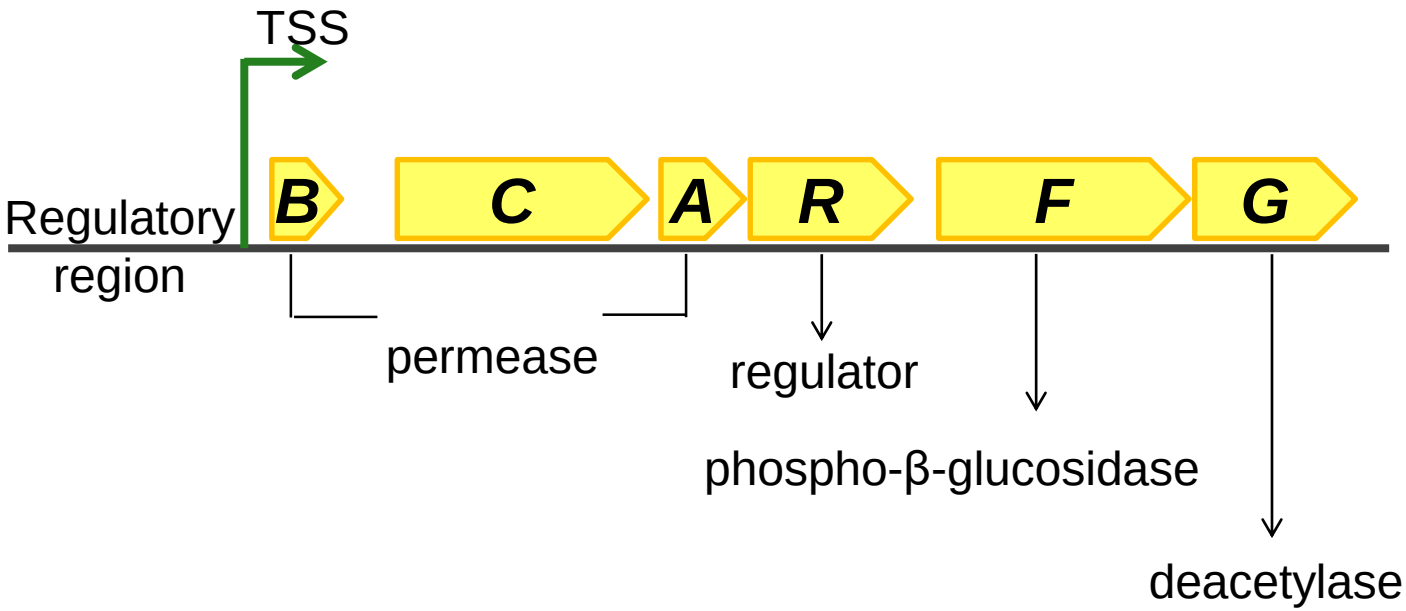


Cellobiose
(Derivative
of cellulose)

Upon prolonged selection on cellobiose, mutations in the cryptic *cel* operon enable cellobiose utilisation

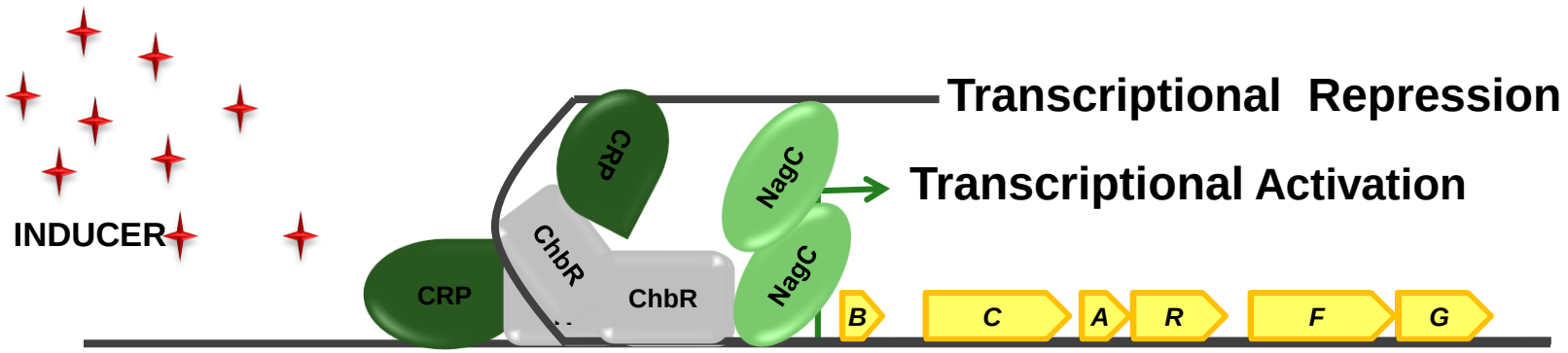
Parker & Hall, 1990

The *chb* operon of *E. coli* enables the utilization of chitooligosaccharides



Keyhani & Roseman, 1997; Thompson et al, 1999; Keyhani et al, 2000; Plumbridge & Pellegrini, 2004; Verma & Mahadevan, 2012

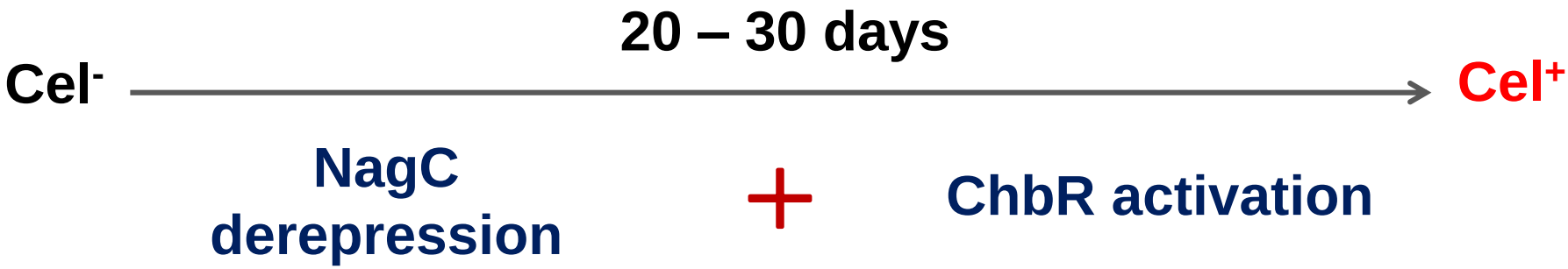
Regulation of the *chb* operon in *E. coli*



Plumbridge & Pellegrini, 2004

Cel$^-$ wild type *E. coli* can mutate to Cel$^+$ in two steps by modification of the *chb* operon

***E. coli* K-12**



The activating mutations in the regulator *chbR* are spread over the ChbR ORF. These mutations in *chbR* allow the recognition of cellobiose as a novel substrate, at the same time retaining the ability to recognise chitobiose.

Kachroo *et al* 2007

Cel$^+$ mutants appear sooner in *Shigela sonnei* compared to *E. coli*

***S. sonnei* AK1**

Cel$^-$ —(**5 – 10 days**)→ **Cel$^+$**

Insertions within *chb* operon regulatory region

IS*600* insertion at $^-$21
Introduction of new putative $^-$35
Distancing of *cis* elements

↓

Constitutive expression of operon
Arb$^+$Sal$^+$Cel$^+$

IS*2* or IS*600* insertion within NagC binding site
NagC derepression
Augments transcription of *chb* operon

↓

Inducible expression of the operon
Arb$^-$Sal$^-$Cel$^+$

Joseph et al 2017

The lessons learned so far

Microorganisms, when confronted by a novel substrate, have the capacity to evolve additional metabolic capability by mutational modification of pre-existing genetic systems that are silent or active.

Often this involves tinkering with regulatory genes that enables response to a new substrate.

Closely related organisms may employ different evolutionary paths to achieve the same goal.

Diversity in the repertoire of transposable elements can impact the evolutionary path adopted.

Evolution of a novel metabolic function need not necessarily involve a trade-off of the original metabolic capability but can increase promiscuity.

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