The genotoxin colibactin

Jean-Philippe Nougayrède

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Molecular and cellular pathogenesis of *E. coli* infections
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22nd Meeting of the French Society of Toxinology, "Toxins: New Targets and New Functions“ 10th - 11th December 2014, Pasteur Institute

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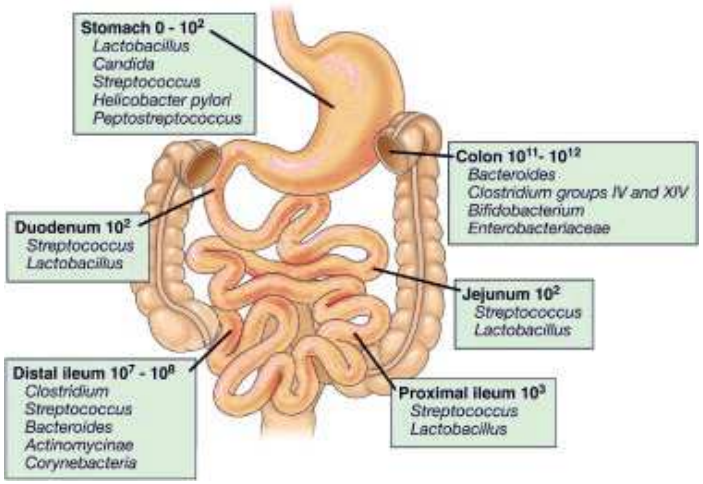
Escherichia coli : a commensal bacterium of the intestinal tract with considerable pathogenic potential

E. coli belongs to the initial microflora colonizing the newborn gut



E. coli is the predominant facultative anaerobe in the adult gut (10^3 to 10^8 /g of feces)

E. coli is a leading cause of infant acute **diarrhea** and the primary cause of travelers' diarrhea.

E. coli is an emerging **foodborne pathogen.**

About 10 to 20 percent of women have had at least one episode of **urinary tract infection** due to *E. coli* in their lifetime.

E. coli causes 10-50% of **nosocomial infections**.

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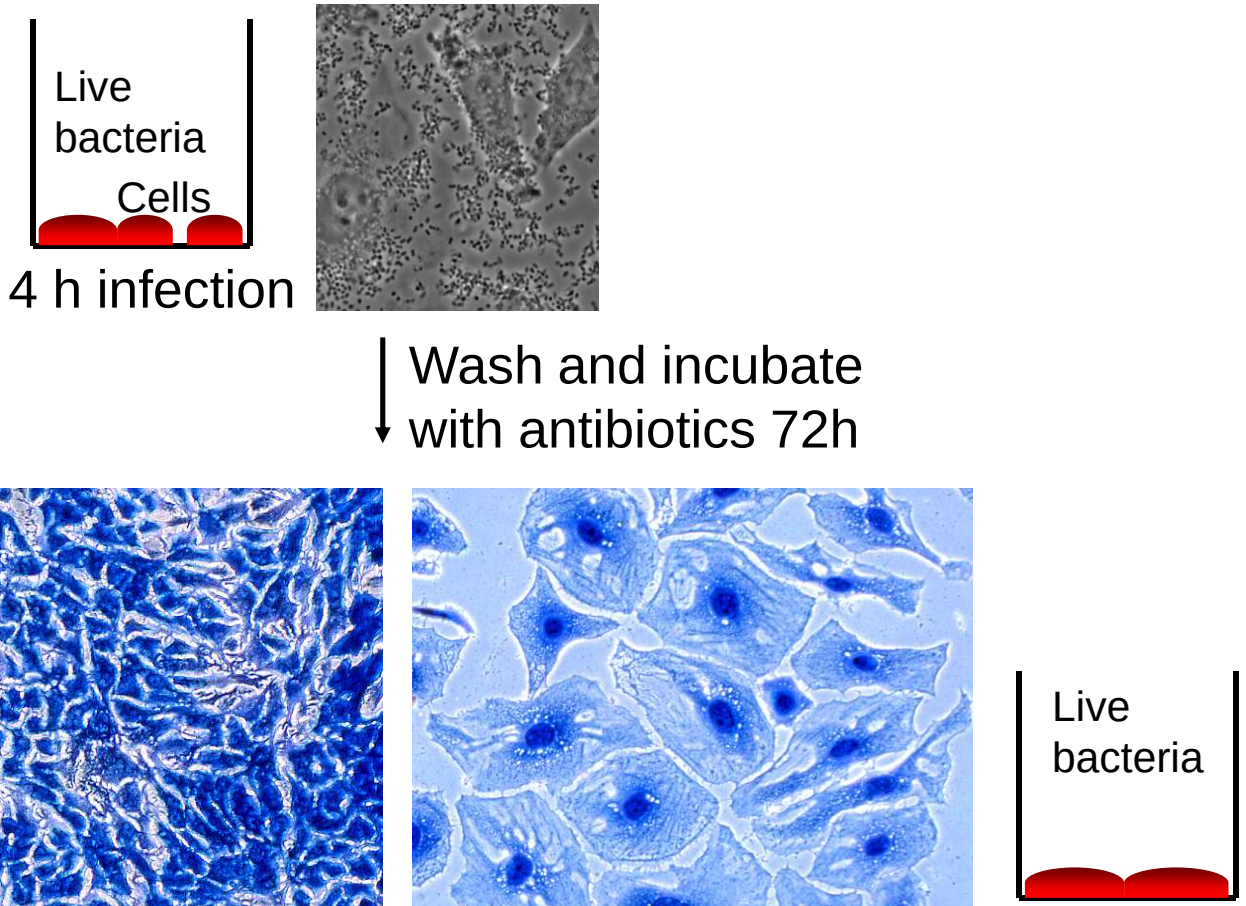
A short infection of cultured cells with extra-intestinal pathogenic *E. coli* induces a "megalocytosis" effect



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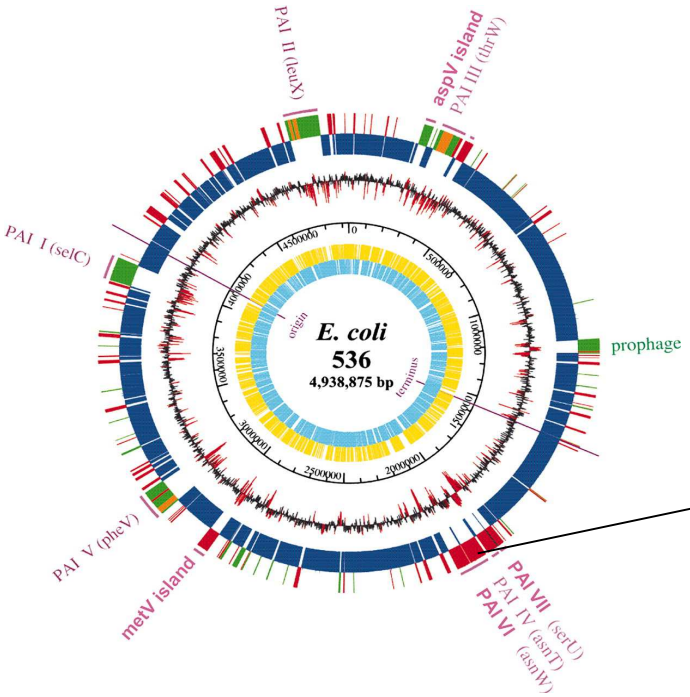
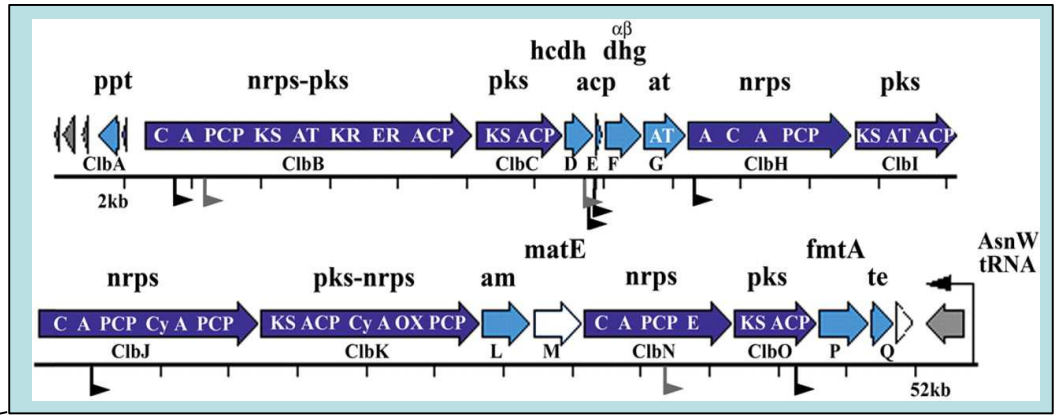
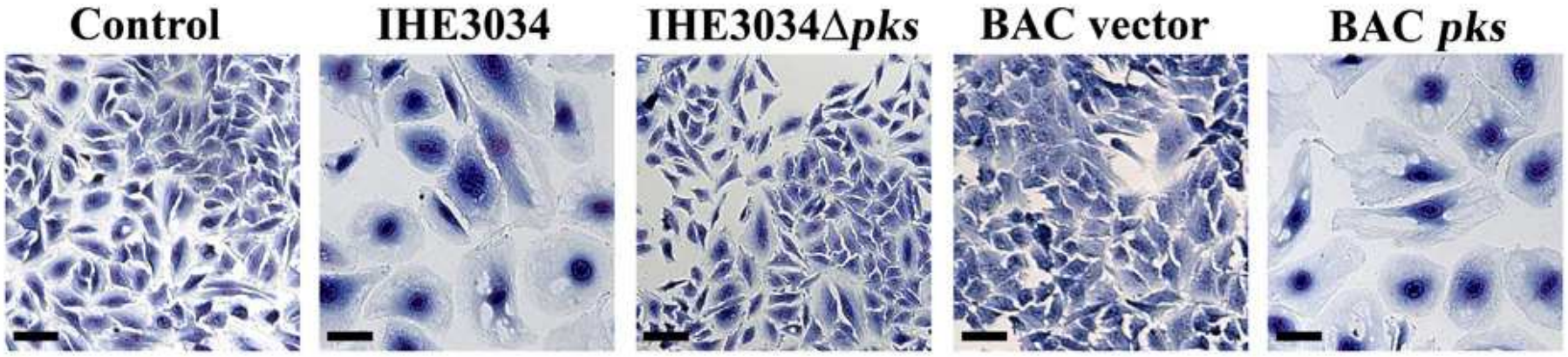
A 52 kb “pks” genomic island confers toxicity







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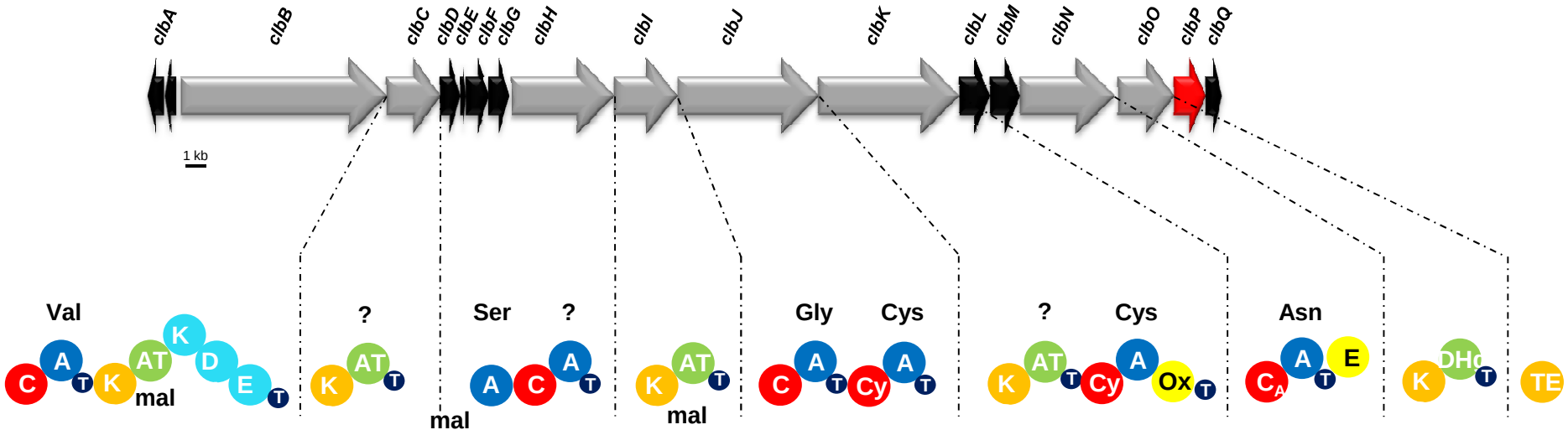
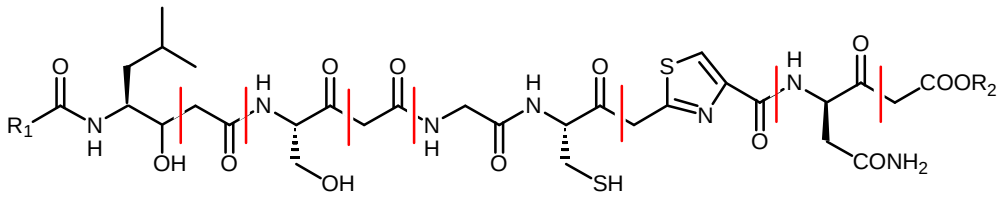
The pks gene cluster codes for synthesis of a peptide-polyketide metabolite



clbBCHIJKNO – Non-ribosomal peptide and polyketide synthases

clbA – PPTase
clbD – acyl-CoA dehydrogenase
clbE – ACP
clbF – short chain acyl-CoA dehydrogenase
clbG – malonyl-CoA transacylase
clbL – amidase
clbM – multidrug transporter
clpP – peptidase
clbQ – type 2 thioesterase



Colibactin *in silico* predicted

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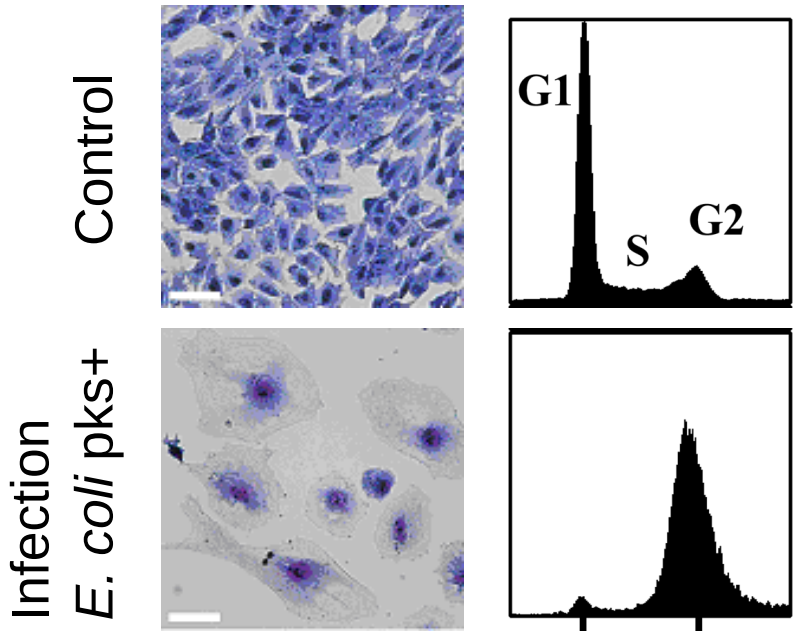
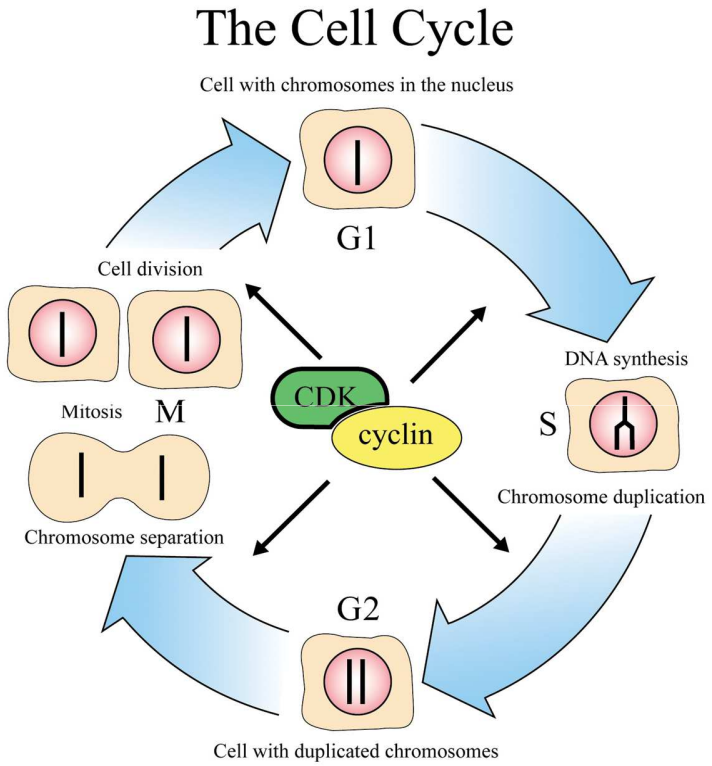
Infection with *E. coli* pks+ induces host cell cycle arrest



Cell cycle (G2) arrest



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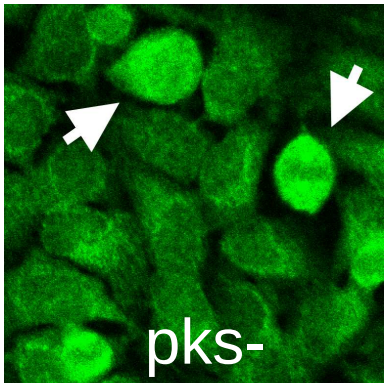
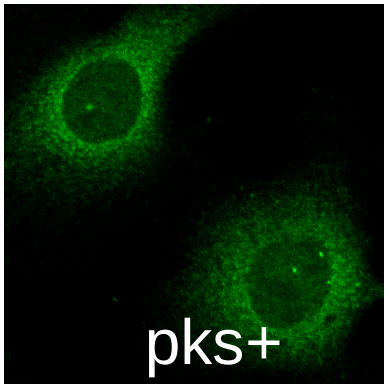
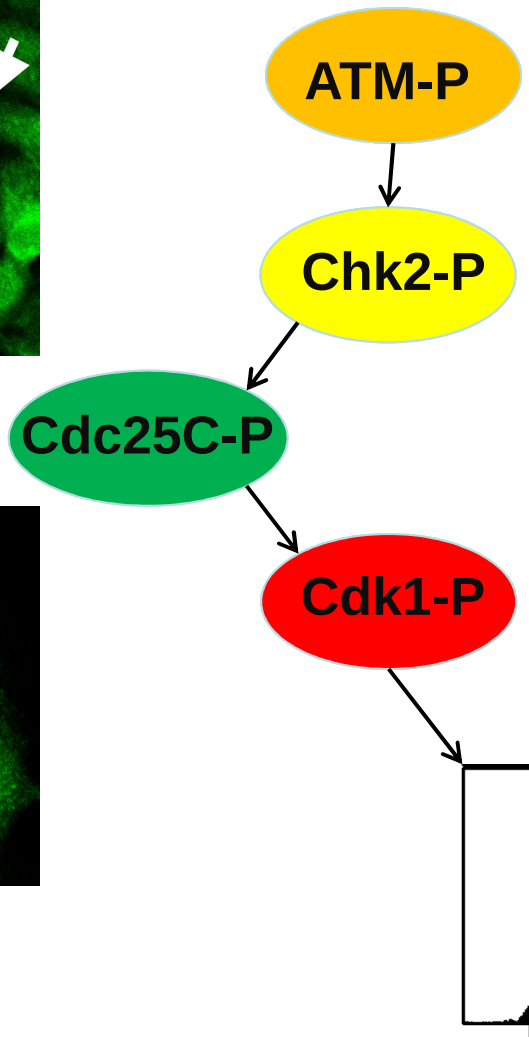
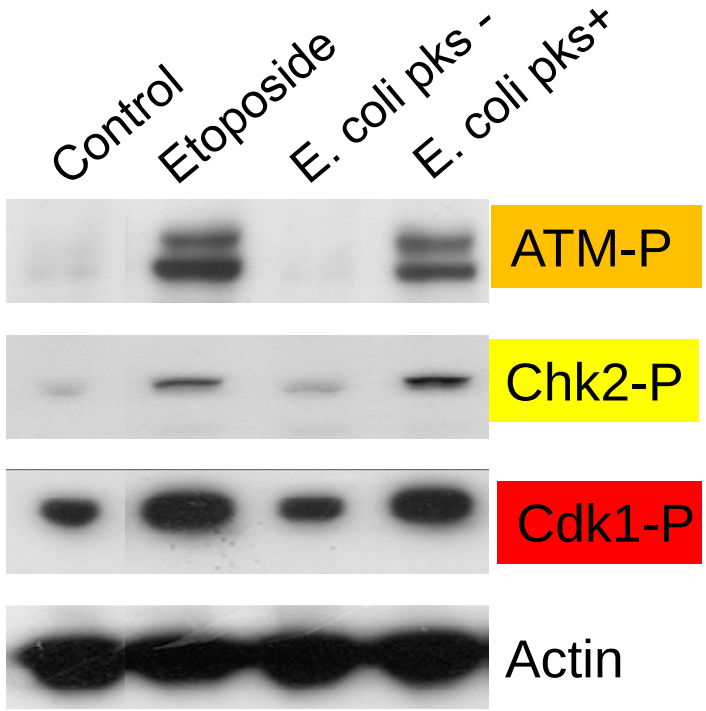
Recruitment of the G2-checkpoint in *E. coli* pks+ infected cells









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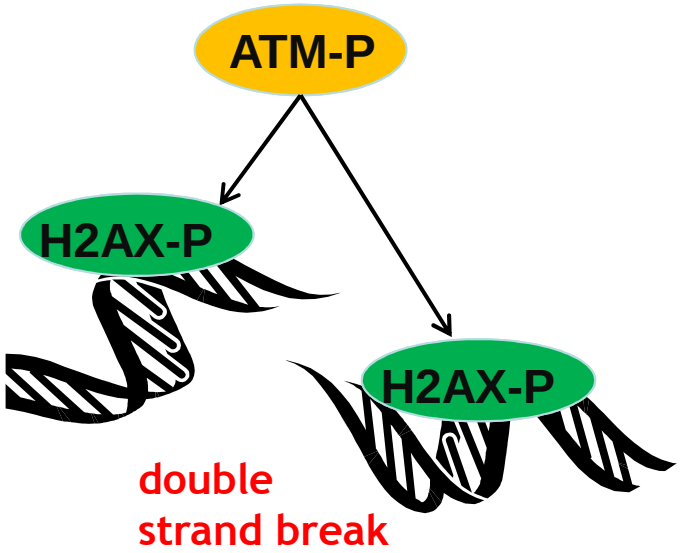
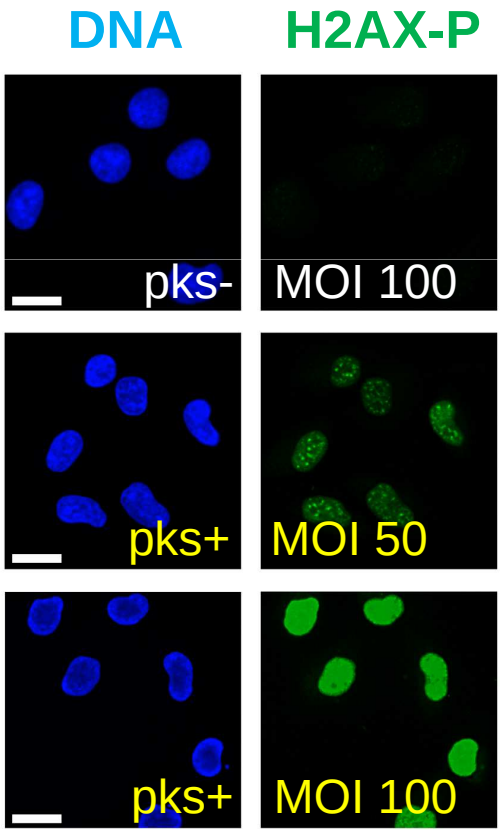
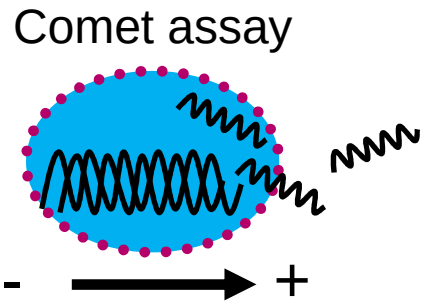
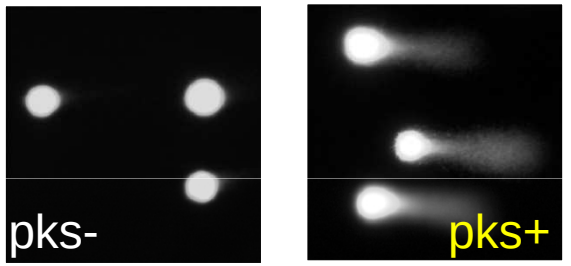
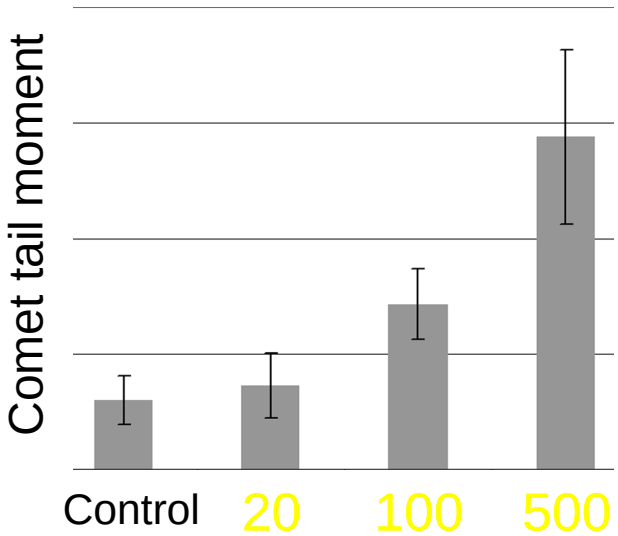
Infection with *E. coli* pks+ induces host DNA double strand breaks











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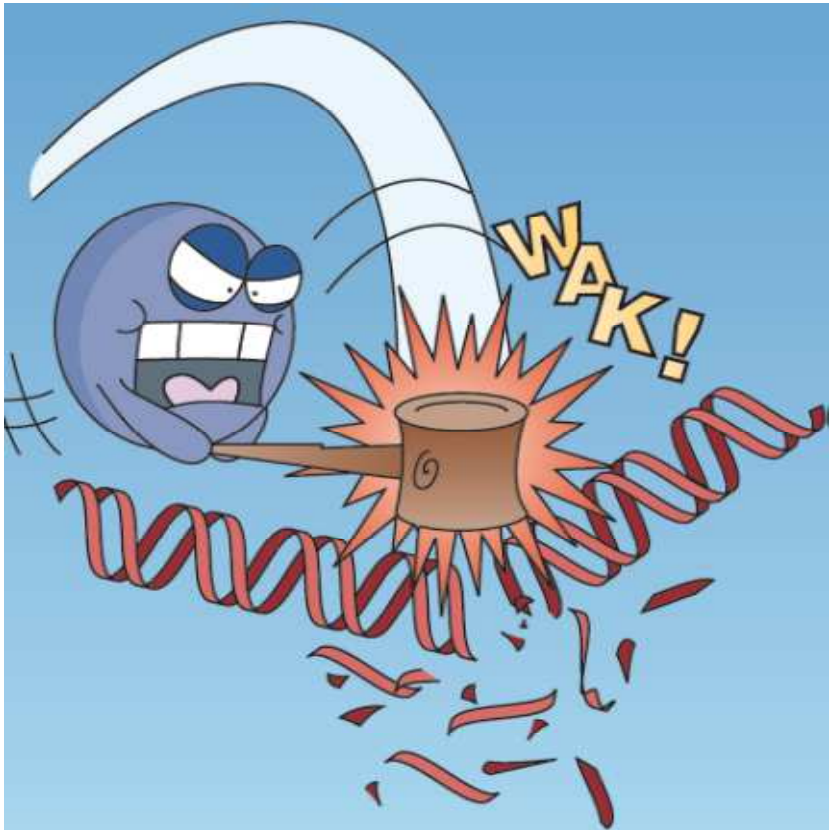
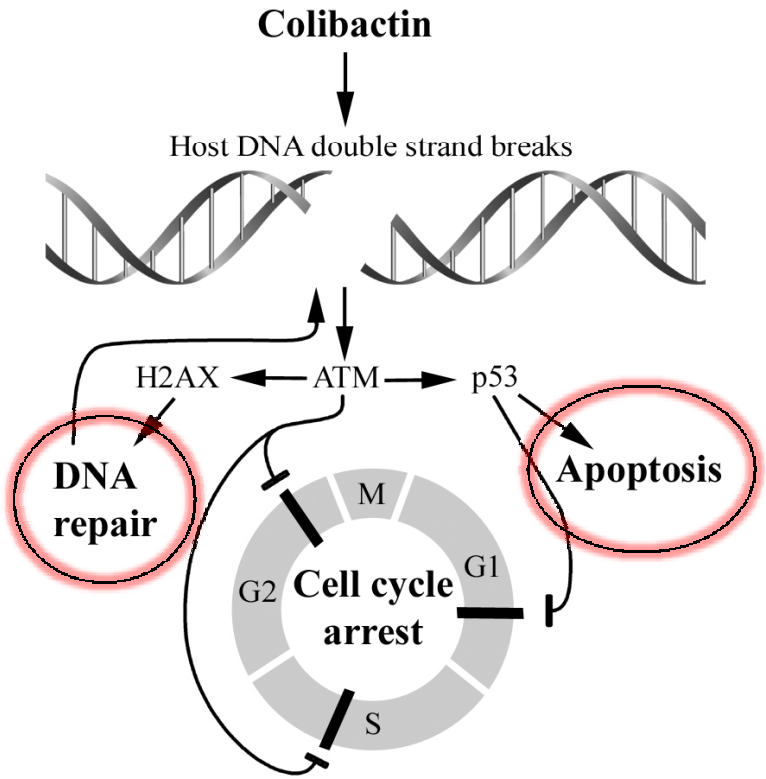


What impact on the host ?



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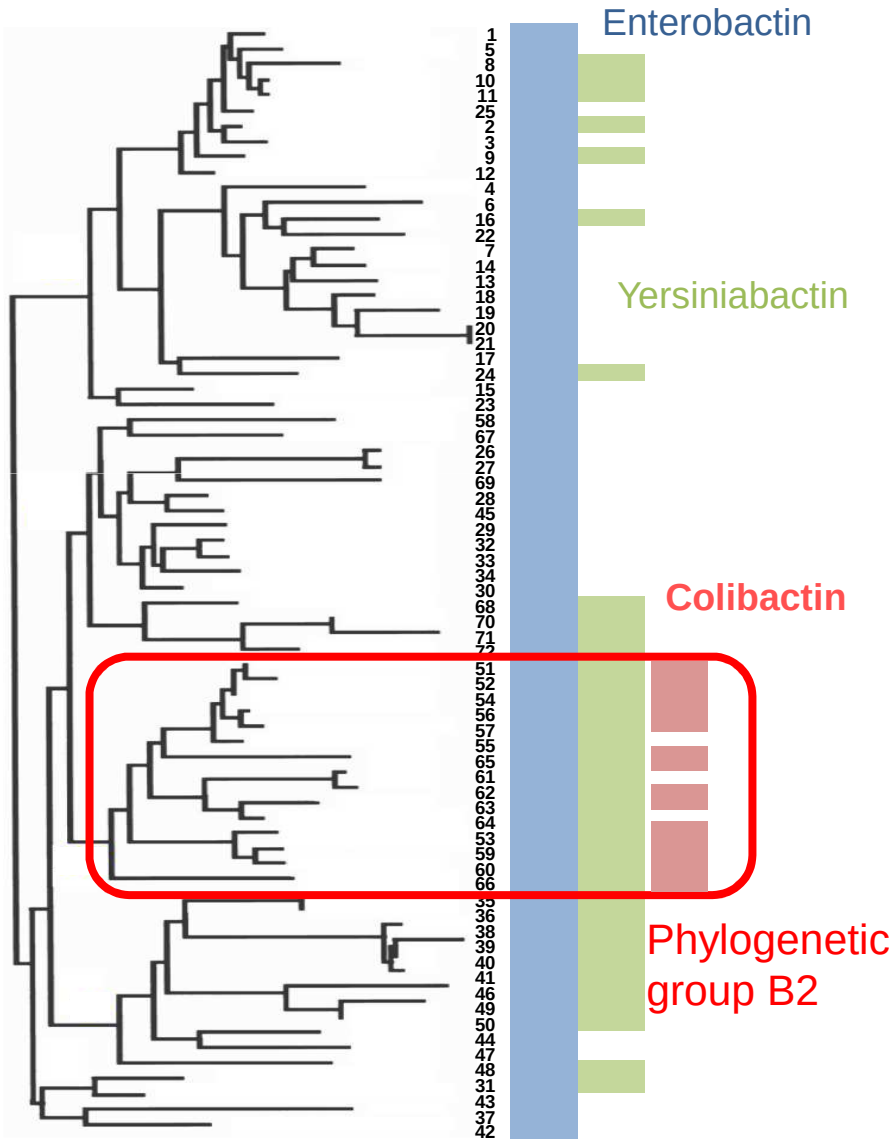
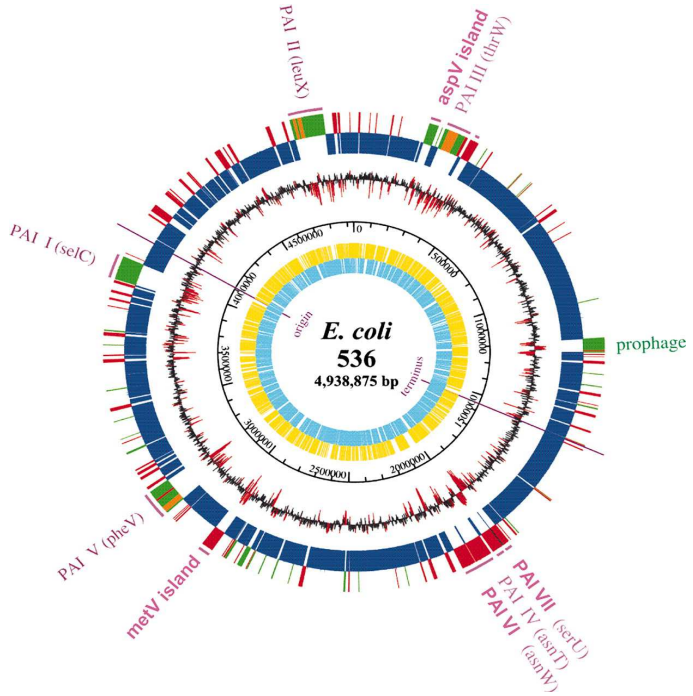
The pks island is frequent in extra-intestinal pathogenic *E. coli*



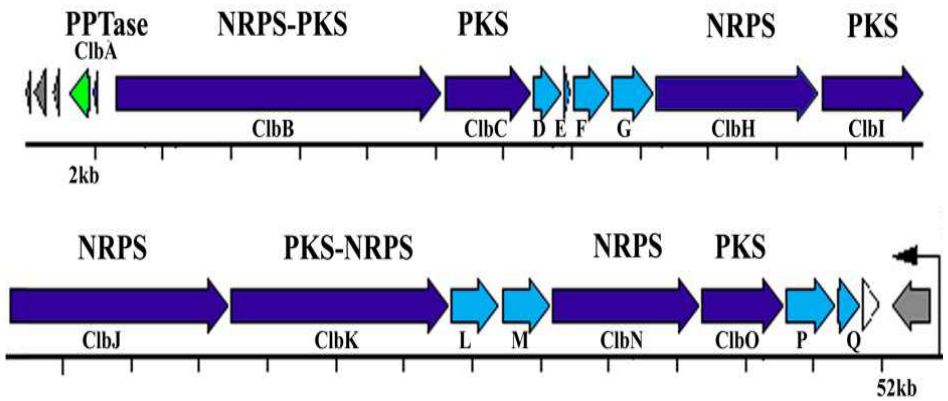
Martin et al, Plos Pathog 2013

ExPEC *pks*+

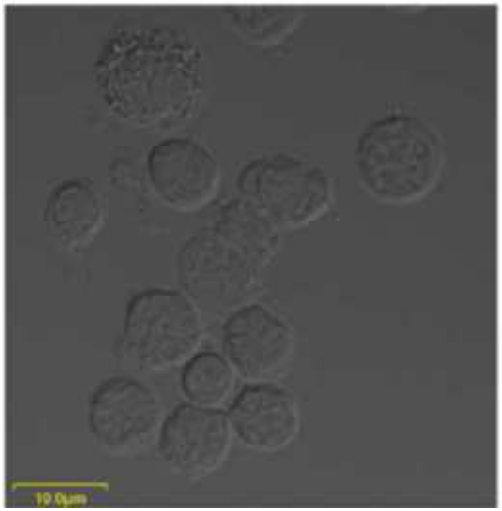
- Nougayrède et al. 2006 (n=97) → 53%
- Johnson et al. 2008 (n=62) → 58%
- Putze et al. 2009 (n=205) → 37%
- Dubois et al. 2010 (n=146) → 32%



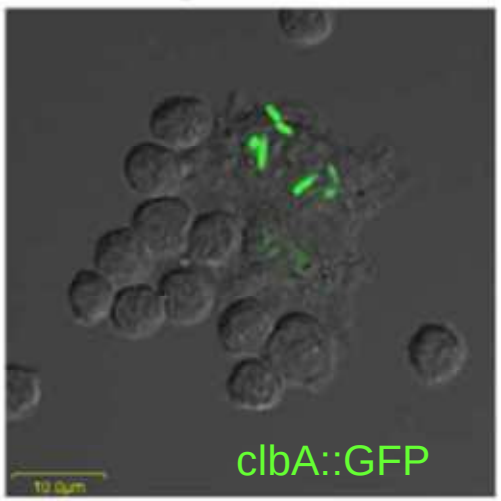
Colibactin expression in a mouse model of sepsis



PBS

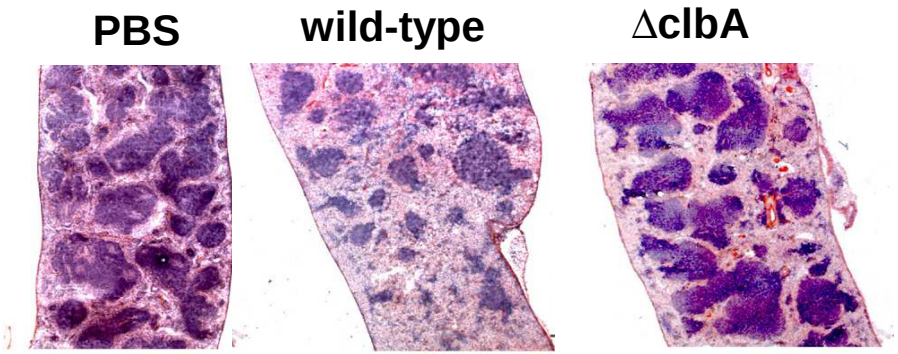


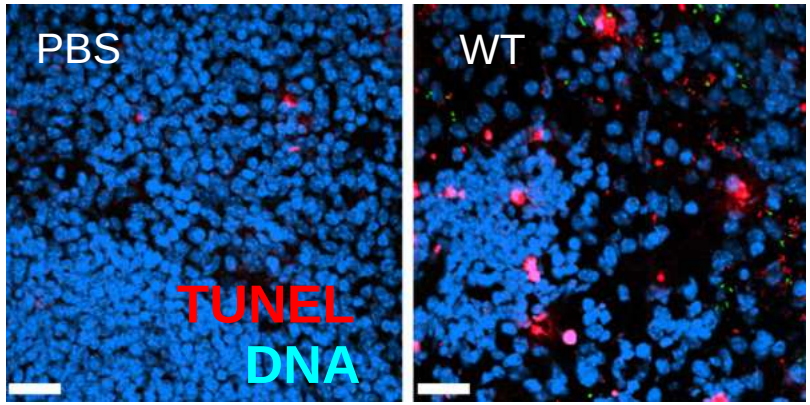
ExPEC *pks*+

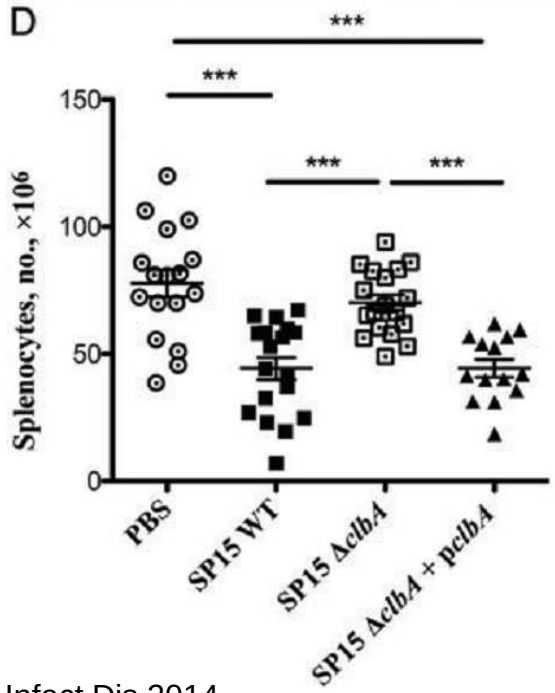


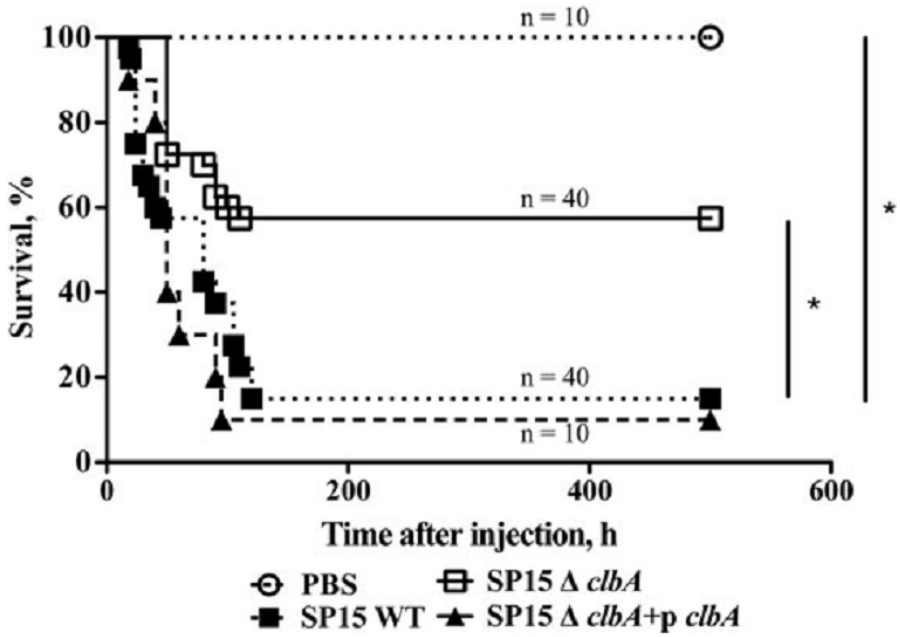
(p. 74-75). Toxicon, 116. Presented at 22nd Meeting of the French-Society-of-Toxinology (SFET), Paris, FRA (2014-12-10 - 2014-12-11). GBR : Elsevier Ltd.

Colibactin production during sepsis exacerbates lymphopenia and decreases mice survival rate









Marcq et al, J Infect Dis 2014

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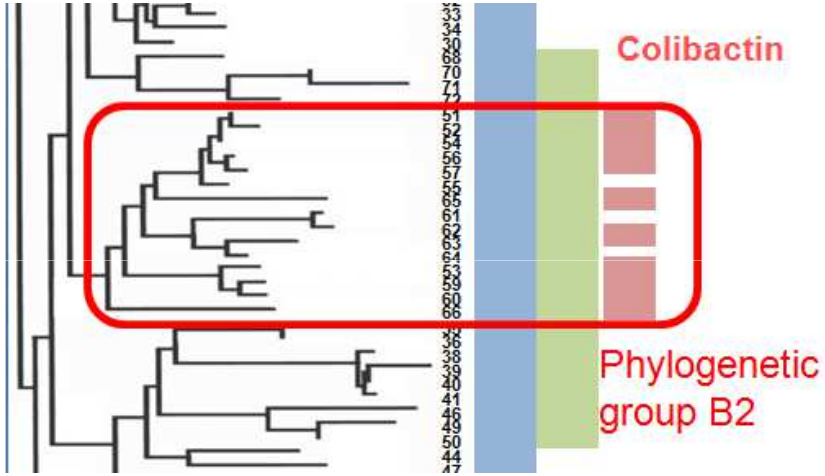
Colibactin is a virulence factor for *E. coli*…
… but the pks island is also frequently found in "commensal" isolates, in adults and infants



"Commensal" isolates *pks*+

Nougayrède et al. 2006 (n=32, B2) → 44%
Johson et al. 2008 (n=69, B2) → 32%
Unpublished (n=99) → 6%
Putze et al. 2009 (n=142) → 19.7%
Dubois et al. 2010 (n=51) → 12%

Payros et al. 2014 (n=184) → 27% of infants colonized with *E. coli* at 3 days of life (15% total)

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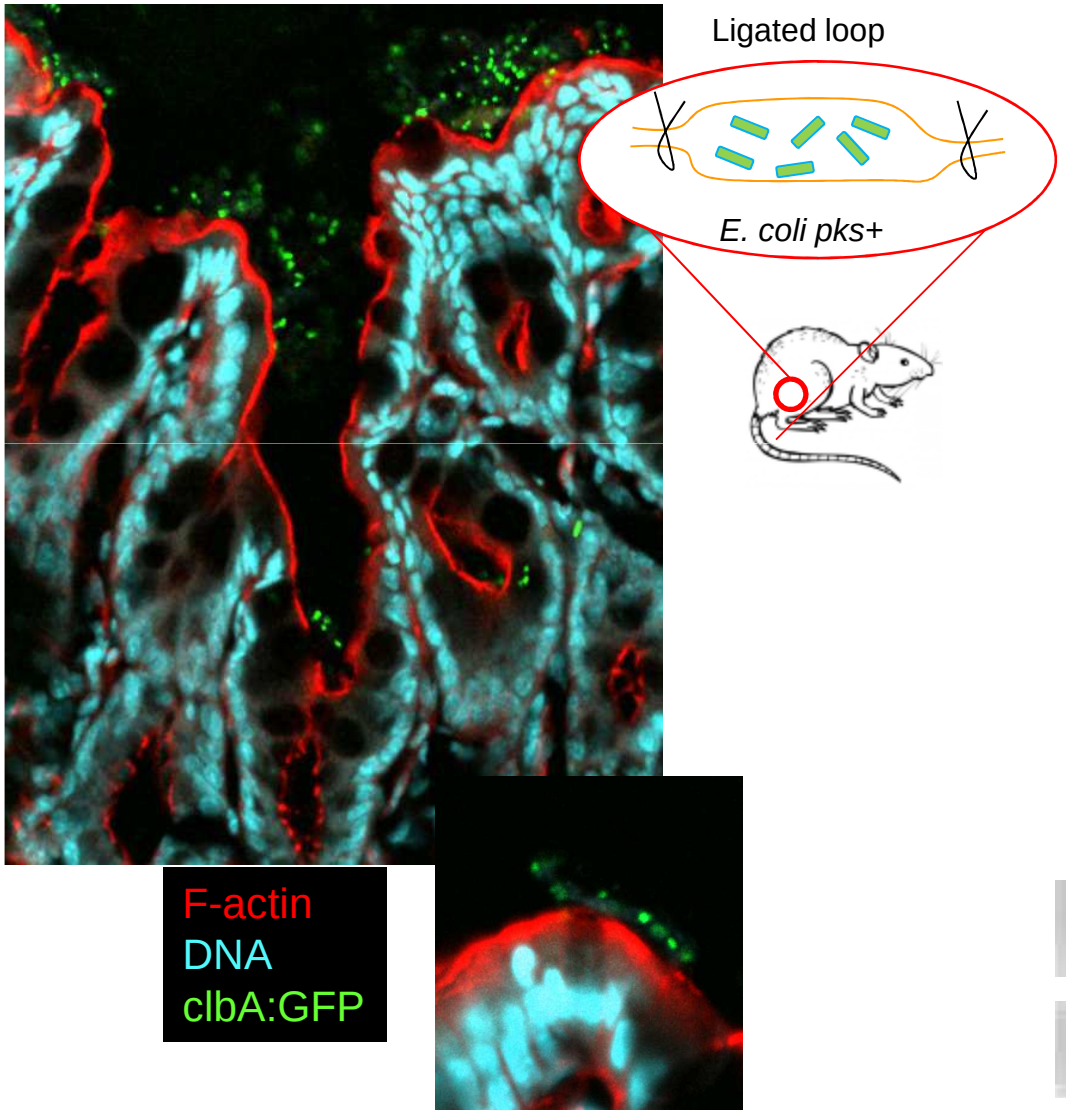
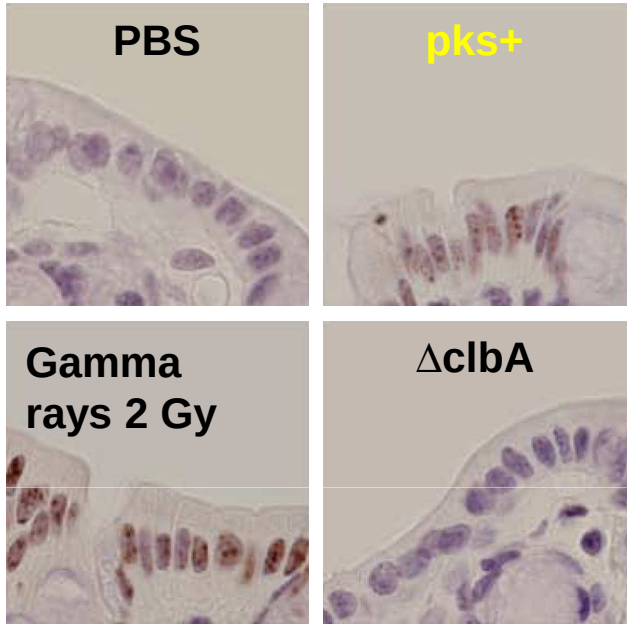
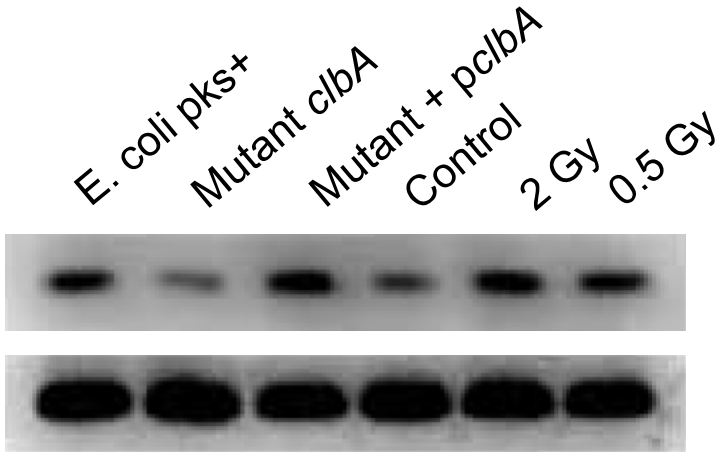
Colibactin is expressed in the lumen and induces DNA damage in enterocytes







Cuevas-Ramos et al, PNAS 2010

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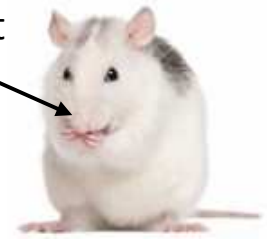
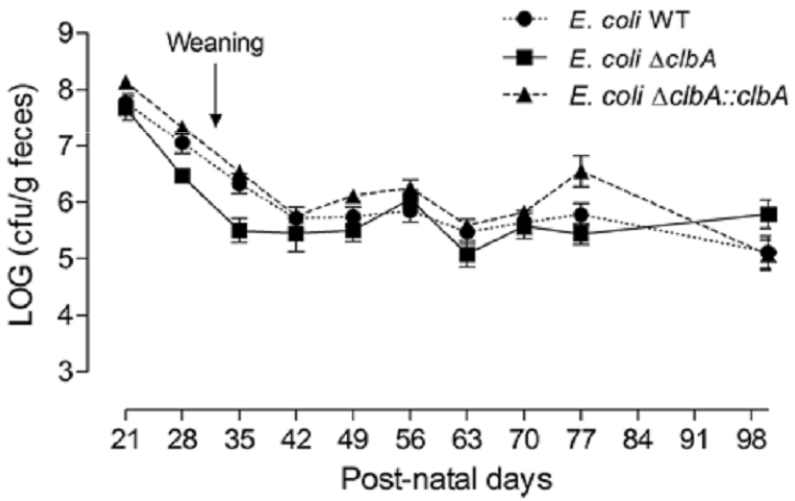
A model of “natural” vertical transfer of maternal *E. coli* to the progeny

Oral gavage of *pks+* or mutant *E. coli* strains



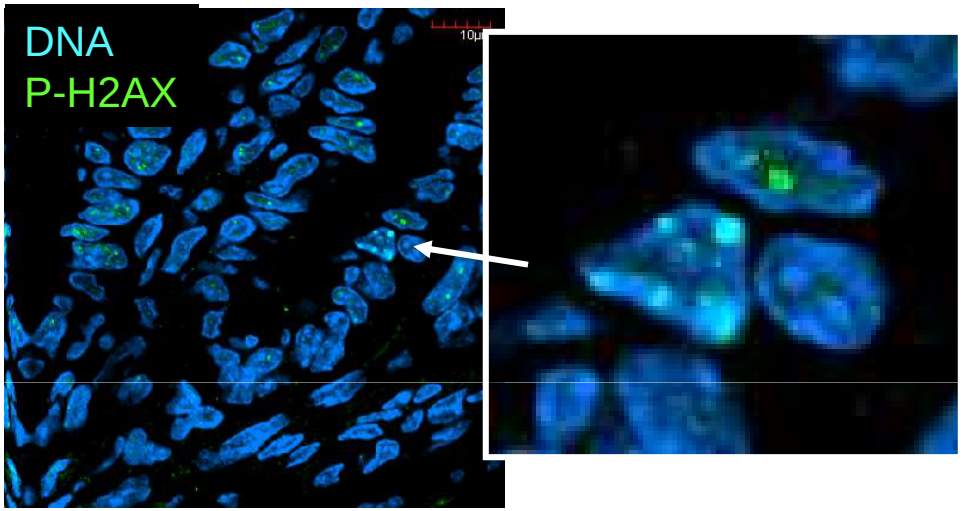
Birth

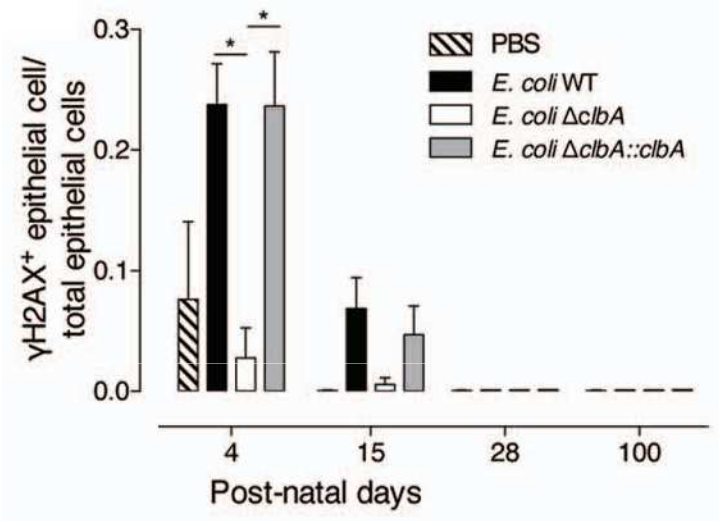
« Natural » vertical transfert of *E. coli* strains

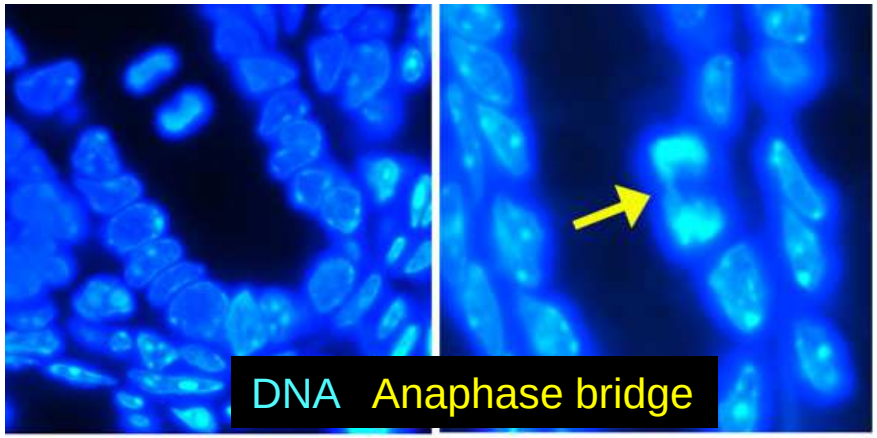


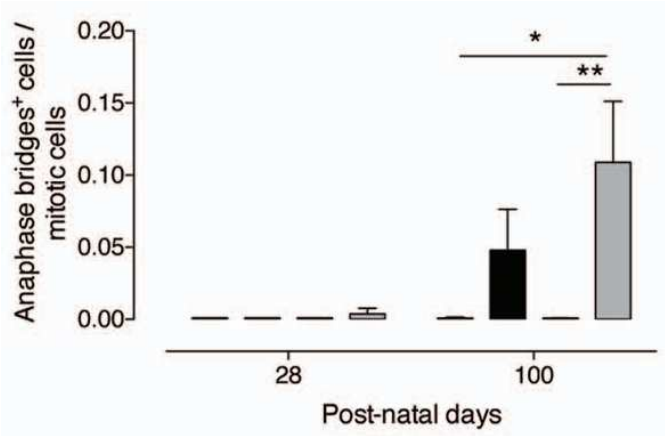
Payros et al, Gut Microbes, 2014

Transient DNA damage and chronic mitotic aberrations in enterocytes following perinatal colonization with a commensal pks+ *E. coli*









Payros et al, Gut Microbes, 2014

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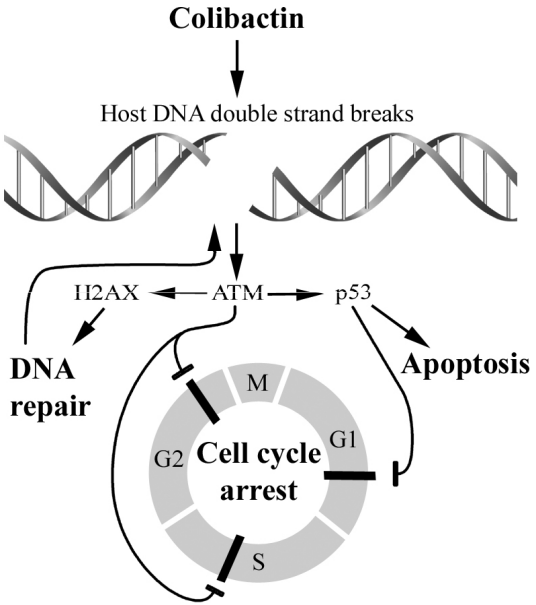
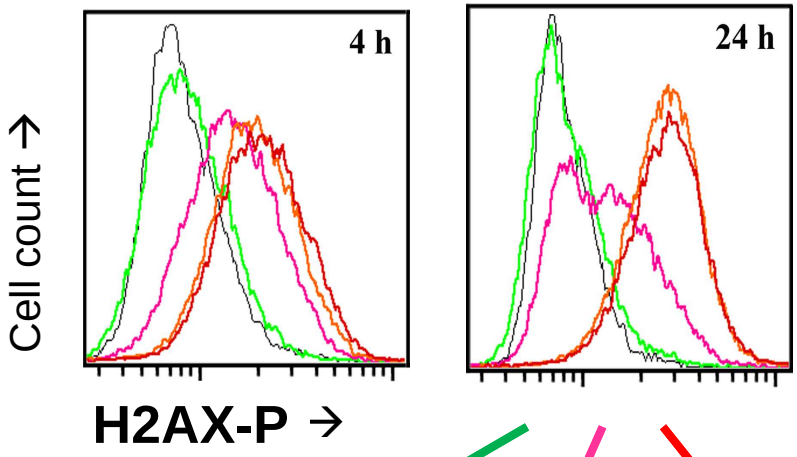
What are the cellular consequences of transient exposure and damage?

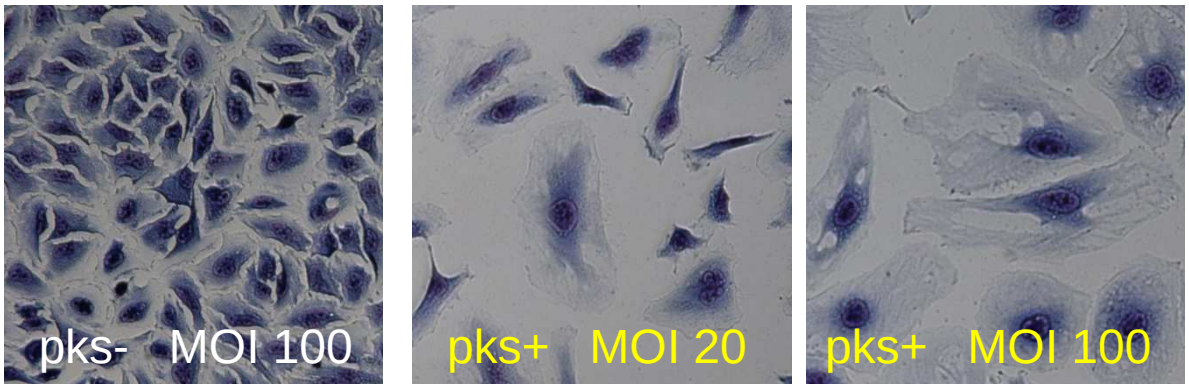




No damage

Moderate damage and repair

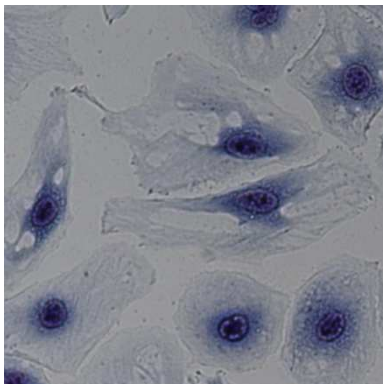
Irreversible damage



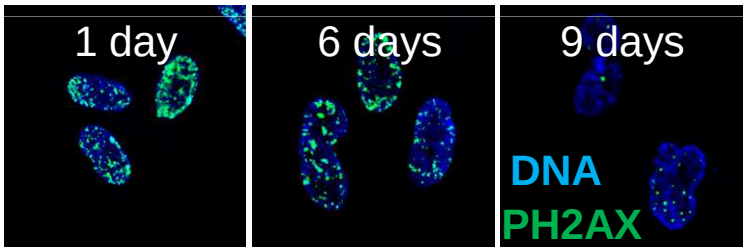
Nougayrede et al 2006
Cuevas-Ramos et al, PNAS 2010

(p. 74-75). Toxicon, 116. Presented at 22nd Meeting of the French-Society-of-Toxinology (SFET), Paris, FRA (2014-12-10 - 2014-12-11). GBR : Elsevier Ltd.

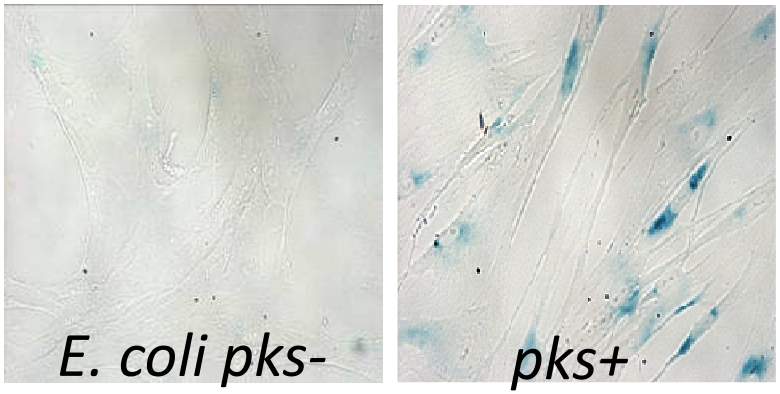
High dose induce cellular senescence and secretion of tumor growth factors

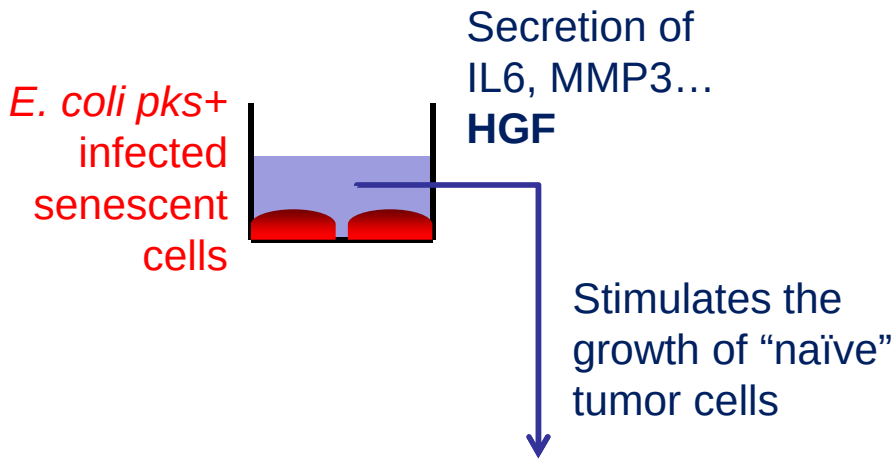


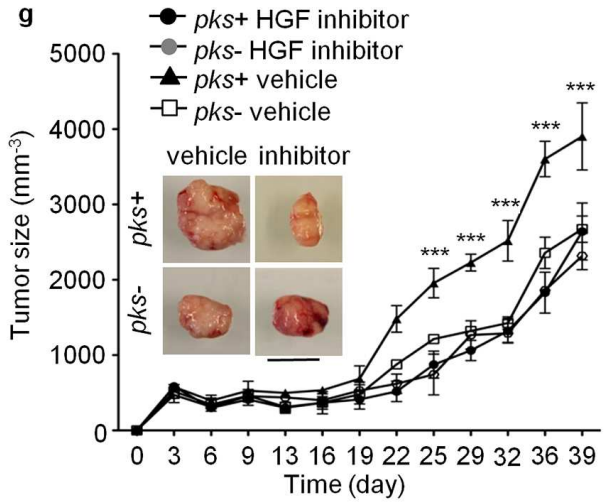
Persistant DNA damage signalling



Senescence-associated β-galactosidase



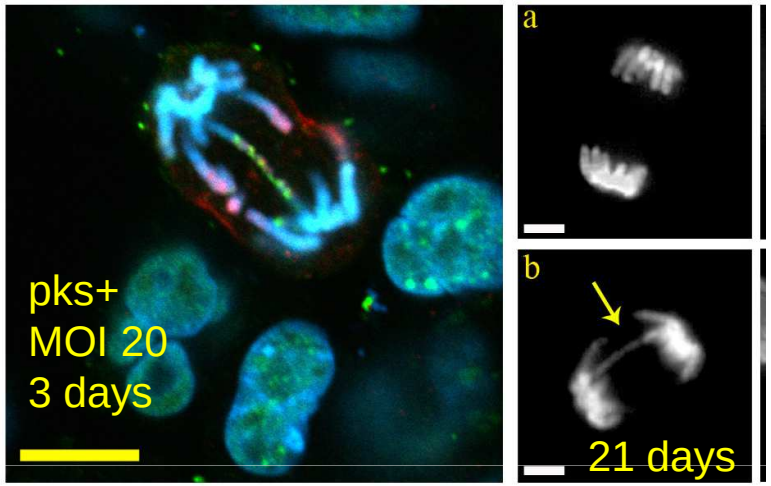


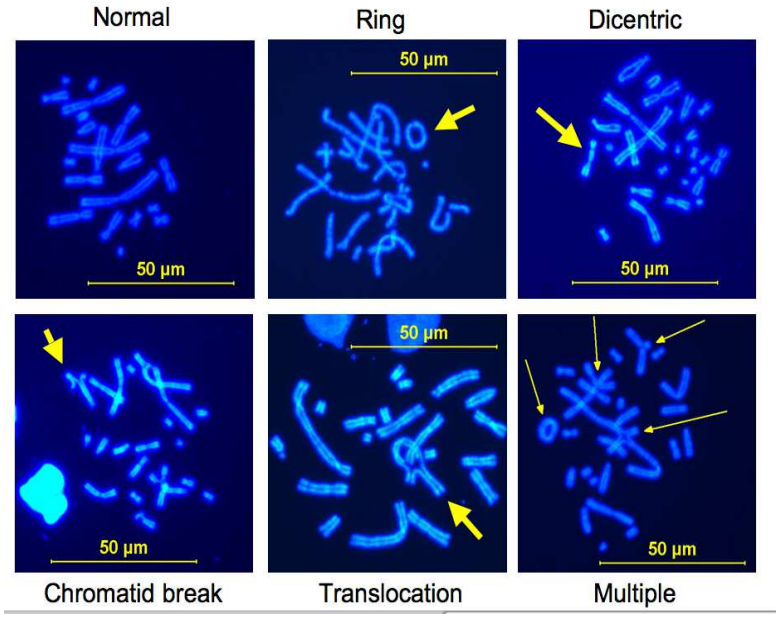


Secher et al, Plos One 2013
Cougnoux et al, Gut, 2014

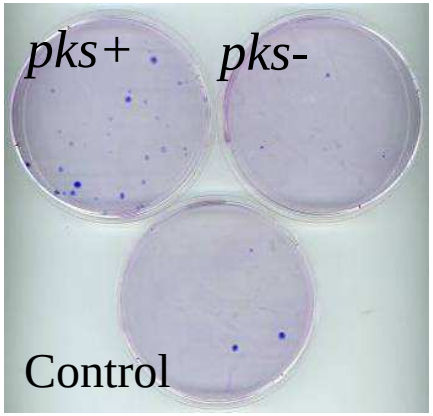
(p. 74-75). Toxicon, 116. Presented at 22nd Meeting of the French-Society-of-Toxinology (SFET), Paris, FRA (2014-12-10 - 2014-12-11). GBR : Elsevier Ltd.

Low dose may result in DNA misrepair, followed by chronic chromosomal aberrations and gene mutation





hprt mutants selected with 6-thioguanine
tk mutants selected with trifluorothymidine



Locus	Cells	Infection	MF ± SE × 10^{-5}
hprt	CHO	Control	1.68 ± 1.17
		E. coli pks–	2.89 ± 2.02
		E. coli pks$^+$	11.40 ± 1.16 *
		E. coli clbA	1.54 ± 1.11
		E. coli clbA + p*clbA*	11.80 ± 1.14*
tk	CHO	Control	31.7 ± 2.44
		E. coli pks–	29.1 ± 3.18
		E. coli pks$^+$	48.3 ± 2.02*
hprt	HCT-116	Control	1.52 ± 0.18
		E. coli pks–	1.52 ± 0.27
		E. coli pks$^+$	3.58 ± 0.20*

Cuevas-Ramos et al, PNAS 2010

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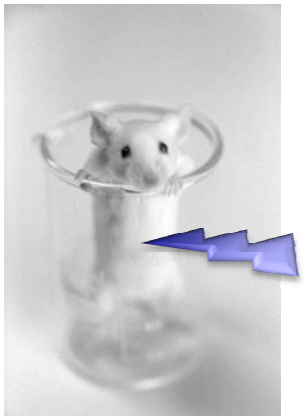
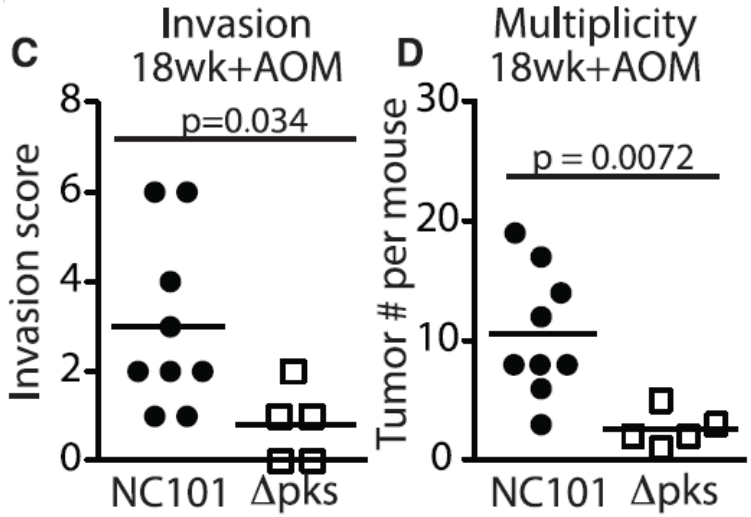
pks+ *E. coli* promote tumourigenesis in inflammatory colorectal cancer mouse models

IL10-/- mice monocolonized with pks+ NC101



6 weekly injection with AOM carcinogen during 18 weeks

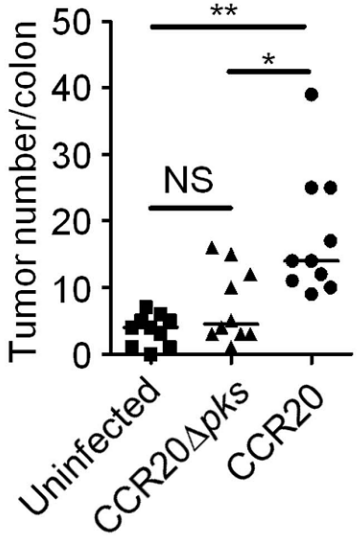
Arthur et al, Science 2012



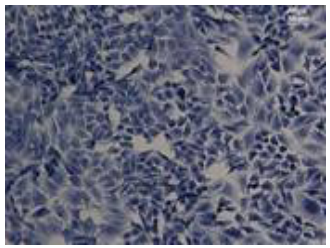
Streptomycin and gavage with pks+ CCR20

1 injection with AOM carcinogen + 2 cycle of DSS

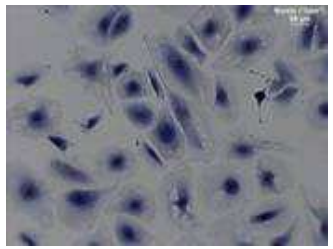
Cougnoux et al, Gut, 2014



The pks island is found in *E. coli* probiotic strain Nissle 1917!

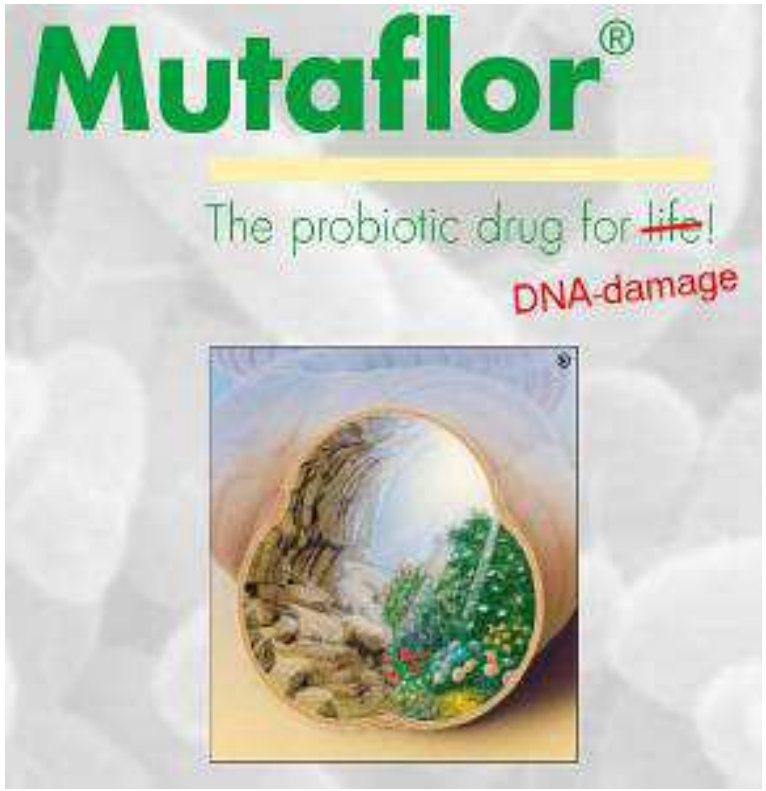


Control



Nissle 1917

« **Mutaflor** is a microbial drug containing live *E. coli* strain **Nissle 1917**. It is the first probiotic drug for which efficacy in maintaining remission of ulcerative colitis was proven by a confirmative clinical study ».



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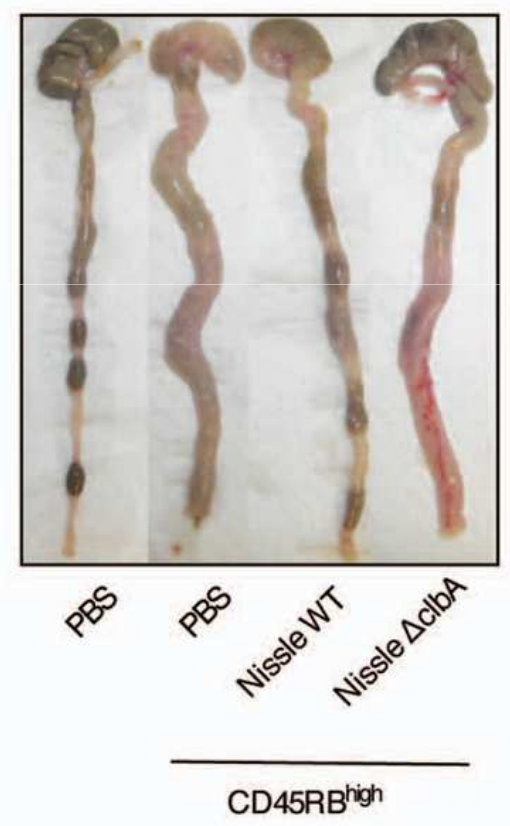
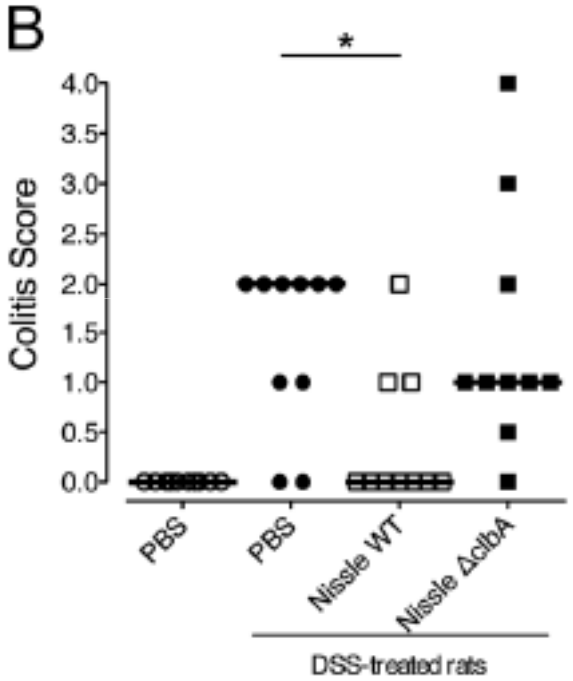
A non-genotoxic Nissle 1917 mutant is impaired for probiotic activity





Olier et al, Gut Microbes 2012

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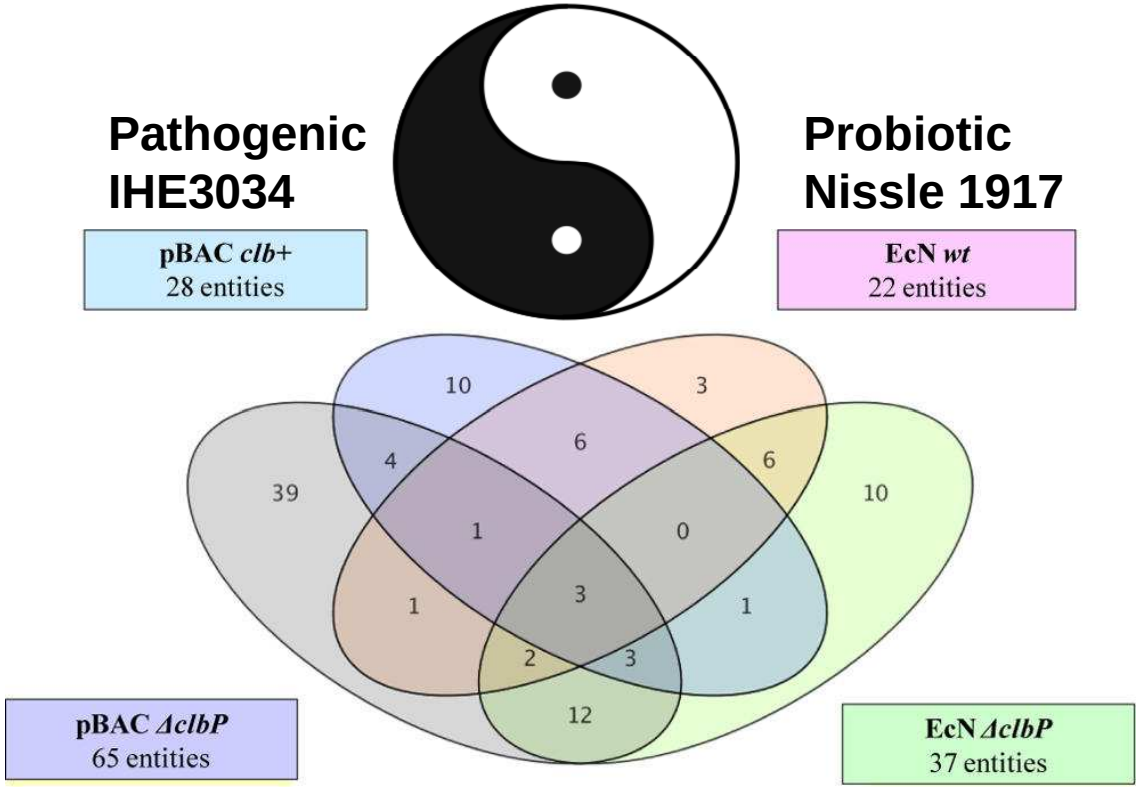
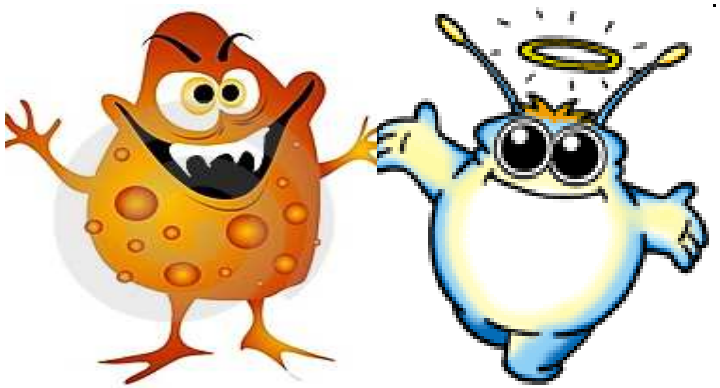
"Colibactin" = a mixture of molecules with various activities ?



Detected Clb-pathway dependant molecules

A number of molecules are specific to each strain, suggesting that "colibactin" represents a diverse catalog of molecules with various activities that could contribute collectively to different phenotypes

J. Am. Chem. Soc., June 2014, 136 (26), pp 9244–9247



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