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Dipartimento di Scienze della Vita
CdS Biologia Molecolare e Cellulare (LM-6)



Genetica dei Procarioti - 4

Prof. *Laura Marri*
ricevimento: previo appuntamento telefonico o e-mail

Detection and Isolation of Mutants

mutations are generally rare
– one per 10^7 to 10^{11} cells



finding mutants requires sensitive detection methods
and/or methods to increase frequency of mutations

Selection

- **Positive (direct) selection** detects mutant cells because they grow or appear different
- **Negative (indirect) selection** detects mutant cells because they do not grow

mutations : one per 10^7 to 10^{11} cells

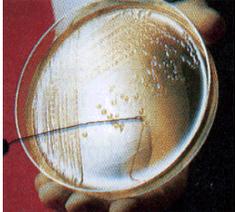
Culture liquide

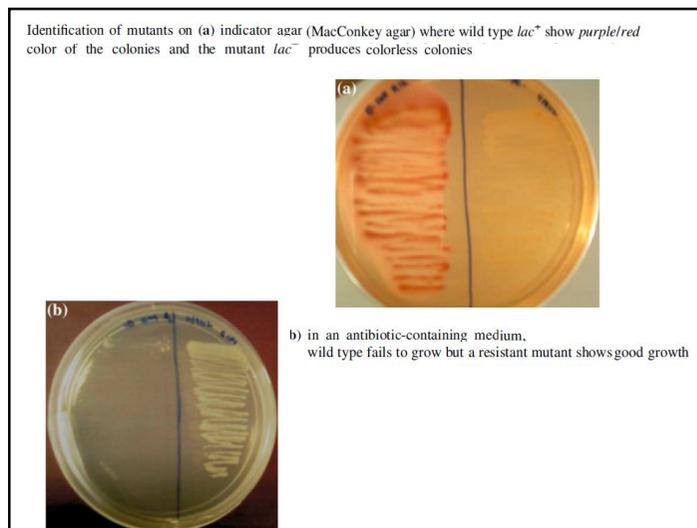
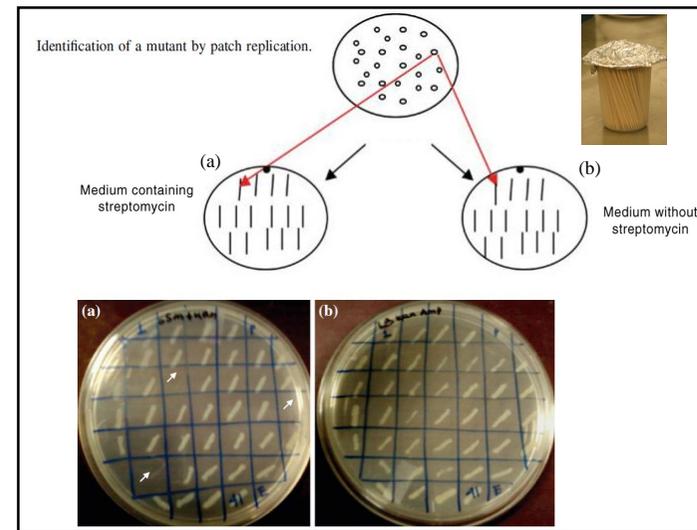
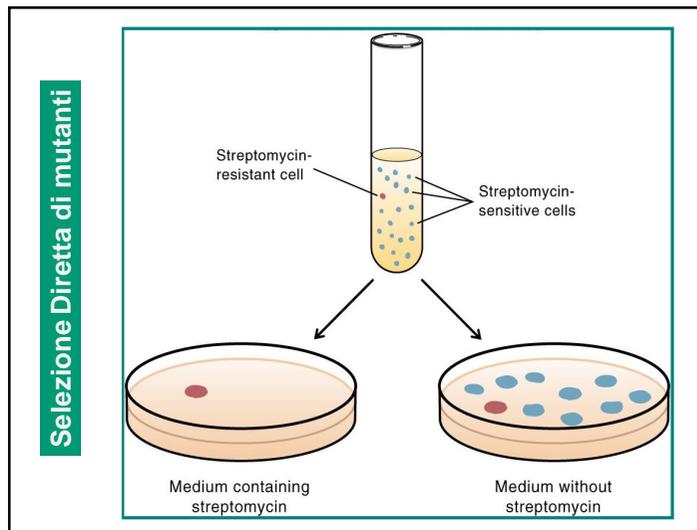
- 10^{10} cellule/ml



Colonie in Agar

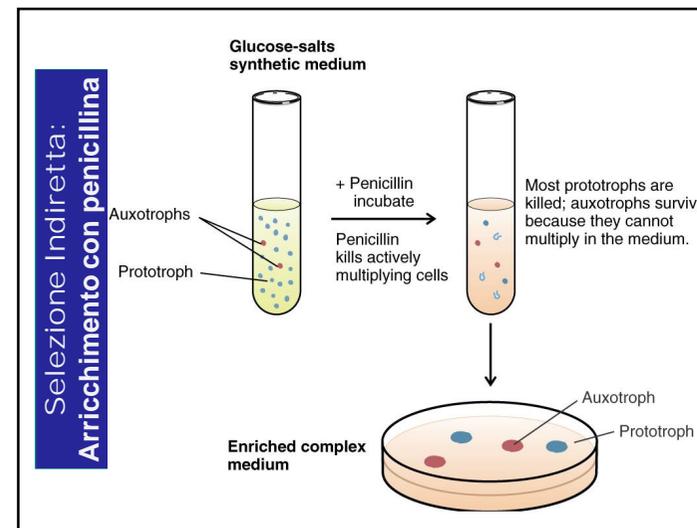
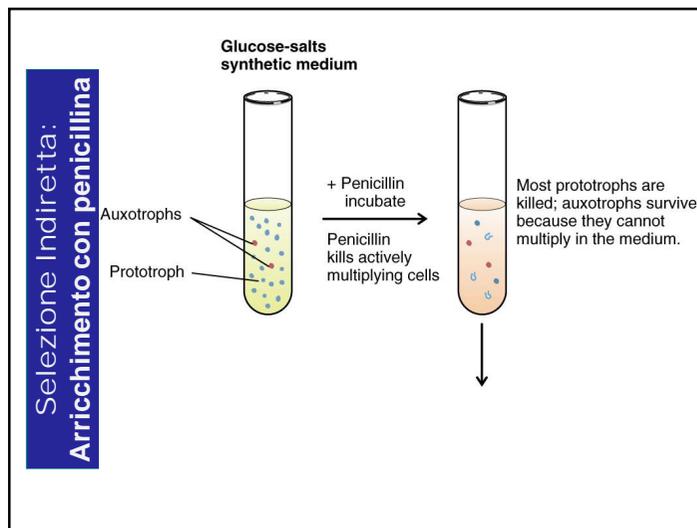
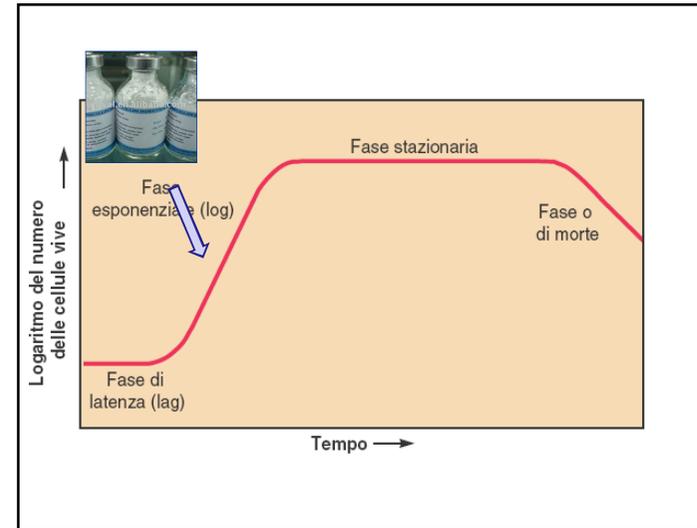
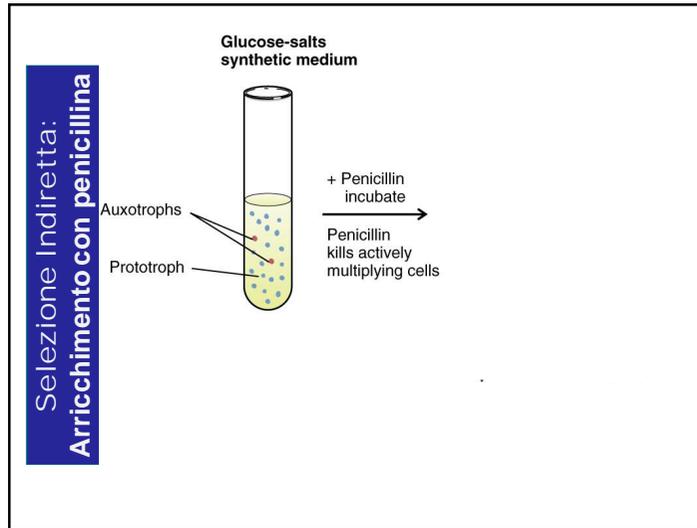
- 10^7 cellule/colonia

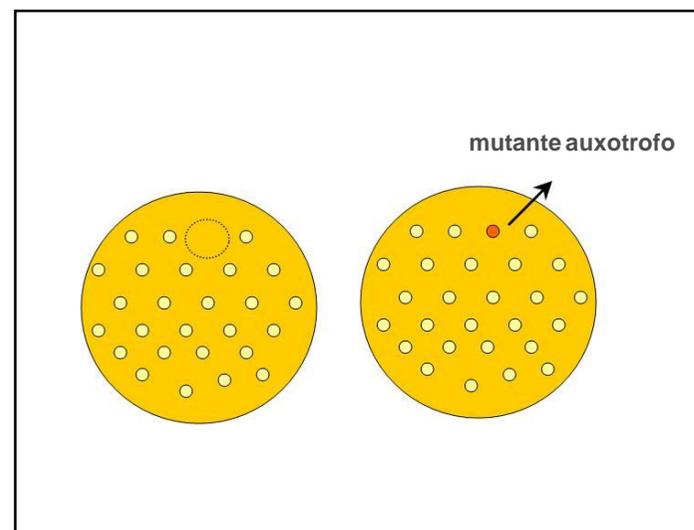
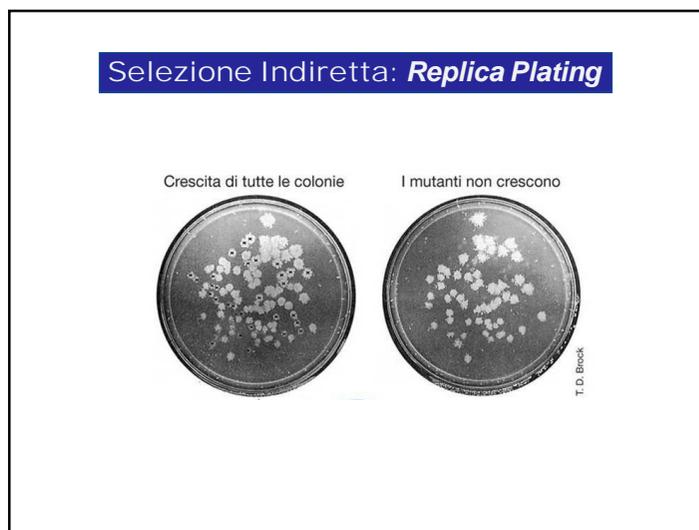
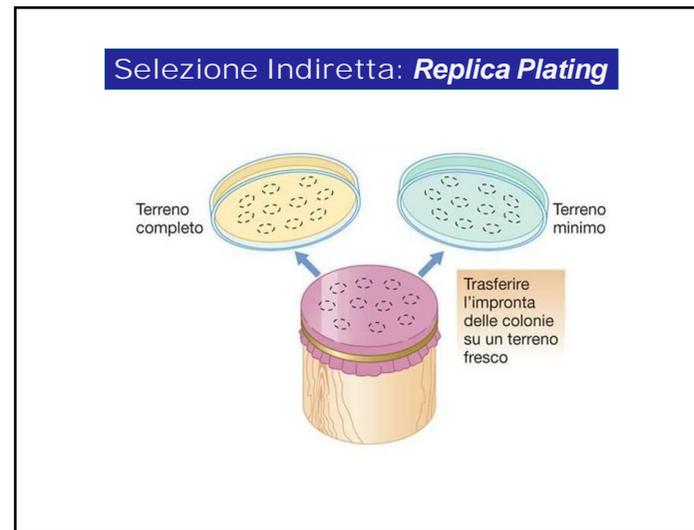
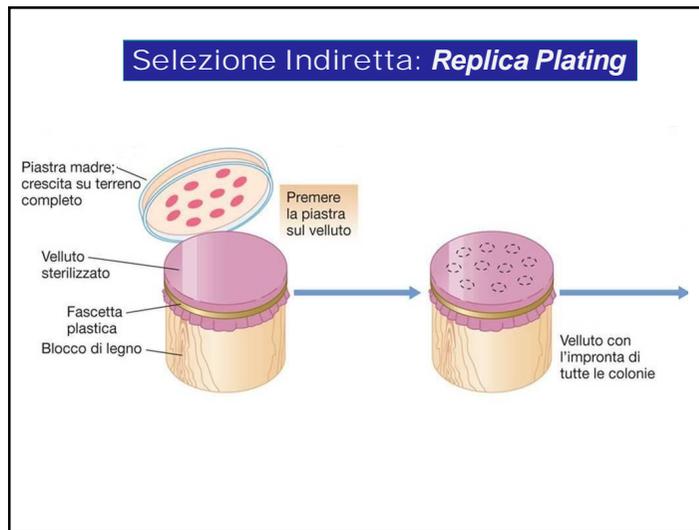




Terreni di coltura per *Escherichia coli*

<p>definito</p> <p>K₂HPO₄ 7 g KH₂PO₄ 2 g (NH₄)₂SO₄ 1 g MgSO₄ 0,1 g CaCl₂ 0,02 g Glucosio 4-10 g Elementi in tracce (Fe, Co, Mn, Zn, Cu, Ni, Mo) 2-10 µg di ognuno 1000 ml di acqua distillata pH 7</p>	<p>compleso</p> <p>Glucosio 15 g Estratto di lievito 5 g Peptone 5 g KH₂PO₄ 2 g 1000 ml di acqua distillata pH 7</p>
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REPLICA PLATING AND INDIRECT SELECTION OF BACTERIAL MUTANTS

JOSHUA LEDERBERG AND ESTHER M. LEDERBERG
 Department of Genetics, College of Agriculture, University of Wisconsin,
 Madison, Wisconsin

Received for publication August 31, 1951



Esther Lederberg in her home in Wisconsin (1958, the day that the Nobel Prize of Joshua Lederberg was announced).

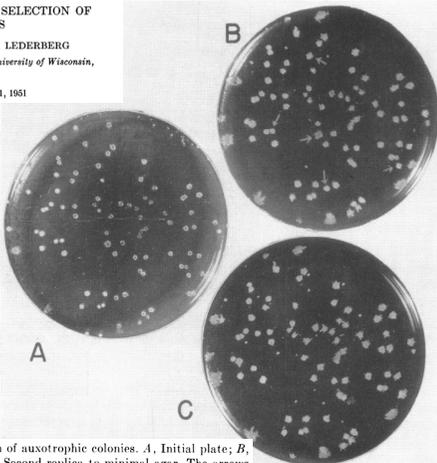


Figure 1. Replica plating for the isolation of auxotrophic colonies. A, Initial plate; B, Replica; both on complete agar medium. C, Second replica to minimal agar. The arrows designate the auxotrophic colonies which fail to grow on minimal medium. The resolution of these replicas is of fair to average quality.

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Selezione Indiretta: Isolamento di Mutanti

Bacteria plated on agar medium and incubated at 25°C

Wild-type bacteria

Conditional mutant

Conditional mutant (temperature-sensitive)

Replica plated onto two plates

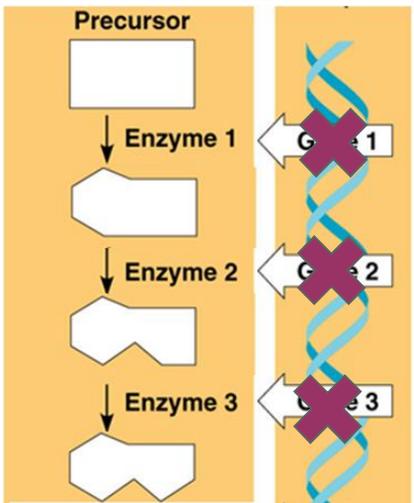
Only wild-type bacteria can grow

Incubate at 25°C

Incubate at 37°C

Esempio di isolamento di mutanti portatori di mutazioni condizionali letali in geni essenziali.

Analisi dei mutanti: Crossfeeding



Precursor

Enzyme 1

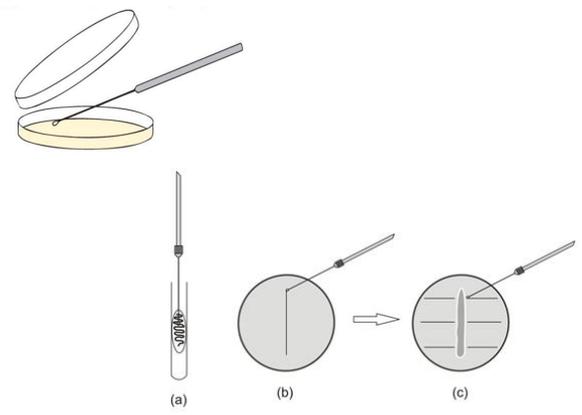
Enzyme 2

Enzyme 3

Gene 1

Gene 2

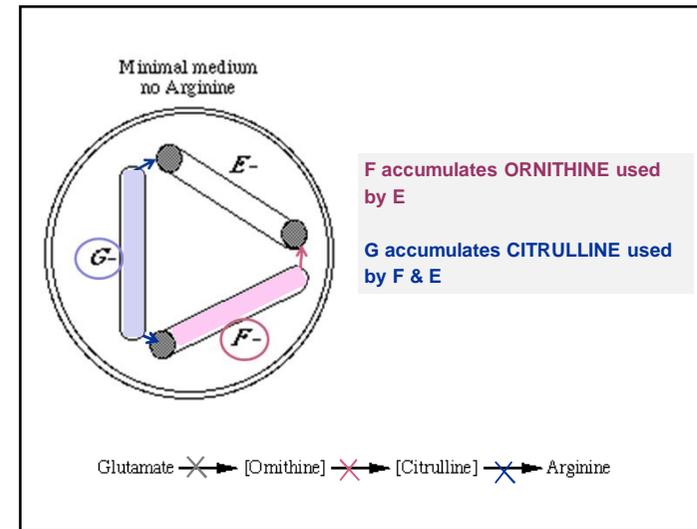
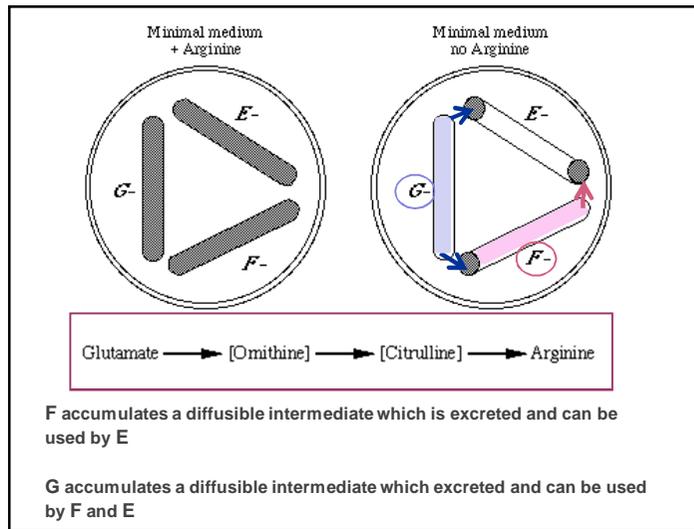
Gene 3



(a)

(b)

(c)



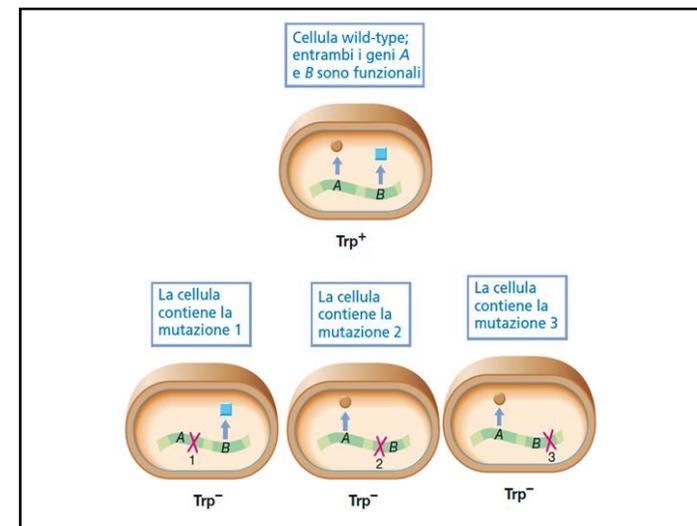
Analisi dei mutanti: **Complementation**

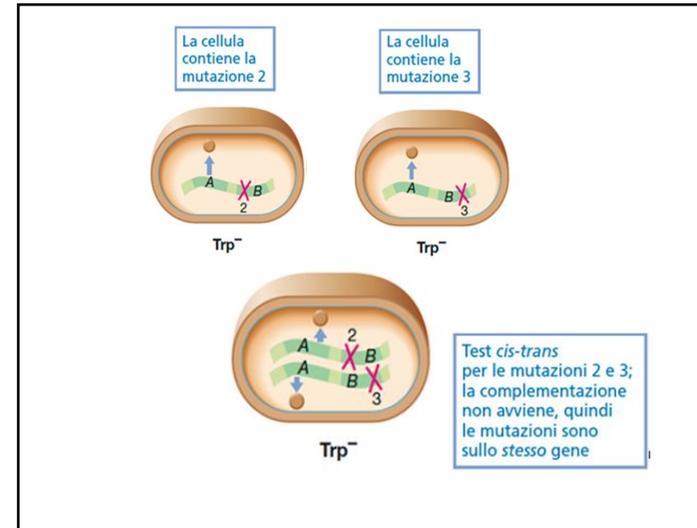
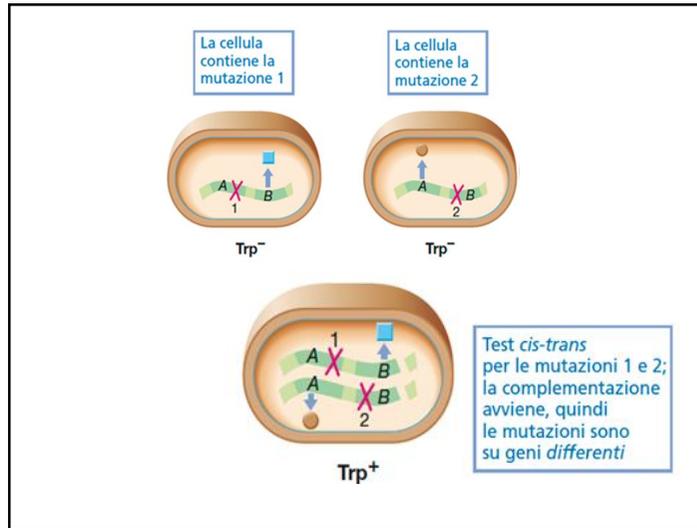
Complementation tests are used to determine if two mutations are in the same or different genes

necessary when different mutations in the same pathway yield the same phenotype

2 mutations on different genes: **trans** configuration

2 mutations on same gene: **cis** configuration





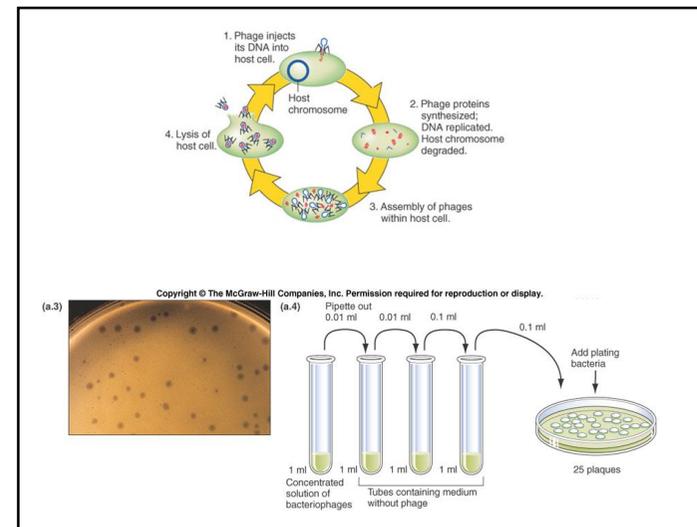
T2, T4 have been enormously useful in genetic studies because:

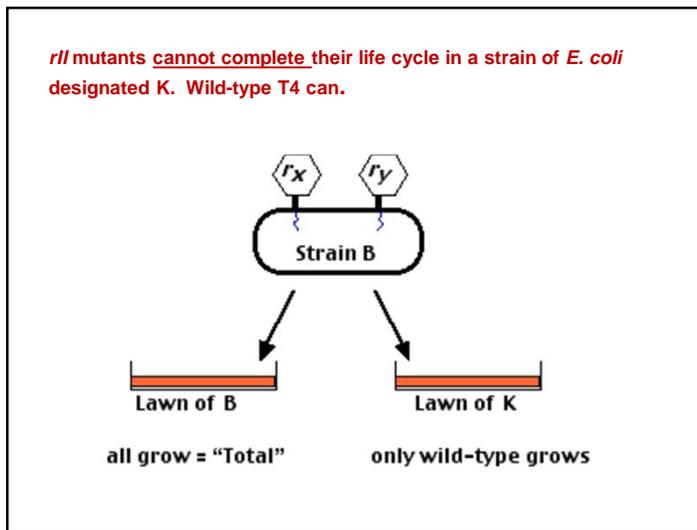
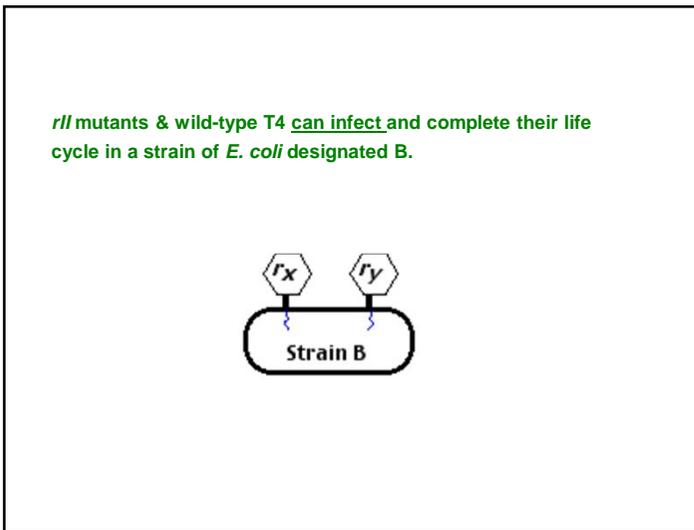
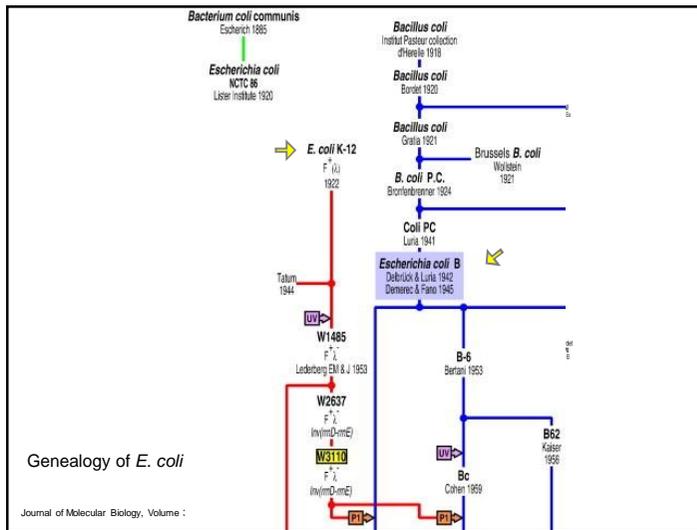
Viruses of two (or more) different genotypes can simultaneously infect a single bacterium.

The DNA molecules of one of the infecting viruses can recombine with that of another forming recombinant molecules.

The huge number of viruses released from a huge number of bacterial hosts enables even rare recombination events to be detected.

200 nm





Phenotypic properties of T4 phage

T4 strain	B	<i>E. coli</i> strain K(λ)
<i>rII</i> ⁻	Large, distinct	No plaques
<i>rII</i> ⁺	Small, fuzzy	Small, fuzzy

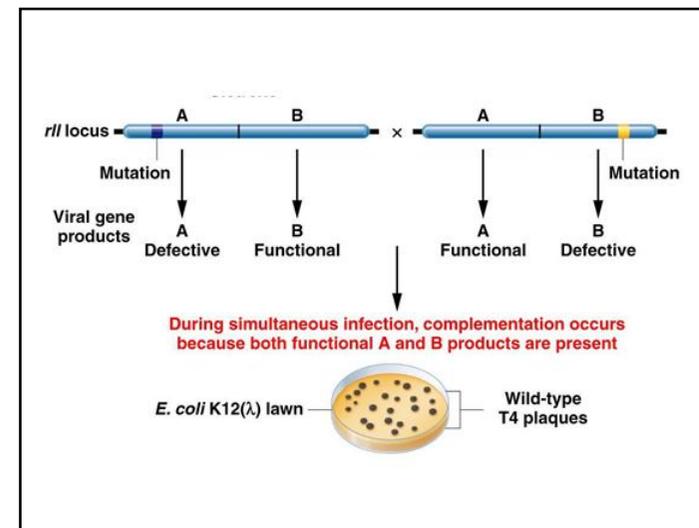
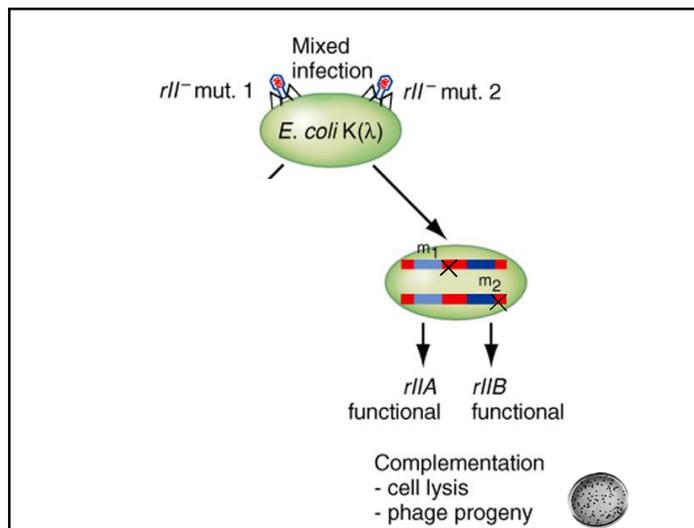
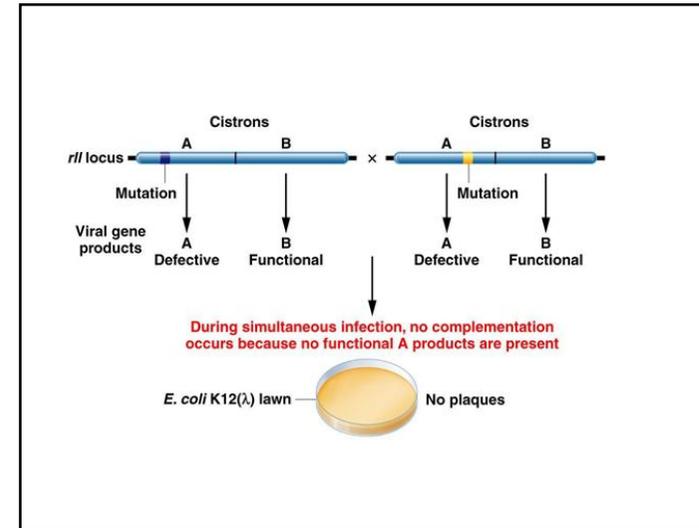
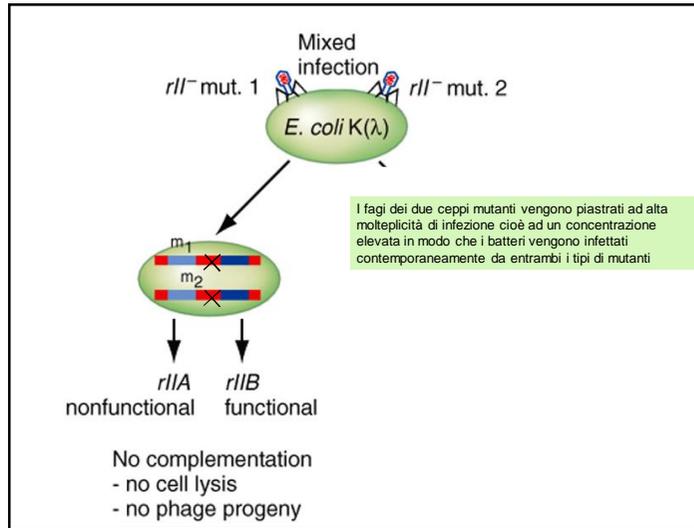
r: rapid lysis

The *rII* protein consists of 2 separate polypeptide chains, coded by the *rIIA* & *rIIB* genes respectively

rIIA
rIIB
Genotype

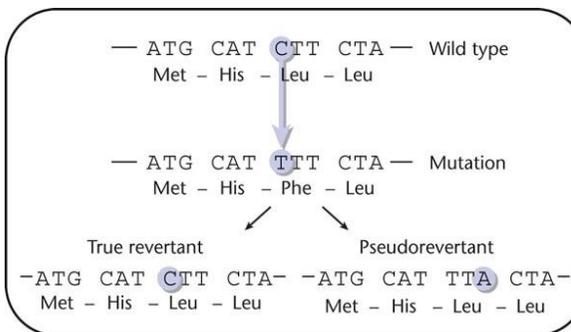
→

rII⁻
Phenotype

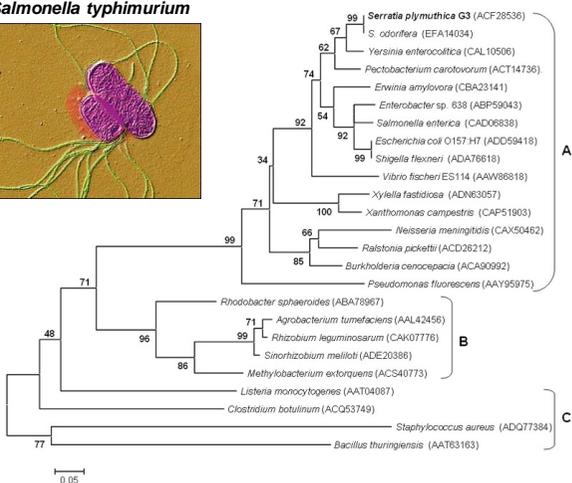
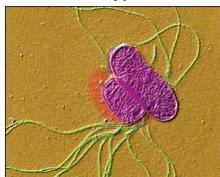


A gene as defined by the *cis-trans* test is called a **cistron** and is equivalent to defining a structural gene as a segment of DNA that encodes a single polypeptide chain

Reversione



Salmonella typhimurium



Proc. Nat. Acad. Sci. USA
Vol. 72, No. 12, pp. 5135-5139, December 1975
Medical Sciences

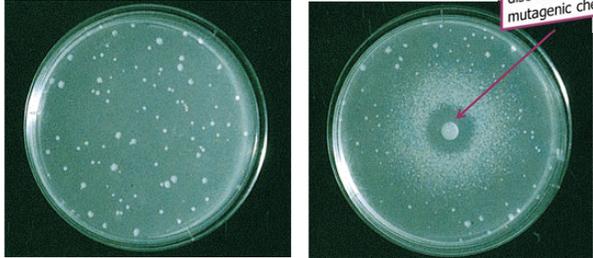
Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals*

(rapid *in vitro* screening/environmental carcinogens and mutagens)

JOYCE McCANN, EDMUND CHOI, EDITH YAMASAKI, AND BRUCE N. AMES



1998-Bruce Ames receives the Medal of Science from President Clinton.

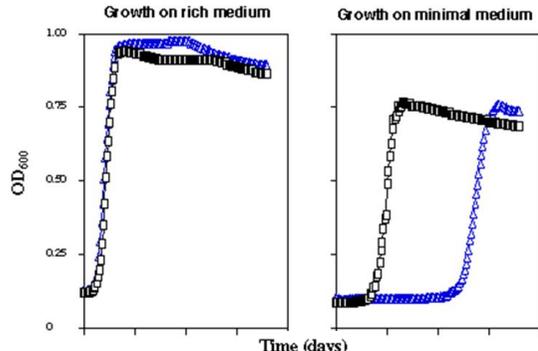
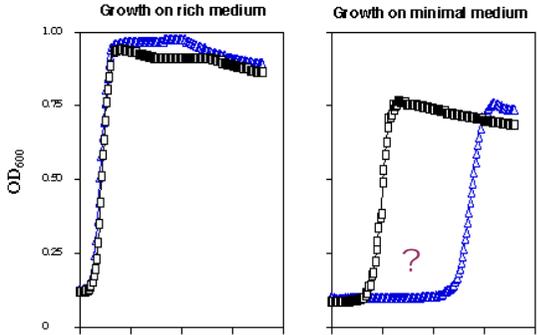


about 10^9 *his⁻* bacteria, $<10^2$ of white colonies derived from single bacteria that have undergone **spontaneous reversion mutations to *his⁺***.

As it diffuses outward, the chemical first kills all the bacteria (clear area), but then at lower concentration is seen to **induce** reversion mutations many revertants (*his⁺*) colonies due to its mutagenic effect.

T. Zahrt isolated a strain of *Salmonella typhimurium* that could grow as well as the wild-type parent on rich medium, but when transferred from rich medium to minimal medium the strain a very long lag phase compared to the wild-type parent.

wild-type : black squares; other strain: blue triangles.

Come si potrebbe determinare se la crescita osservata dopo la fase Lag dipende:

- da una seconda mutazione che consente la crescita in terreno minimo
- da un periodo più lungo di adattamento al terreno minimo

Frequenza di mutazione

La frequenza con cui avvengono i diversi tipi di mutazioni è estremamente variabile. Errori nella replicazione del DNA ricorrono con una frequenza di 10^{-6} 10^{-7} per coppia di basi per singolo ciclo di replicazione.



Aumenta in fase stazionaria: mutazione di una cellula ogni 300-400

operone criptico

sequenze di DNA fenotipicamente silenti normalmente non espresse durante il ciclo vitale dell'organismo

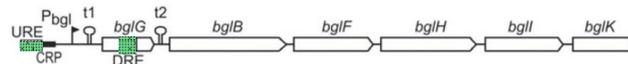


Sistemi criptici in *Escherichia coli* K12

- bgl* metabolismo arbutina e salicina
- cel* metabolismo arbutina, salicina, cellobiosio
- arbT* assunzione arbutina
- asc* metabolismo arbutina, salicina, cellobiosio

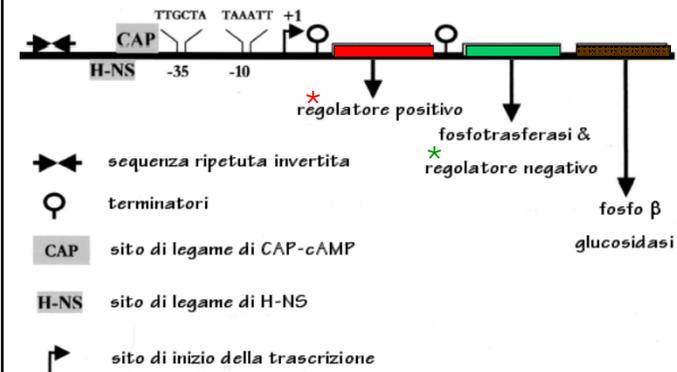
operone criptico *Bgl*

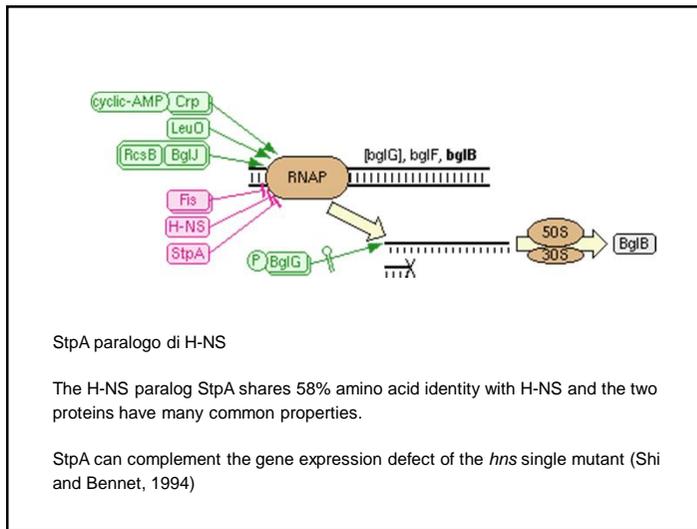
utilizzo beta-glucosidi aromatici



Scheme showing the *bgl* operon with the promoter (P_{bgl}), the CRP binding site (CRP), the Rho independent terminators (t1 and t2) and the structural genes *bglG*, *bglB*, *bglF*, *bglH* and *bglK* (*bglG*, transcriptional antiterminator, *BglB*, phospho β -glucosidase, *bglF*, EII permease, *bglH*, outer membrane porin, *bglI* xylanase, *bglK*, isomerase). The binding sites in URE (upstream regulatory element) and DRE (downstream regulatory element) are indicated with vertical hatched bars.

Operone *bgl*

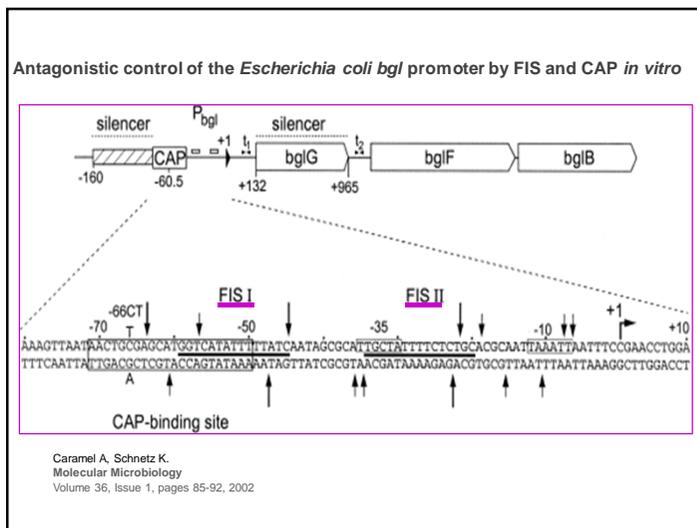




Proteina	Struttura	Massa molecolare del monomero (kDa)	Copie per cellula
Dps	α_{12}	19	20 000 ^a
Fis	α_2	11,2	100 ^a 10 000 ^b
H-NS	α_2 e oligomeri di ordine maggiore	15,4	20 000
HU	$\alpha_2, \beta_2, \alpha\beta$	9,2 (α);	50 000 ^b
Lrp	$\alpha_2-\alpha_6$	19	3000

Repressore globale della trascrizione di centinaia di geni. Regolatore della ricombinazione. I mutanti hanno effetto pleiotropico. E' anche una RNA chaperone. Il livello di espressione è costante con piccolo incremento in fase stazionaria. Forma eteromeri con altre proteine di *E. coli* e con altri paraloghi e ortologhi. Lega in regioni del DNA ricche in A + T.

^a In fase stazionaria.
^b In fase esponenziale.

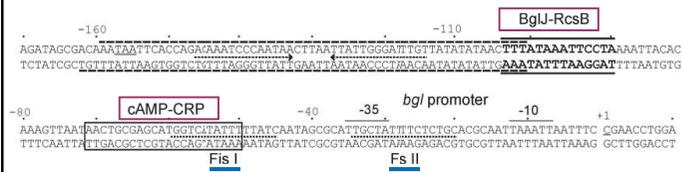


nature 293, 625 - 629 (22 October 1981)

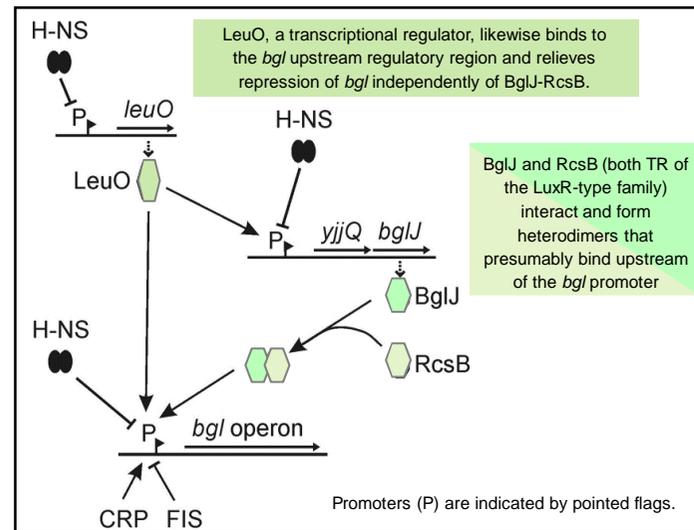
Insertion of DNA activates the cryptic *bgl* operon in *E. coli* K12
Ann E. Reynolds, Jeffrey Felton & Andrew Wright

Spontaneous mutations which activate the cryptic *bgl* operon of *Escherichia coli* K12 are caused by insertion of DNA at a site, *bglR*, within the operon. Two insertion elements, IS1 and IS5, have been observed to effect this activation. Once the activating insertion has occurred the operon is inducible by glucosides in a cyclic AMP-dependent manner.

Sequence of the *bgl* promoter and upstream regulatory region.

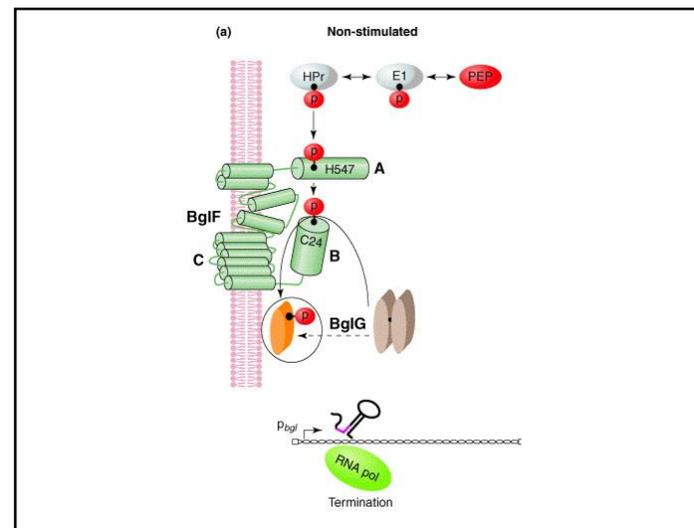
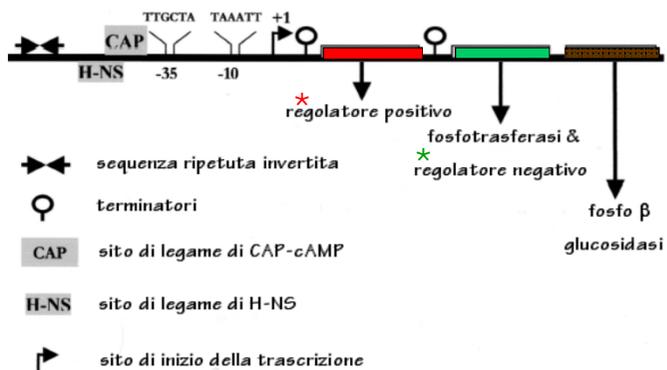


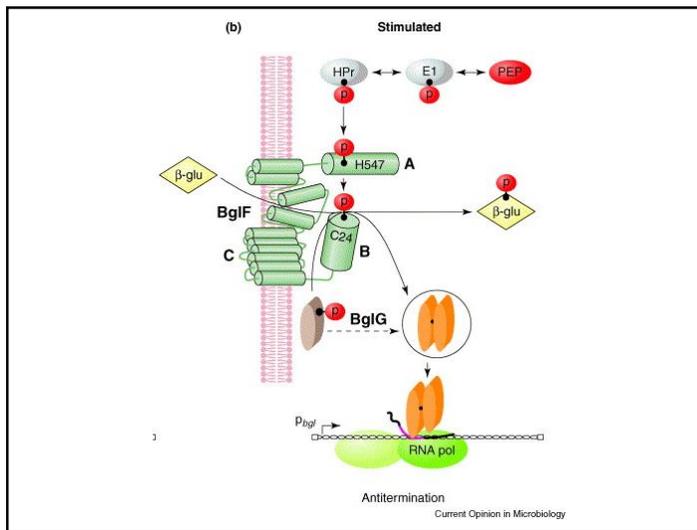
Indicated are the -35, -10, and transcription start sites of the promoter; the **CRP** binding site (boxed); and the **Fis** binding sites (dotted lines), as well as the **BglJ-RcsB** (solid lines) binding sites.



Promoters (P) are indicated by pointed flags.

Operone *bgl*





Ruolo biologico ed evolutivo dei geni criptici

- Secondo il modello di Hall *et al.* (1987), un gene di una popolazione microbica può trovarsi in tre stati:
 - Allele funzionale;
 - allele criptico;
 - allele non-funzionale.

L'allele *wild type* è favorevole in certi ambienti, mentre in ambienti alternativi, con risorse limitate, l'allele criptico è vantaggioso.

La distribuzione di alleli criptici/funzionali per il metabolismo di β -glucosidi varia all'interno delle *Enterobacteriaceae* in relazione alla nicchia ecologica occupata

<i>Klebsiella</i> spp. Gruppo I	}	fermentano β -glucosidi aromatici e cellobiosio
<i>Escherichia coli</i> , <i>Shigella sonnei</i> Gruppo II		
	}	non possono fermentare β -glucosidi, ma possono mutare acquisendo indipendentemente un fenotipo Arb ⁺ , Sal ⁺ o Cel ⁺

Il mantenimento di alcuni sistemi criptici rappresenta un sistema di regolazione a lungo termine per funzioni raramente utilizzate

In ambienti multi-risorsa, un allele criptico attivato è svantaggioso, ma selettivamente favorevole in particolari condizioni di stress (es. digiuno, risposta immunitaria dell'ospite)

PROCEEDINGS OF THE ROYAL SOCIETY B

Hydrolysis of aromatic β -glucosides by non-pathogenic bacteria confers a chemical weapon against predators

rsob.royalsocietypublishing.org

Robert Sonowal¹, Kirithi Nandimath¹, Sucheta S. Kulkarni², Sandhya P. Koushika^{2,3}, Vidyanand Nanjundiah³ and S. Mahadevan¹

Bacteria present in natural environments such as soil have evolved multiple strategies to escape predation. We report that natural isolates of *Enterobacteriaceae* that actively hydrolyze plant-derived aromatic β -glucosides such as salicin, arbutin and esculin, are able to avoid predation by the bacteriovorous amoeba *Dictyostelium discoideum* and nematodes of multiple genera belonging to the family *Rhabditidae*. This advantage can be observed under laboratory culture conditions as well as in the soil environment. The aglycone moiety released by the hydrolysis of β -glucosides is toxic to predators and acts via the dopaminergic receptor Dop-1 in the case of *Caenorhabditis elegans*. While soil isolates of nematodes belonging to the family *Rhabditidae* are repelled by the aglycone, laboratory strains and natural isolates of *Caenorhabditis* sp. are attracted to the compound, mediated by receptors that are independent of Dop-1, leading to their death. The β -glucosides-positive (Bgl⁺) bacteria that are otherwise non-pathogenic can obtain additional nutrients from the dead predators, thereby switching their role from prey to predator. This study also offers an evolutionary explanation for the retention by bacteria of 'cryptic' or 'silent' genetic systems such as the *bgl* operon.