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Survival of digested DNA in the gut and the potential of genetic transformation of resident bacteria

FSA Project Code FSG 01007, 1.6.1998 - 1.10. 2001, Report
Flintetal.pdf

Three significant sentences from the summary:

The survival of DNA in the human mouth in vivo was only 6 seconds, and the con

We did not detect transformation in vitro using linear DNA that possessed only a single region of matching sequence, which is, arguably, the most likely state of GM DNA in food.

We did however detect transformation of genes that were flanked on both sides by sequences that match the bacterial chromosome.

Table 1. Effect of saliva and food supplements on degradation of pVACMC1 plasmid DNA

Food Type	DNA Concentration (ng/μl)¹		DNA Degradation
	t = 0 sec	t = 10 sec	(% of t = 0 sample remaining after 10 sec)
Saliva alone	6.3	3.3	52
Skimmed Milk	4.6	1.3	71
Semi-skimmed Milk	0.9	0.2	74
Full Fat Milk	3.5	1.5	57
Powdered Milk	2.5	3.8	152
Low Fat Yoghurt	NE ²	0.5	-
Biological Yoghurt	2.5	NE ²	-
Egg	NE ²	NE ²	-
Olive Oil	NE ²	NE ²	-

¹ All results are means of triplicate competitive PCR's² NE = No extraction of DNA possible

Figure 1 - Fate of Free pVACMC1 DNA in the Human Mouth

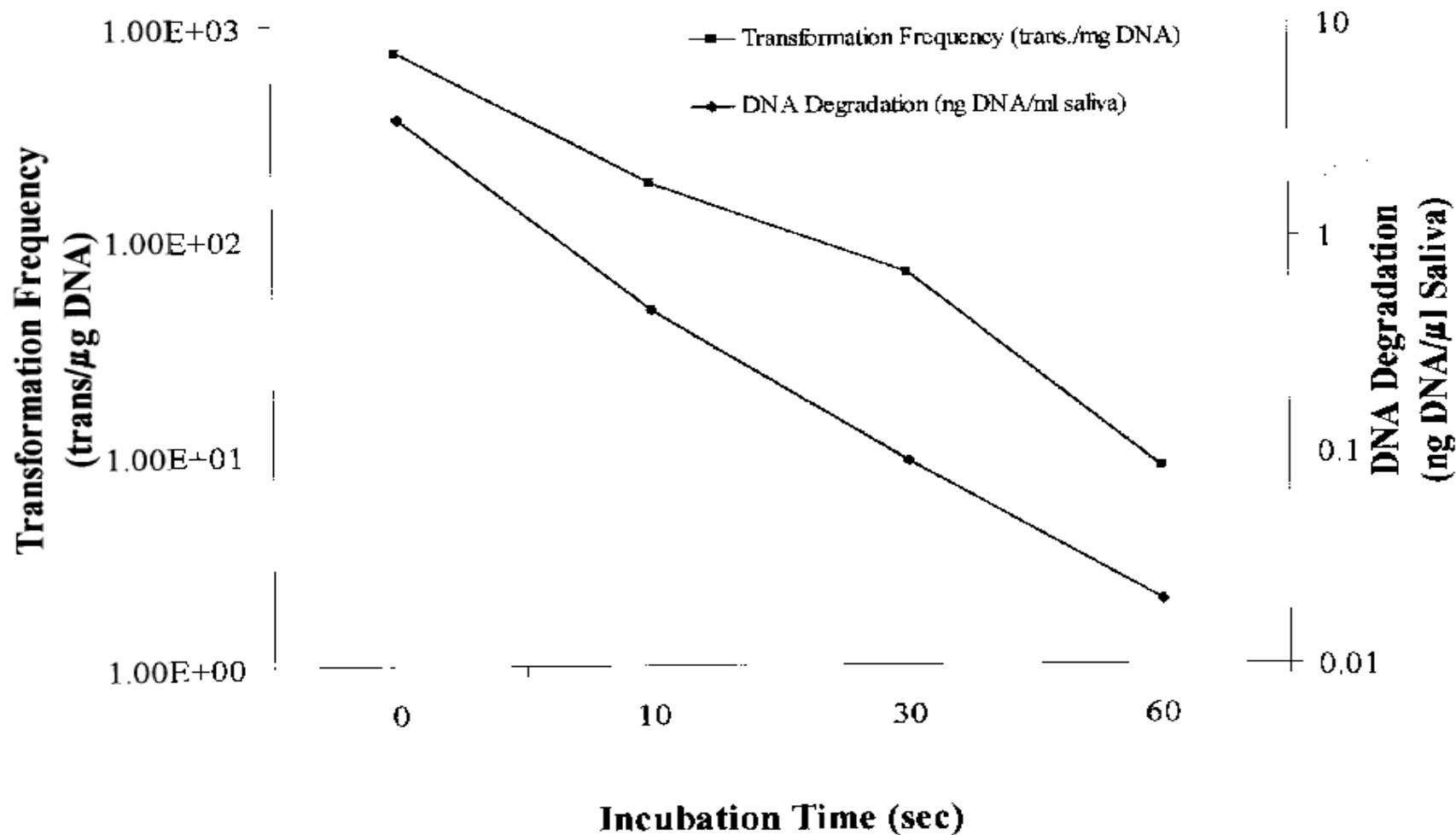


Figure 2 - Proposed Mechanism of Chromosomal Integration using Plasmid DNA

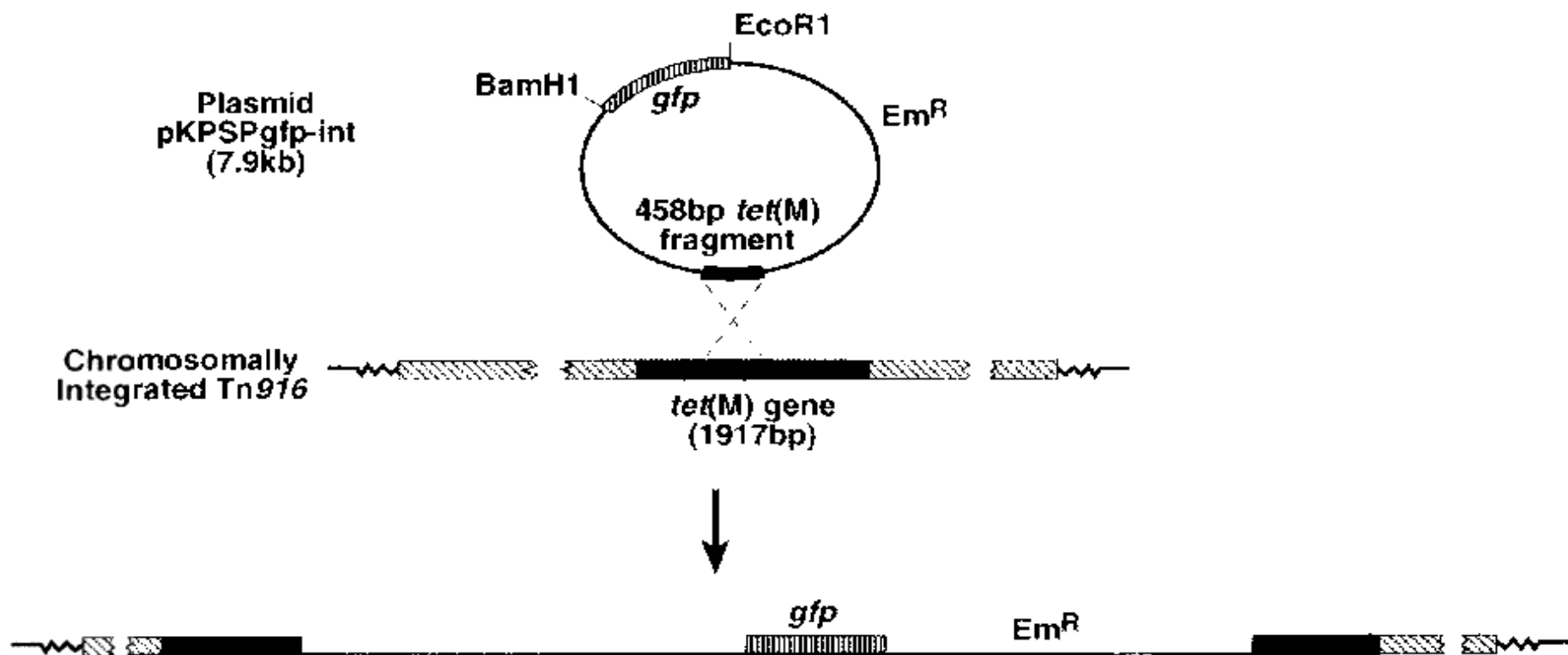


Table 2 - Comparison of Transformation Efficiencies for Different DNA Types

Transforming DNA	Transformation Efficiency (tr./parent cell)
pVACMC1	2.9×10^{-4}
pKPSPgfp-int	1.2×10^{-2}
<i>E. faecalis</i> /Tn916/gfp	5.1×10^{-5}
Chromosomal DNA	

Figure 3 - Proposed Mechanism of Chromosomal Integration using Chromosomal Transforming DNA

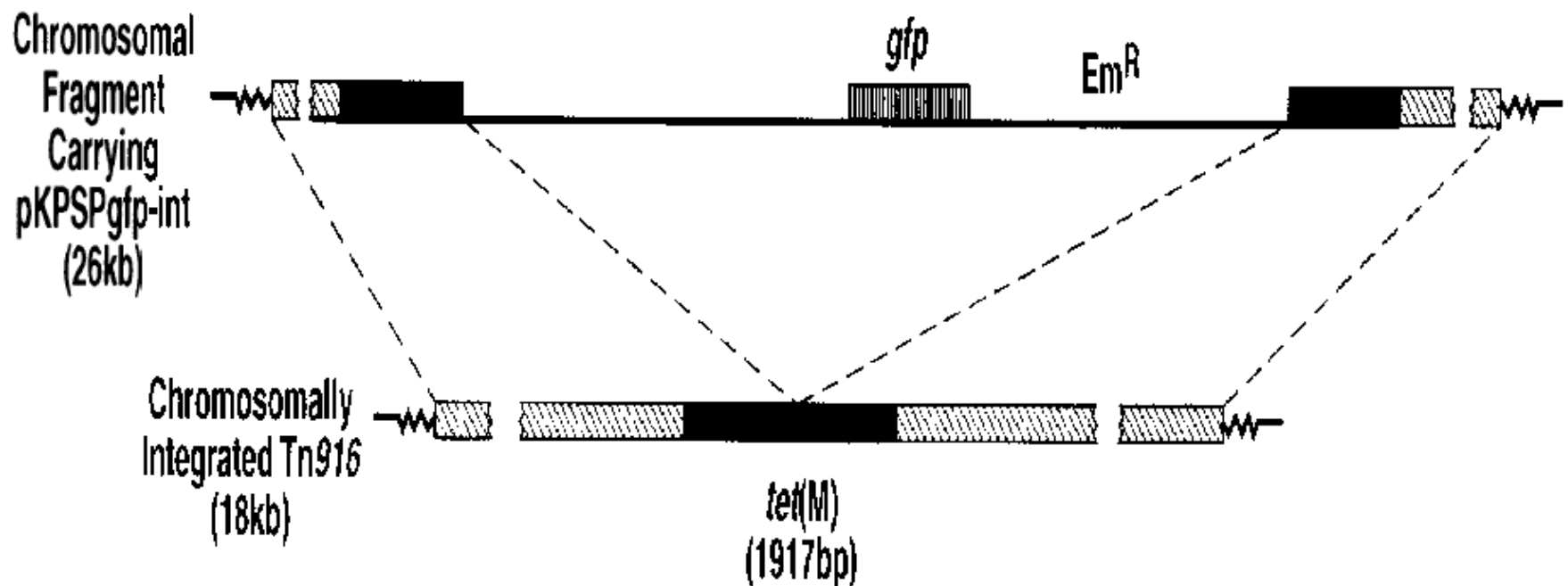
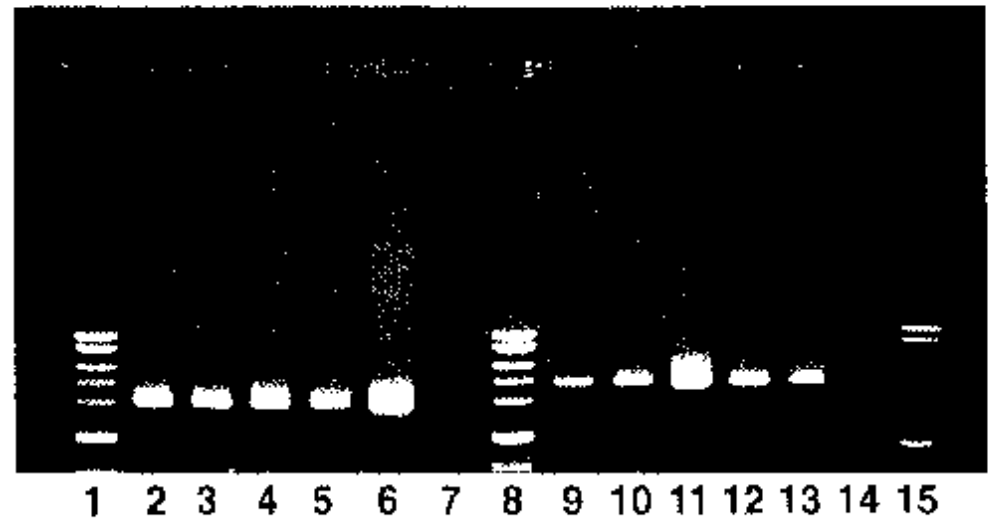


Table 3 - Effect of Restriction Digestion on Transformation

Transforming DNA	Transformation Efficiency (tr./parent cell)
pKPSPgfp-int	2×10^{-4}
pKPSPgfp-int/ <i>EcoRI</i> (or <i>EcoRV</i> or <i>BamH1</i>)	0
<i>L. lactis</i> /Tn916/ <i>gfp</i> chromosomal DNA	4×10^{-5}
<i>L. lactis</i> /Tn916/ <i>gfp</i> chromosomal DNA/ <i>NotI</i>	0

**Figure 4 - PCR
Analysis of the
Integration of
pKPSPgfp-int
into the
Chromosome of
S. gordonii
DL1/Tn916**

(a)



(b)

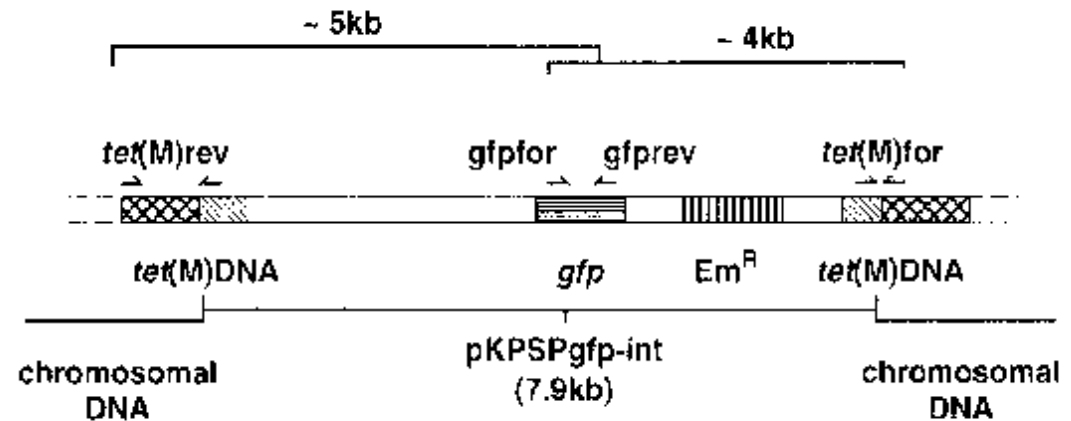


Figure 5 - Sequence Homology Between *tet(M)*, *tet(O)* [76.9% homology] and *tet(W)* [69.7% homology]

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tetM      GCTCATGTTGATGCGGGAAAACTACCTTAACAGAAAGCTTATTTATATAACAGTGGAGCG 60
tetO      GCTCACGTTGACCGCAGGAAAGACAACATTAACGGAGAGTTTATTGTATACCAGTGGTGCA 60
tetW      GCCCATGTAGACGCTGGAAAGACGACCTTGACGGAGAGCCTGCTATATGCCAGCGGAGCC 60
          ** ** * ** * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
tetM      ATTACAGAATTAGGAAGCGTGGACAGAGGTYACAACGAAAACGGATAAATACGCTTTTAGAA 120
tetO      ATTGCAGAACCAGGGAGCGTAGATAAAGGCACAACAAGGACAGATACAATGAATTTGGAG 120
tetW      ATTTCAGAACCAGGGAGCGTCAAAAAGGGACAACGAGGACGGACACCATGTTTTTTGGAG 120
          *** ***** ** * ** * * * * * * * * * * * * * * * * * * * * *
tetM      CGTCAGAGAGGAATTACAATTCAGACGGCGATAACCTCTTTTCAGTGGAA-AAATACTAA 179
tetO      CGTCAAAGGGCAATCACTATCCAGACAGCAGTGCATCTTTTCAGTGGGA-GGATGTAAA 179
tetW      CCGCAGCGTGGGATTACCATTCAGCGGCAAGTCACTTCCCTCCAGTGGCACAGATG-TAA 179
          ** ** * ** * * * * * * * * * * * * * * * * * * * * * * *
tetM      GGTGAACATCATAGACACGCCAGGACATATGGATTTTTTAGCAGAAGTATATCGTTCATT 239
tetO      AGTCAACATTATAGATACGCCAGGCCATATGGATTTTTTGGCGGAAGTATACCGTTCATT 239
tetW      AGTTAACATTGTGGATACGCCCGGCCACATGGATTTTTTGGCGGAGGTGTACCGCTCTT 239
          ** ***** * ** ***** ** * * * * * * * * * * * * * * * * *
tetM      ATCAGTATTAGATCGGGCAATTCTACTGATTTCTGCAAAAGATGGCGTACAAGCACAAAC 299
tetO      ATCCGTATTAGACGGAGCAGTATTATTAGTTTCTGCAAAGGATGGCATAACAGCCACAGAC 299
tetW      GGCTGTTTTAGATCGGGCCATCTTGGTGTCTCCGCTAAAGATGGCGTGCAGGCCAGAC 299
          * ** ***** ** * * * * * * * * * * * * * * * * * * * * *
tetM      TCGTATATGTTTTCATGCACCTTAGGAAAATAGGTATTCCACAACTTTTTTTTATCAATAA 359
tetO      CCGTATACTGTTTTCATGCACCTACAGACAATGAAGATTCCGACAATTTTTTTTATCAATAA 359
tetW      CCGTATTCGTTCATGCCCTGCGGAAAATGAACATTCCACACCCTATCTTTATCAACAA 359
          ***** ** * * * * * * * * * * * * * * * * * * * * *
tetM      GATTGACCAAATGGAATTGATTTATCAACGGTTTATCAGGATATTAAAGAGAAACTTTC 419
tetO      AATTGACCAAGAGGGGATTGATTTGCCAATGUTATATCGAGAAATGAAGCAAAGCTTTC 419
tetW      GATCGACCAGGCTGGCGTTGATTTCCAGAGCGTGGTTCAGTCTGTTCCGGGATAACCTCTC 419
          ** ***** ** * * * * * * * * * * * * * * * * * * * * *
tetM      TCGGAAAATTGTAATCAAACAGAAAGGTAGAACTGCATC 457
tetO      TTCGAAAATTATAGTGAAGCAAAGGTTGGGCAGCAT- 456
tetW      CGCCGATATTATCATCAAGCAGACGGTGTCCCTGTCC 457
          * ** * * * * * * * * * * * * * * * * *

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Figure 6: Effect of simulated gut contents on transformation of competent cells of *S. gordonii* DL1/Tn916 with pKPSPgfp-int

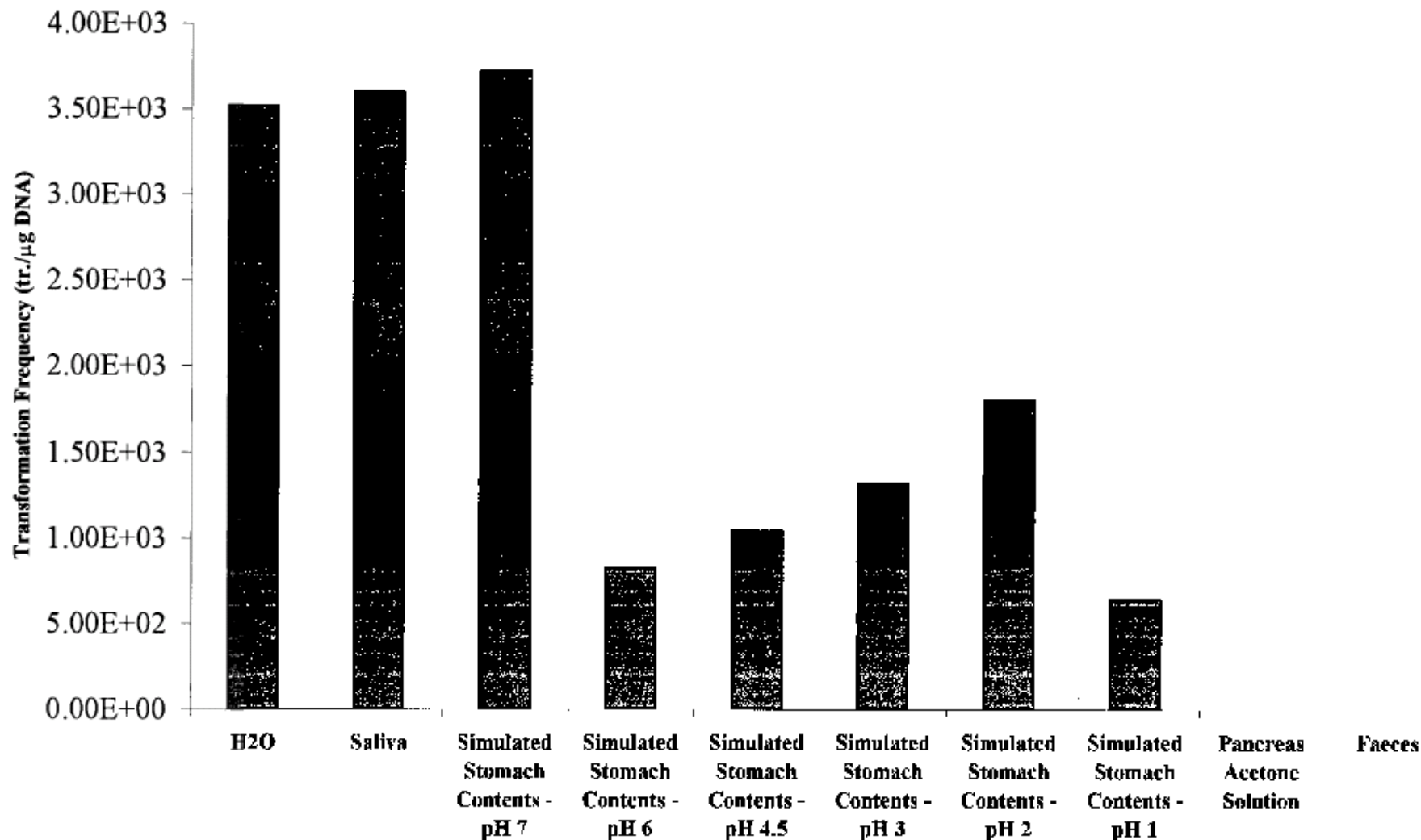


Figure 7. Competence development and transformation of *S. bovis* NCTC11436 with pKPSPgfp-int during exponential growth

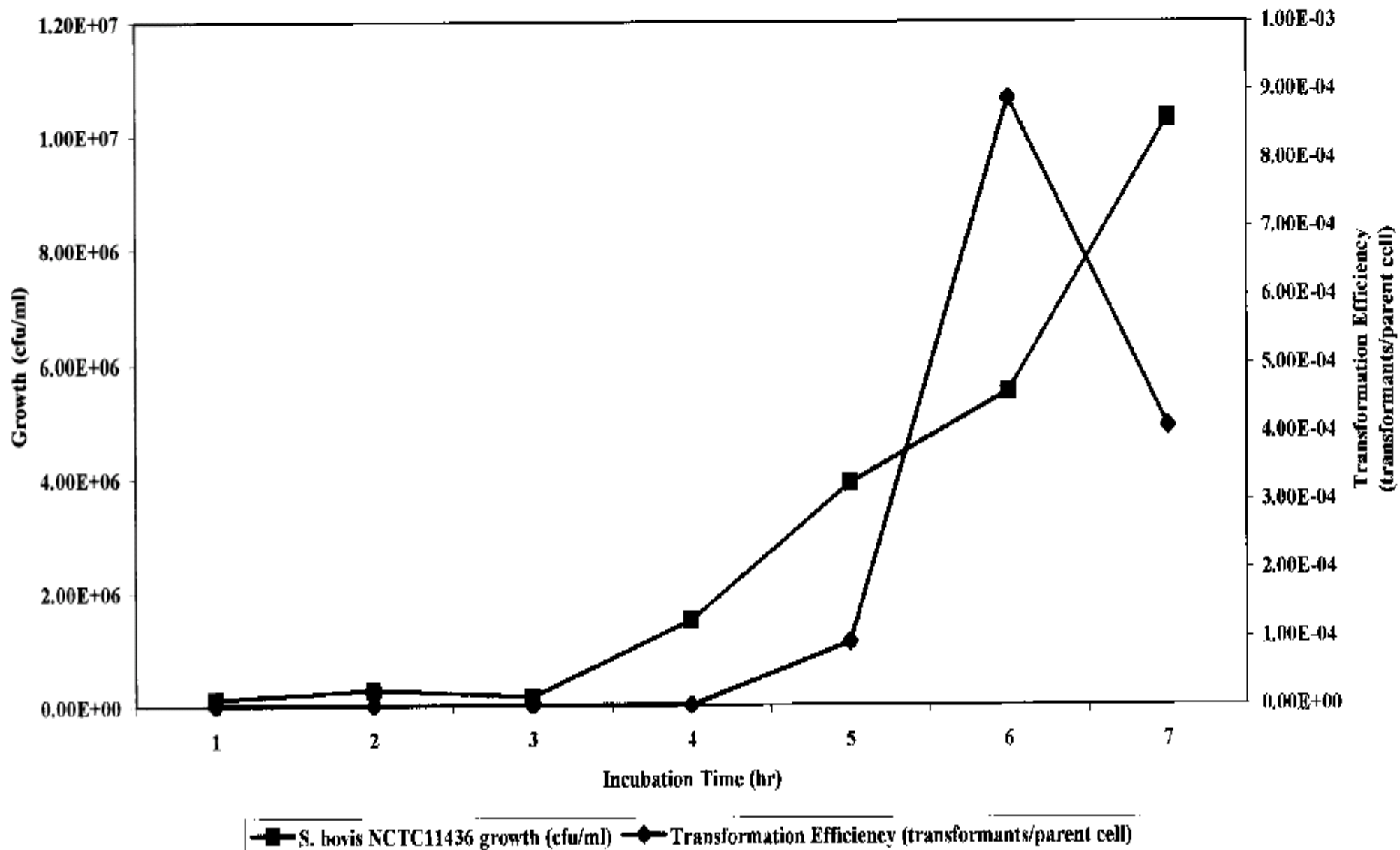


Table 4. Transformation of *Streptococcus bovis* NCTC11436

DNA	Transformation Frequency (trans/μg DNA)	Transformation Efficiency (trans/parent cell)
pVACMC1	4.96×10^2	4.13×10^{-5}
pKPSPgfp-int	4.88×10^3	4.08×10^{-4}
pKPSPgfp- int(O)	0	0
pKPSPgfp- int(W)	0	0

Figure 8. Effect of the Presence of Different Compounds on the Transformation of *S. bovis* NCTC11436

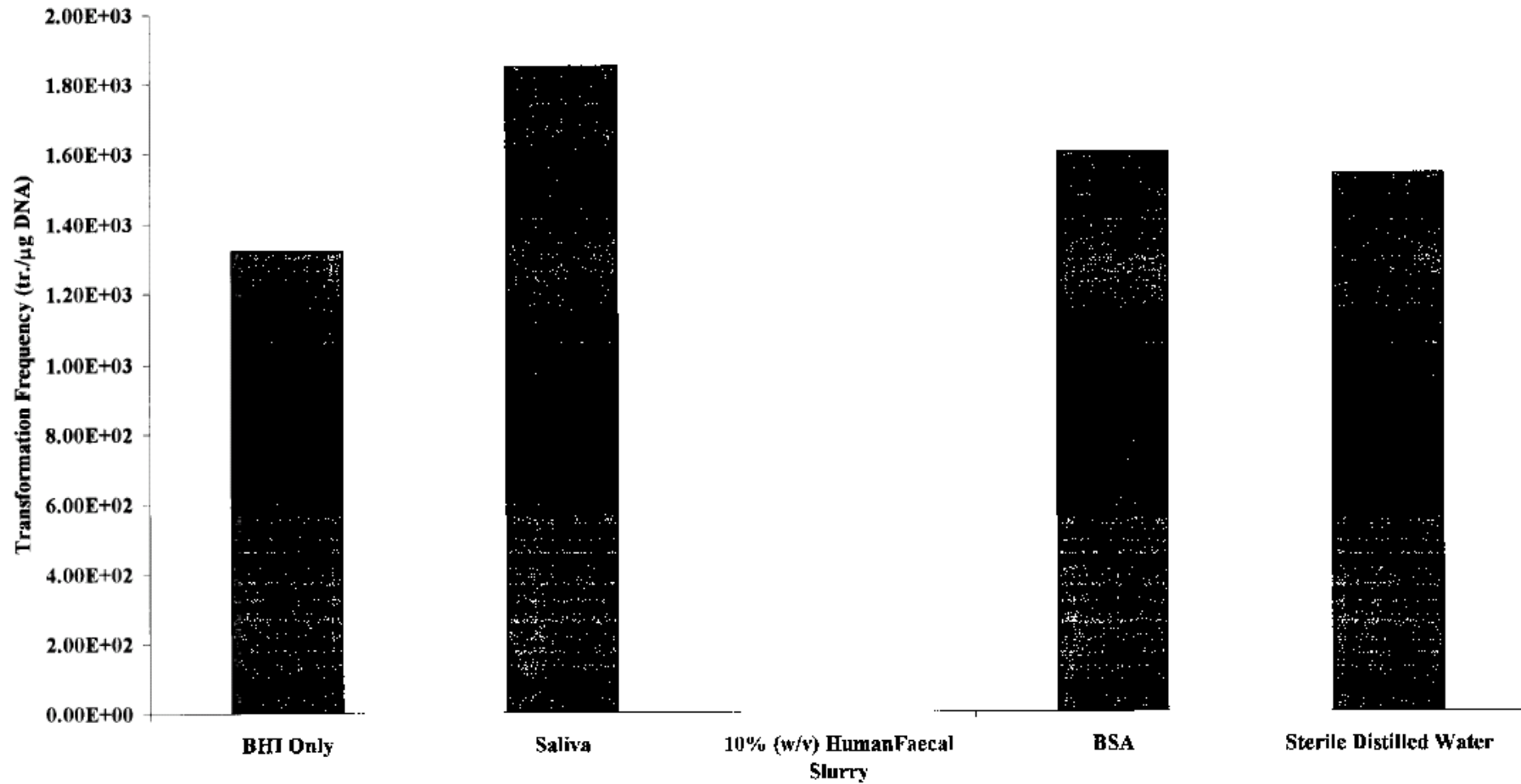


Figure 9. Effect of Competence Inducers on the Growth and Transformation Frequency of *S. bovis* NCTC11436

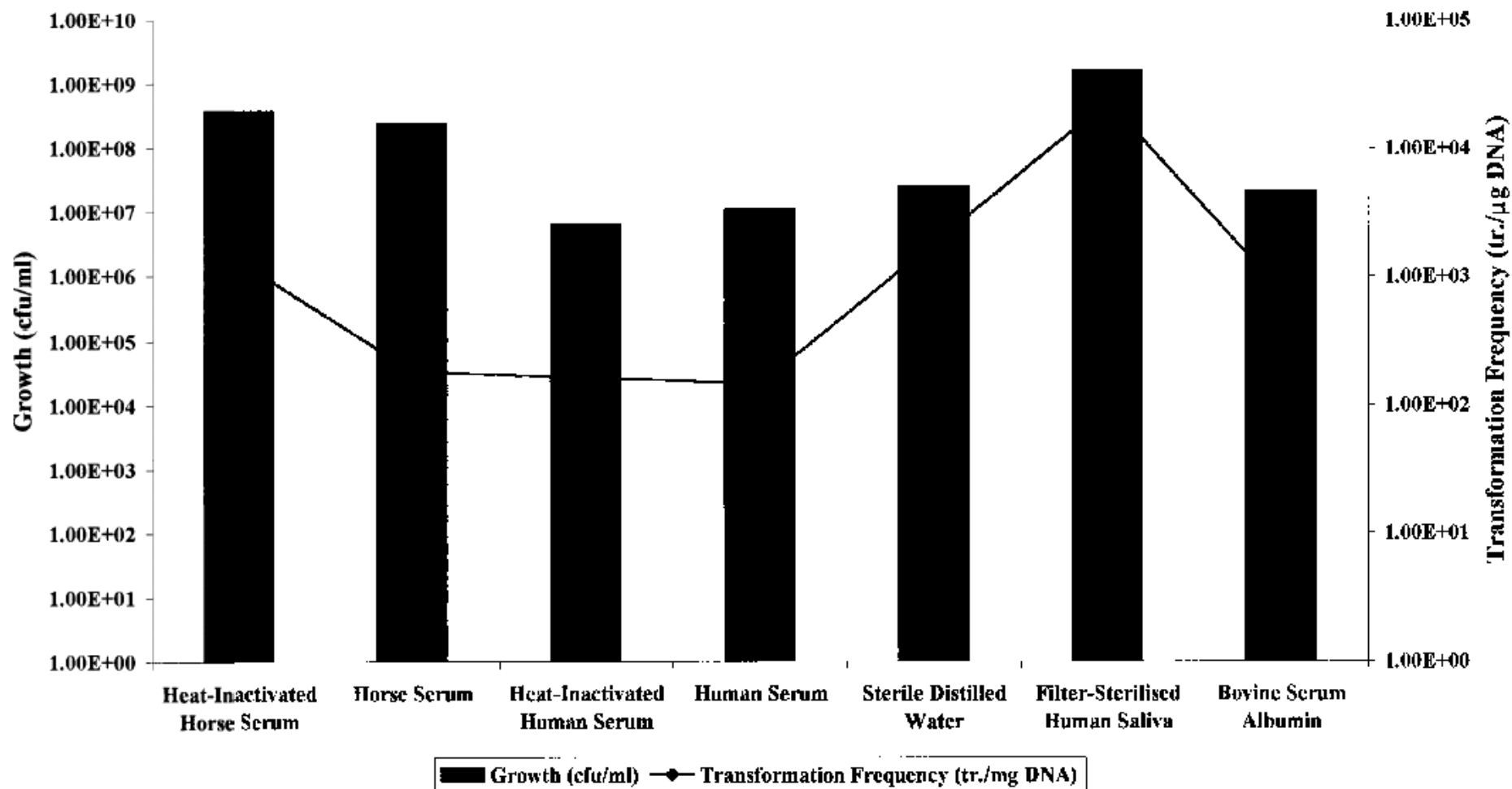


Figure 10. Effect of the presence of foodstuffs and saliva on the transformation frequency of competent cells of *S. gordonii* DL1/Tn916 & *S. bovis* NCTC11436

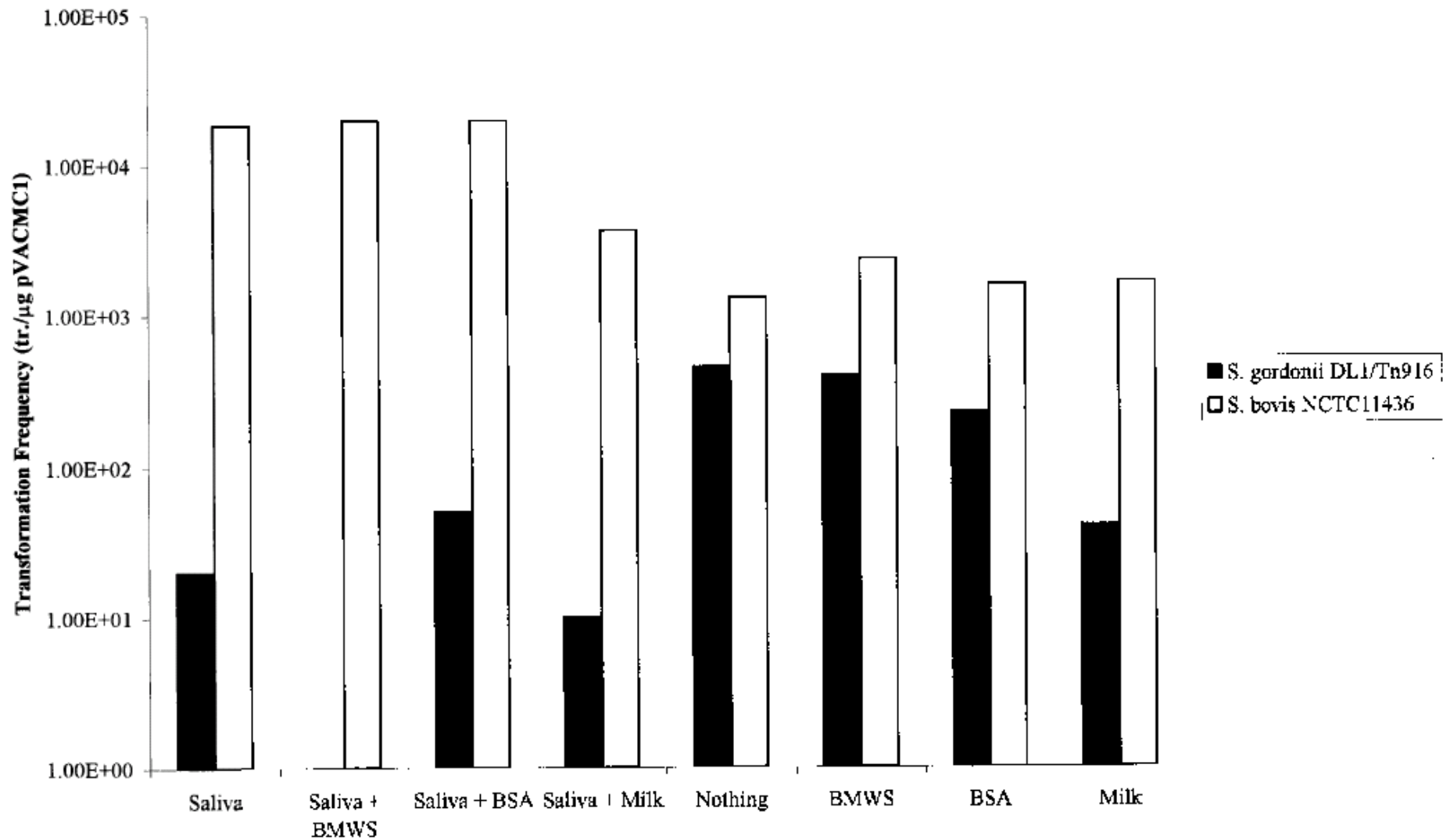


Figure 11. Effect of foodstuffs on the induction of competence and transformation of *S. gordonii* DL1 and *S. bovis* NCTC11436

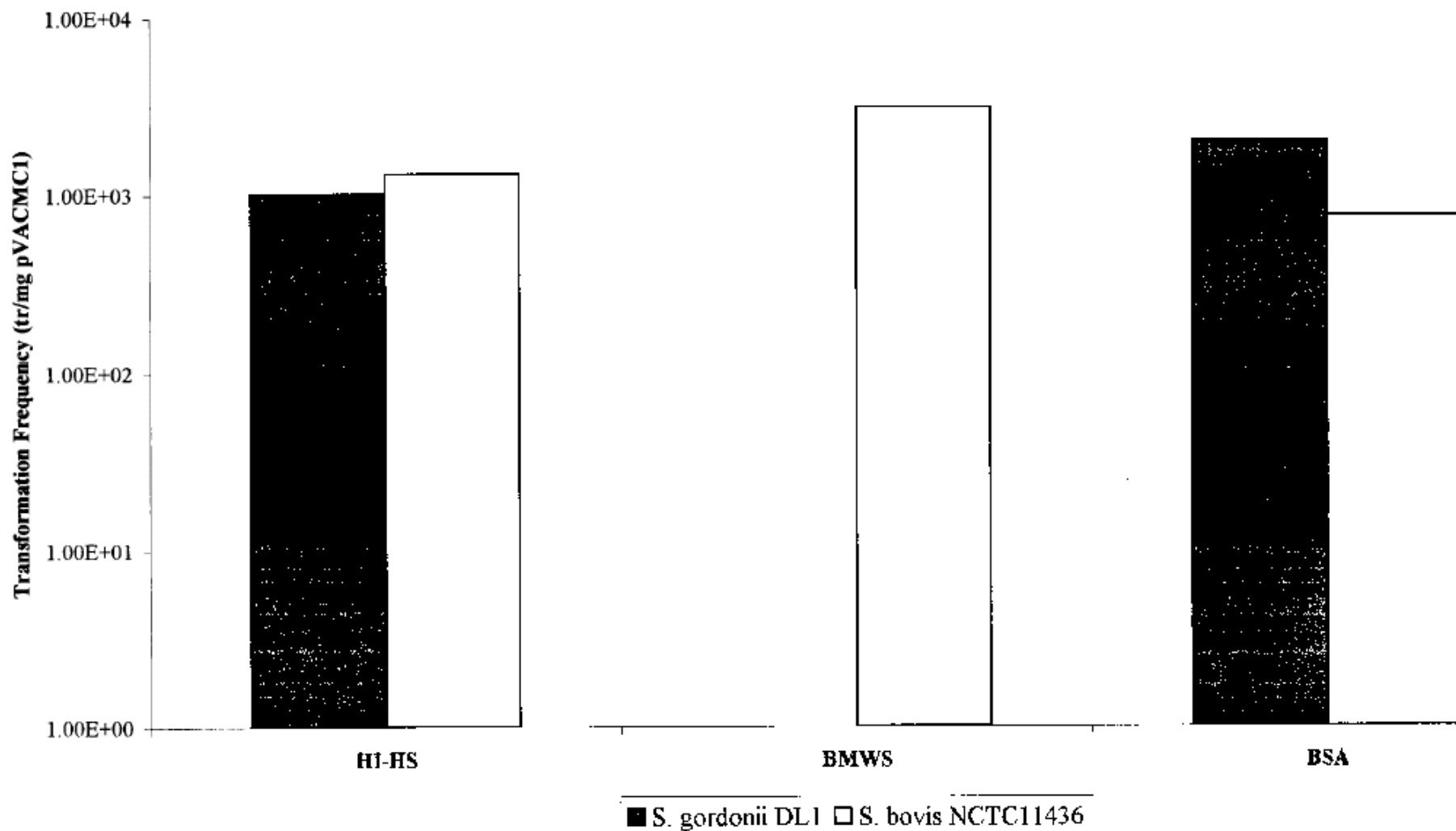


Figure 12. Effect of the presence of food components on the transformation of *S. gordonii* DL1, *S. gordonii* DL1/Tn916 & *S. bovis* NCTC11436

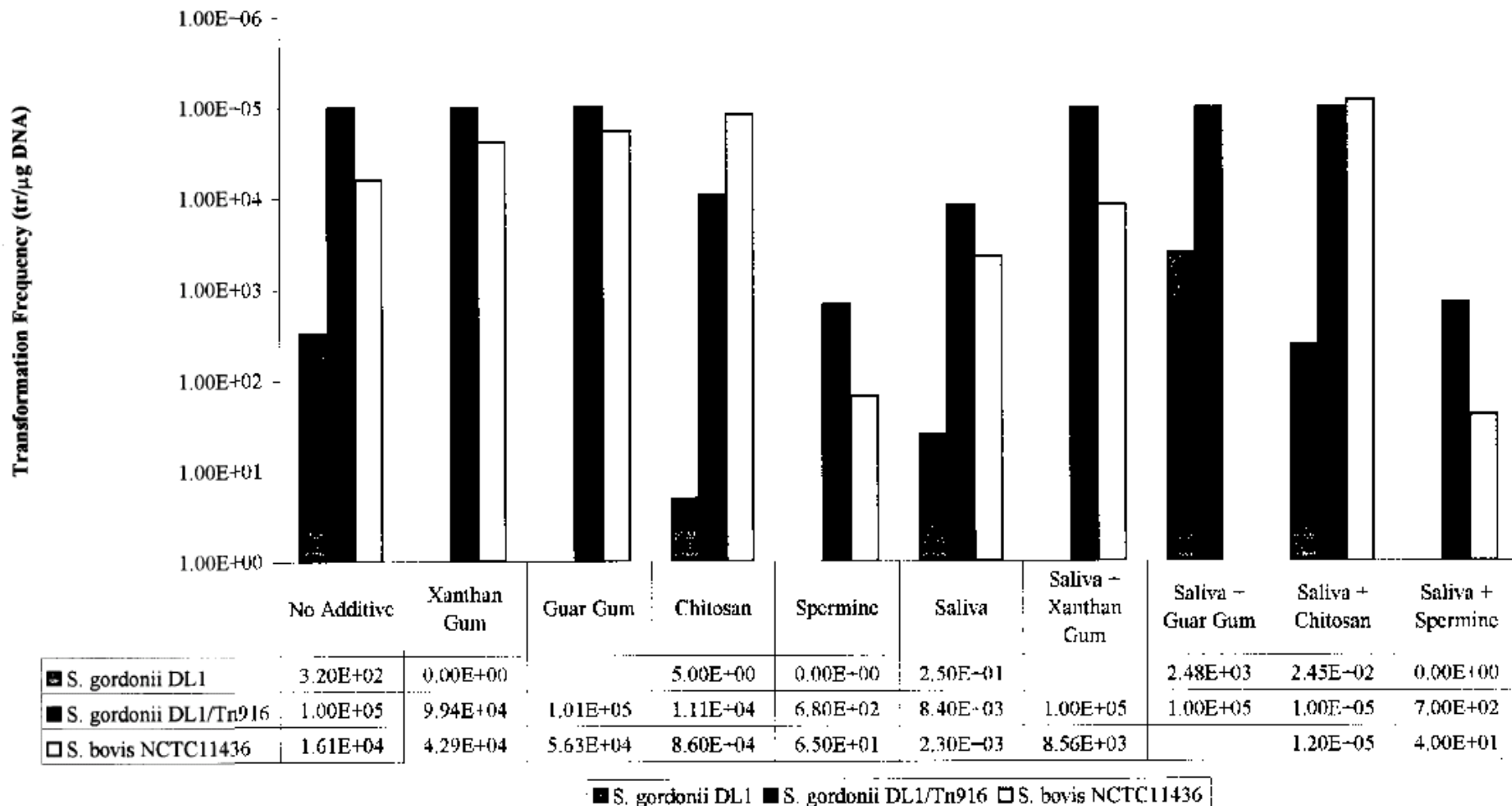


Table 5 - Transformation of a Commensal Human Gut *E. coli* Isolate

Competence Inducer	Transformation Frequency (trans/ μ g pUC18)
0-2 mM CaCl ₂	0
5 mM CaCl ₂	2.1 x 10 ²
10 mM CaCl ₂	5.1 x 10 ⁴
>15 mM CaCl ₂	0
Whole Saliva (1.5 mM CaCl ₂)	0
Filter-sterilised Saliva (1.5 mM CaCl ₂)	0
Filter-sterilised Saliva + 10 mM CaCl ₂	??
Filter-sterilised Saliva + Semi-skimmed Milk (19 mM CaCl ₂)	0

Figure 13 - Survival of *L. lactis*/Tn916/gfp and *E. faecalis*/Tn916/gfp in a Human Colon Fermentor

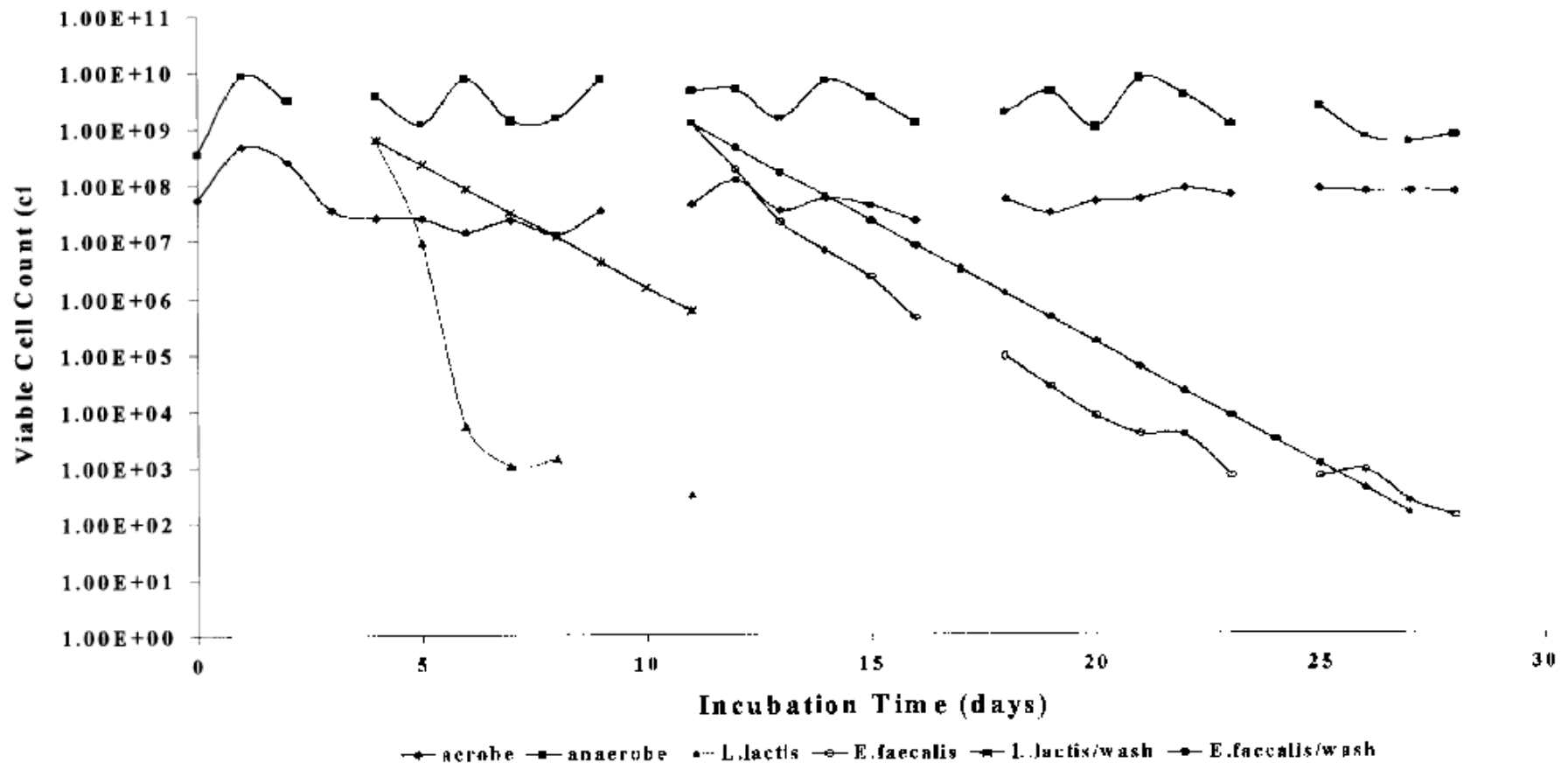


Figure 14 - Survival of *L. lactis*/Tn916/gfp in a Human Colon Fermentor

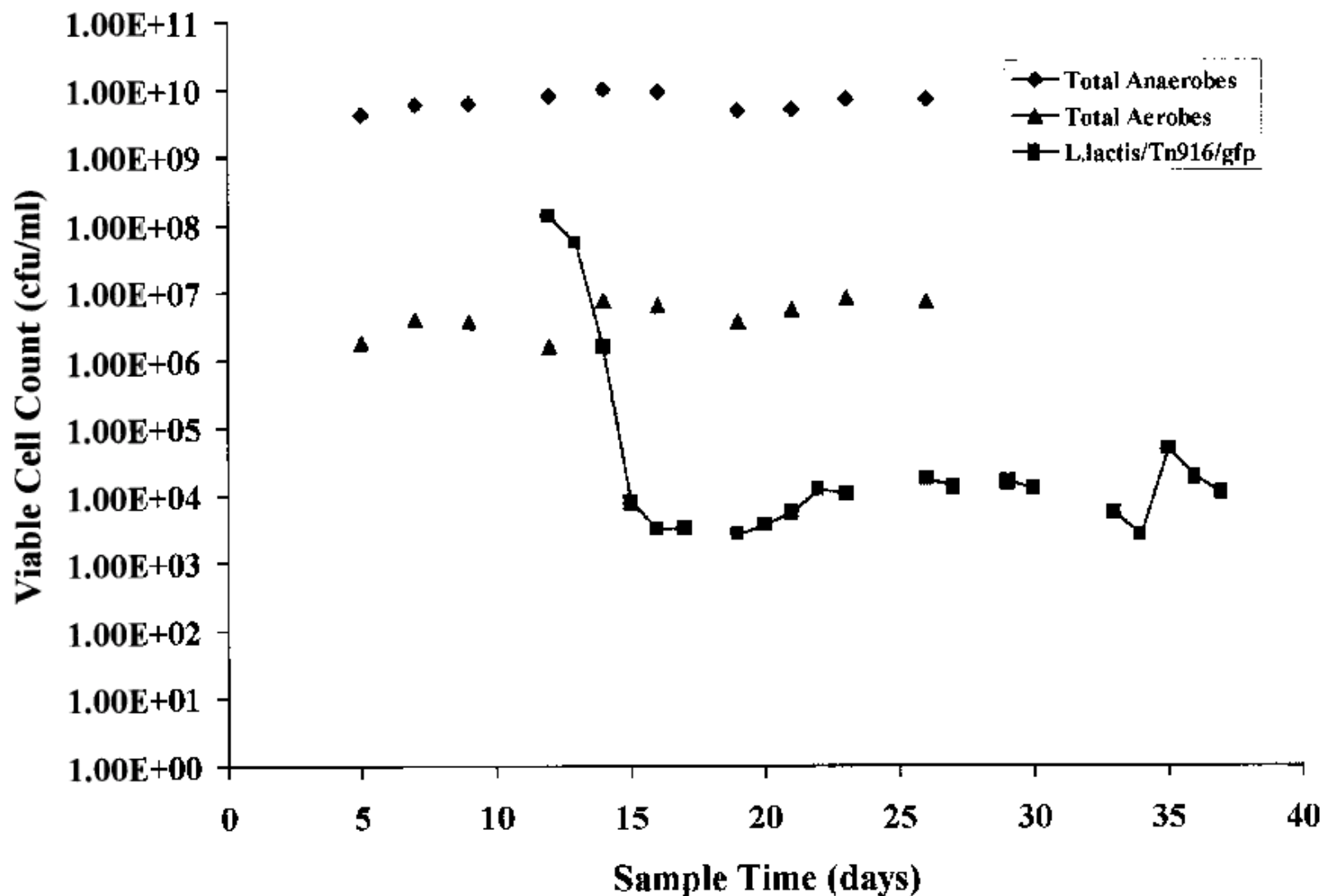


Figure 14 a: Growth of *L. lactis* at pH=6.5

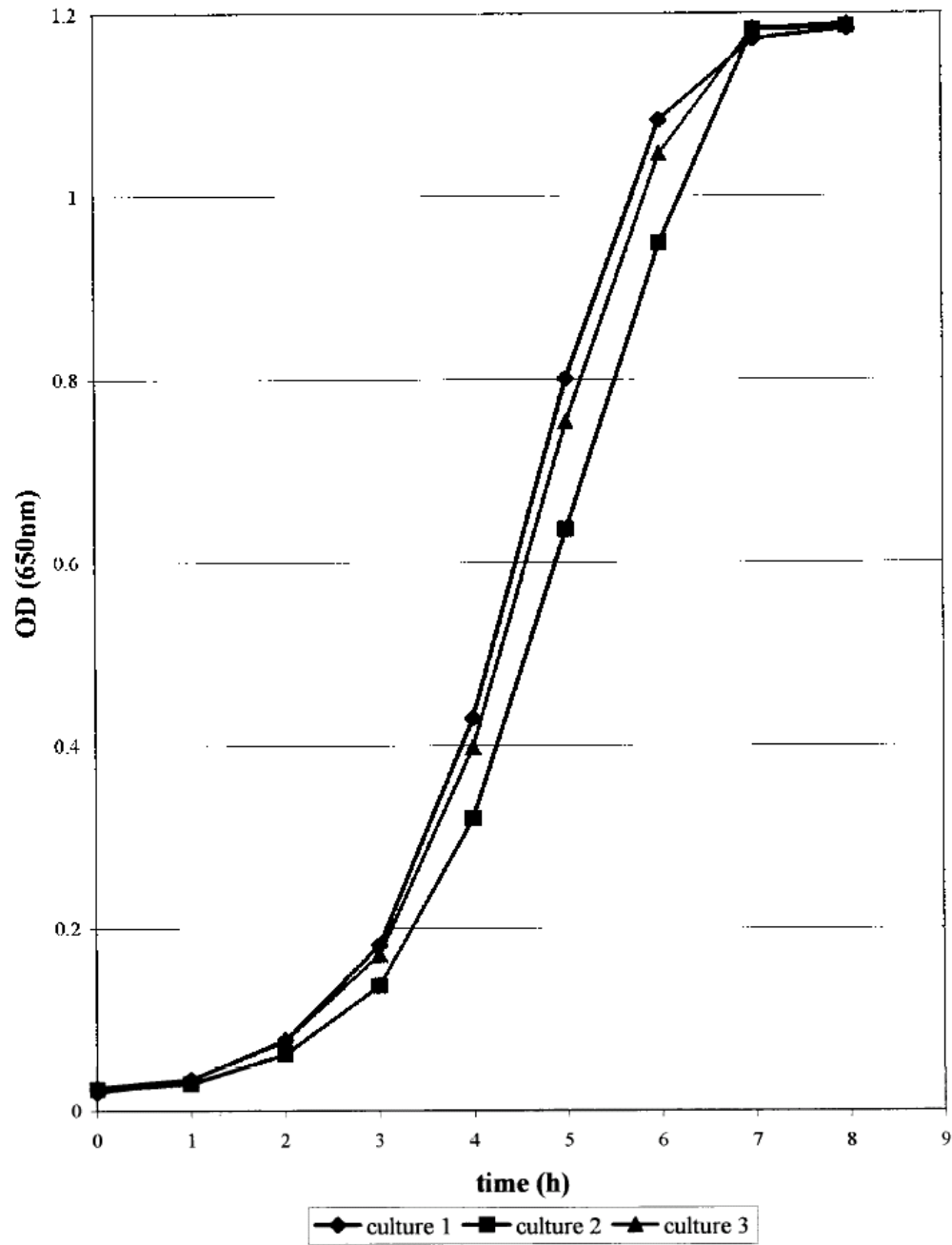


Figure 14b: Growth of *L. lactis* at 40°C

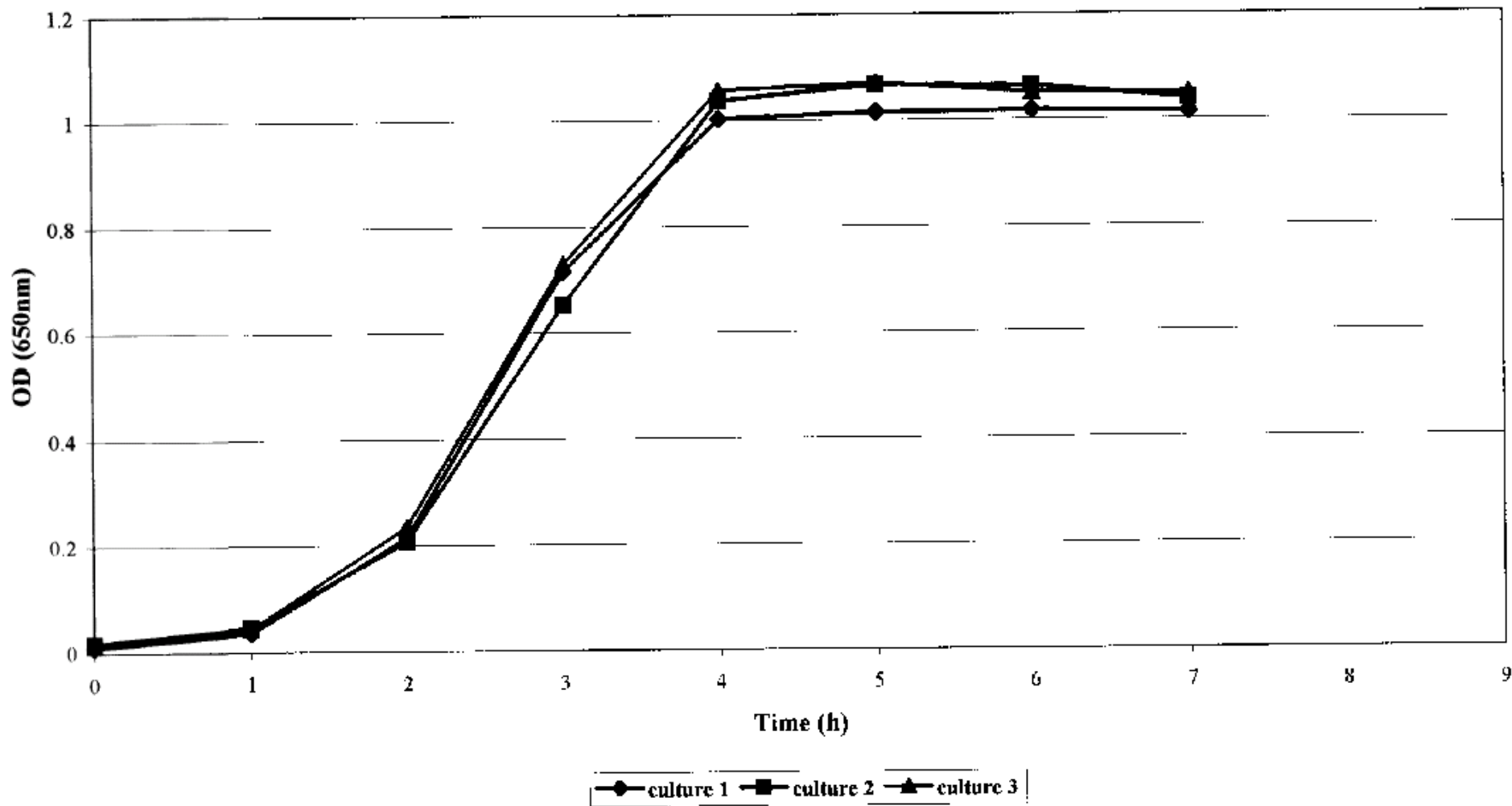


Figure 15 - Survival of *E. faecalis*/Tn916/gfp in the Rat Gut

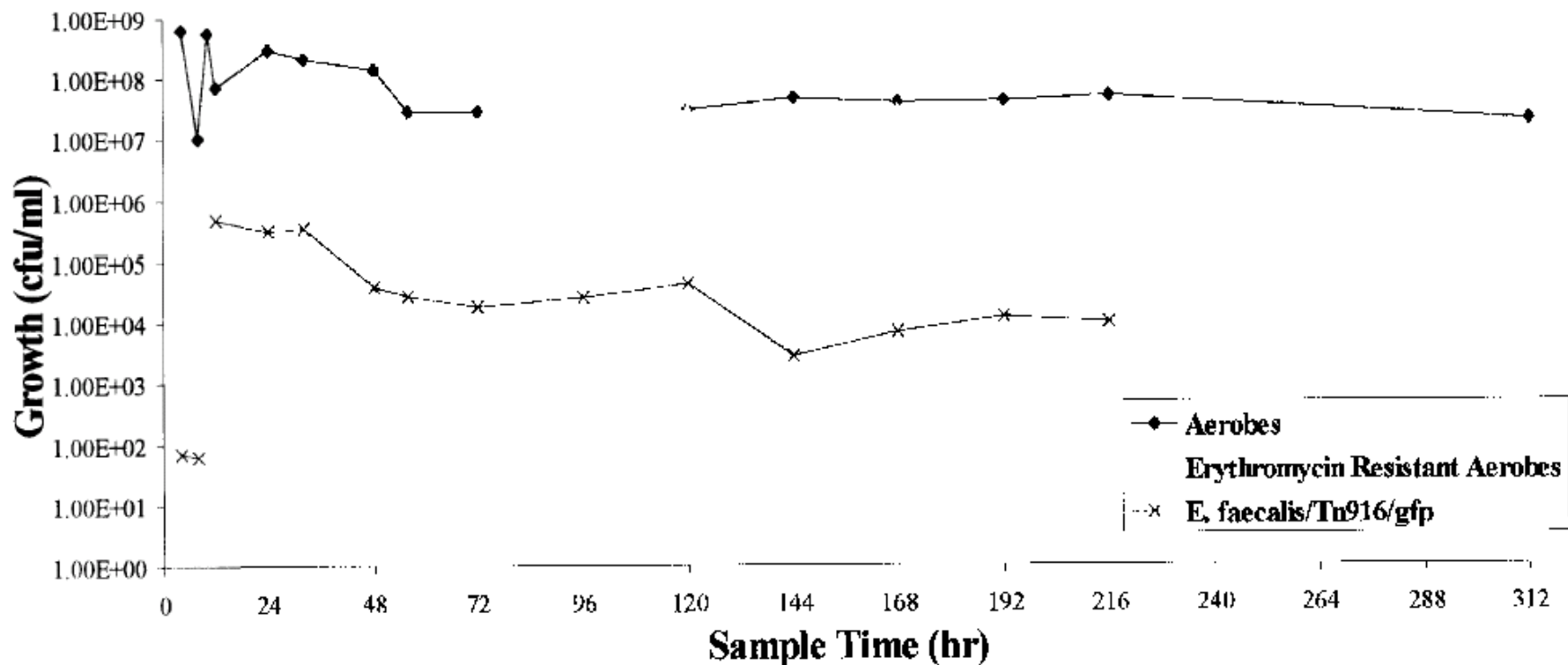


Figure 16 - Survival of *E. faecalis*/Tn916/*gfp* in the Rat Gut 24 Hours after Inoculation

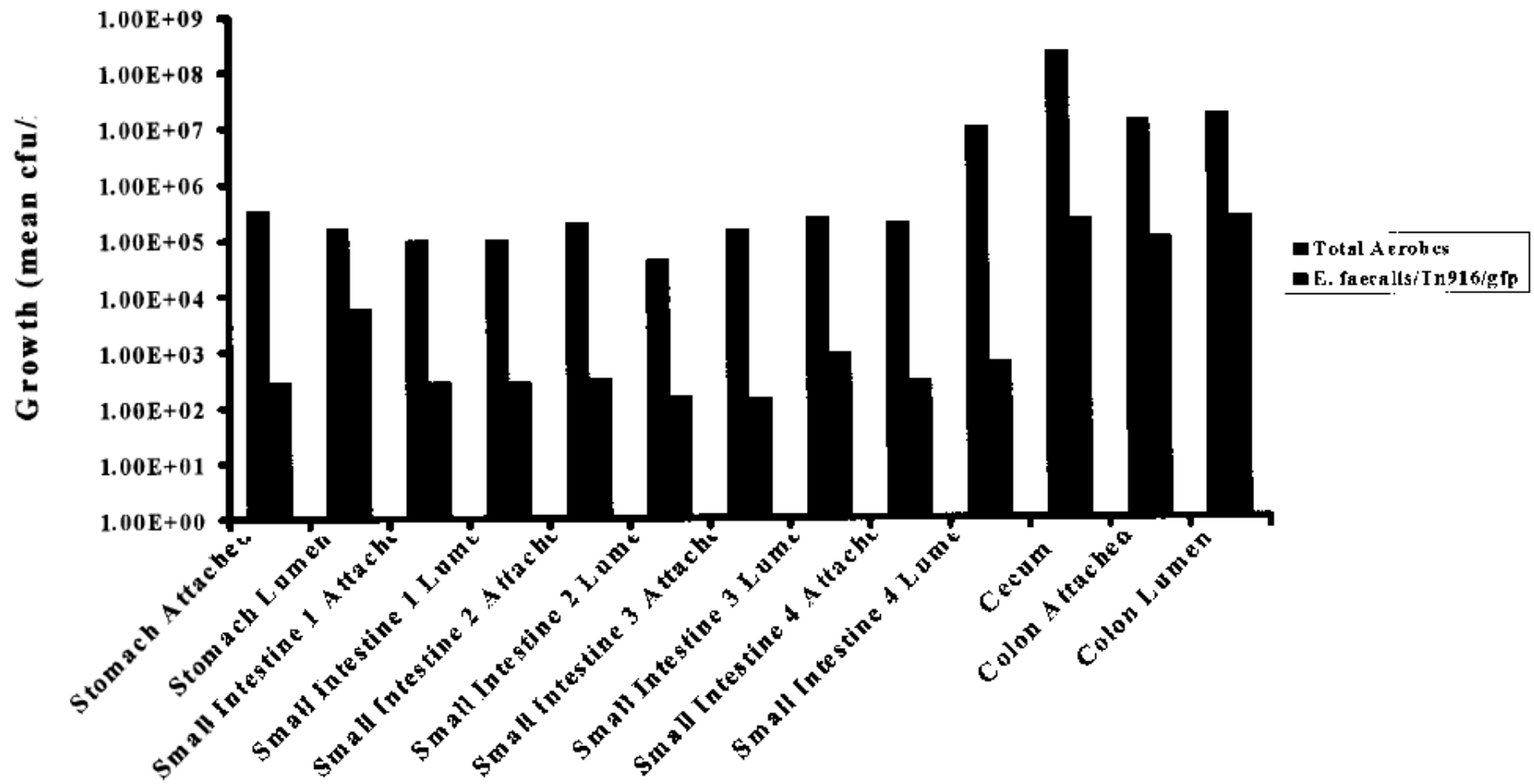


Table 6: Survival of a 610 bp fragment of the *gfp* gene in the faeces of a rat fed 150 μ g pKPSPsgfp

Sample Time (hours)	Supernatant	Microbial Fraction	Debris Fraction
0	-	-	-
8	+	+	+
24	-	+	+
32	-	+	+
48	-	-	-
56	-	-	-

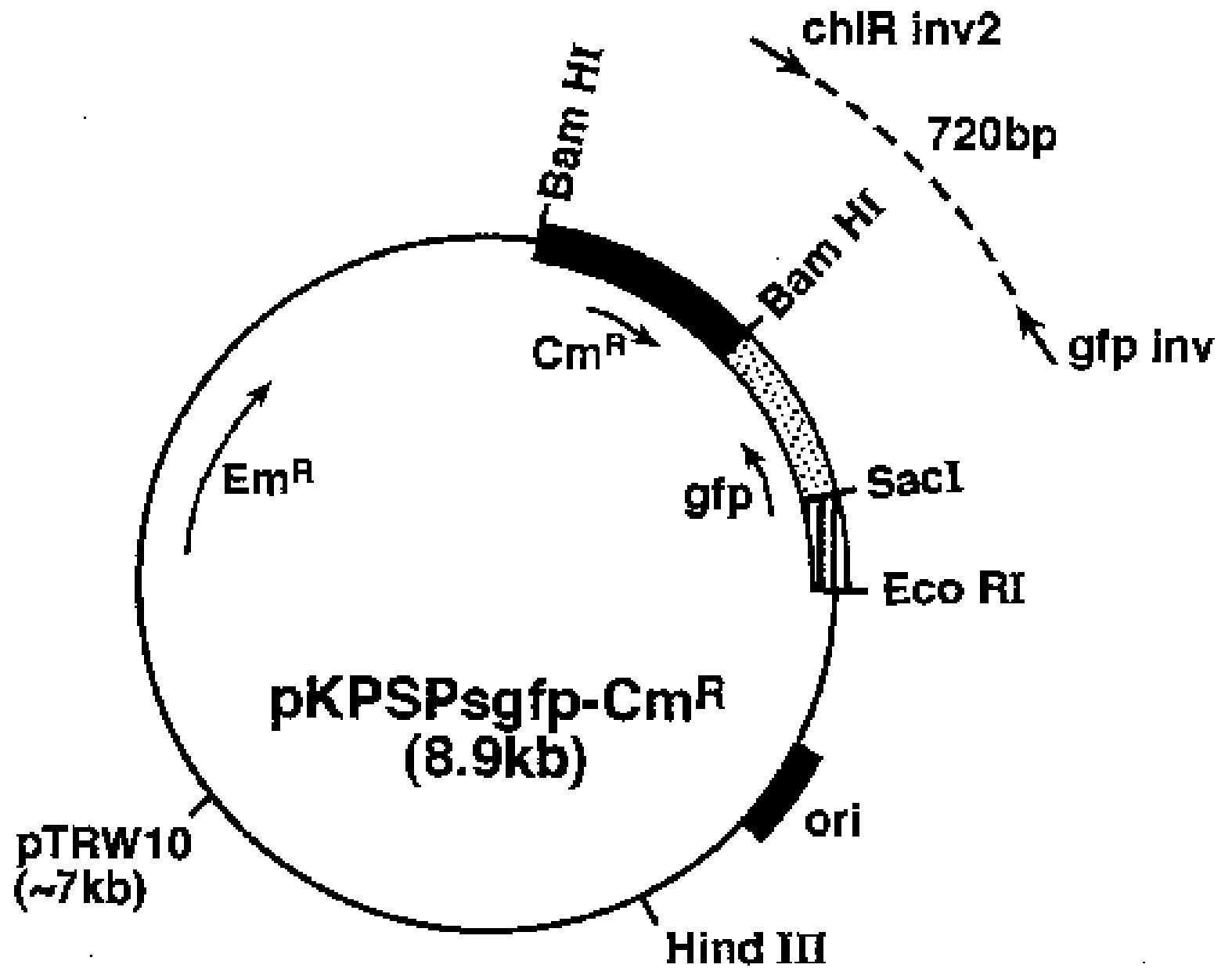


Figure 18: Aerobic growth of rat faecal bacteria (Rat 4) on BHI agar

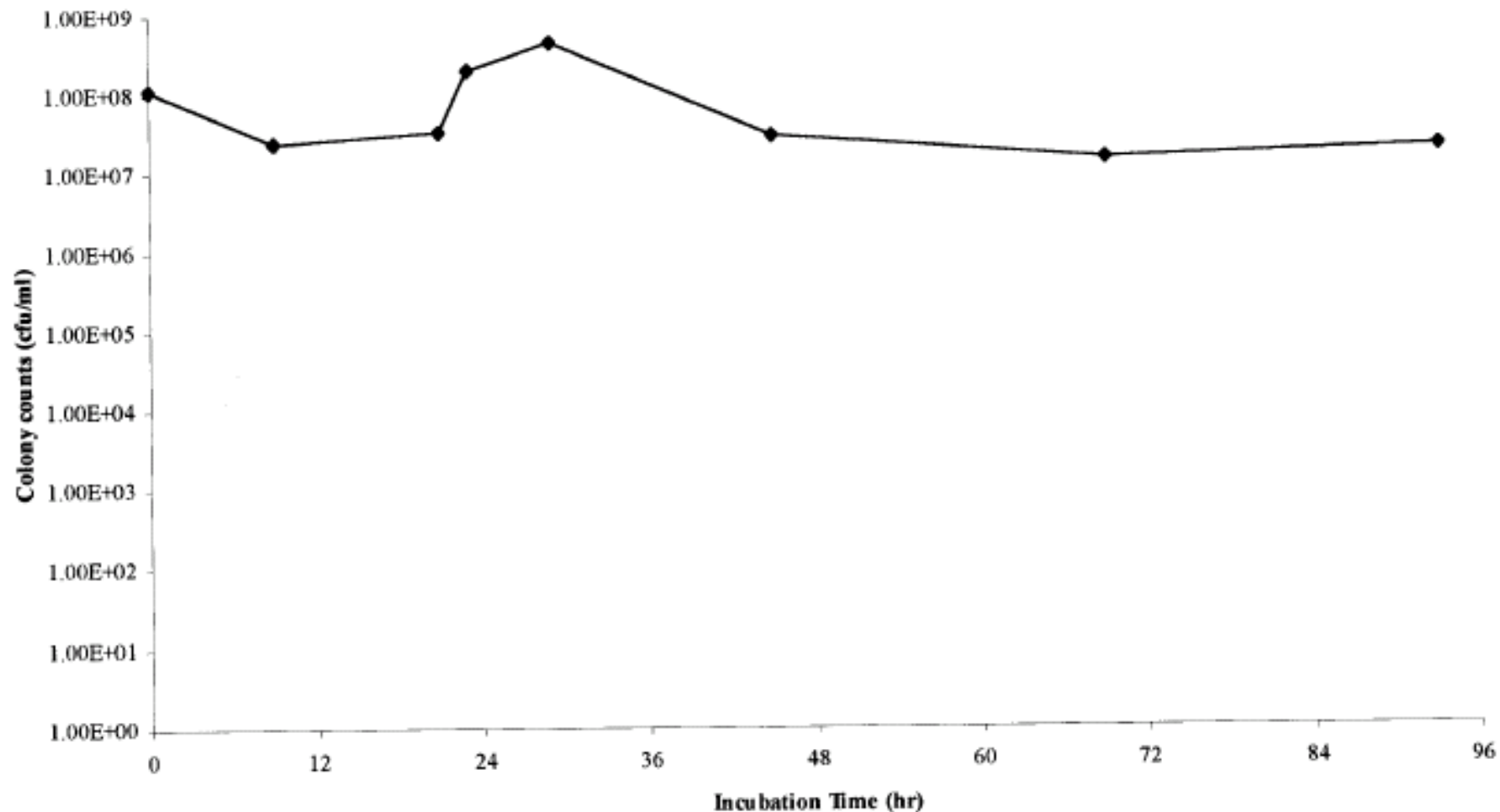


Table 7: Aerobic growth of rat gut content samples on BHI agar

	Rat 1	Rat 2	Rat 3	Rat 4
Small Intestine	2.88×10^9	1.70×10^8	1.49×10^8	2.81×10^7
Caecum	1.36×10^9	1.36×10^9	1.24×10^8	3.32×10^7
Colon	1.48×10^8	2.68×10^9	3.68×10^7	4.54×10^7

Table 8: Antibiotic resistance profiles of rat faeces from rats dosed with 150 µg pKPSPgfpCmR

Incubation Time (hr)	Rat 1		Rat 2		Rat 3		Rat 4	
	BHIC(10) ³	BHIC(5)E(50) ⁴	BHIC(10) ¹	BHIC(5)E(50) ²	BHIC(10) ¹	BHIC(5)E(50) ²	BHIC(10) ¹	BHIC(5)E(50) ²
Uninoculated	0	0	0	0	0	0	0	0
9	NS ⁵	NS ³	NS ³	NS ³	NS ³	NS ³	0	0
21	0	2.00E+03	0	2.00E+03	2.00E+02	1.20E+04	5.00E+02	1.00E+03
23	Sacrificed		8.64E+04	1.70E+03	NS ³	NS ³	5.00E+03	6.08E+04
29			NS ³	NS ³	6.00E+02	0	5.00E+03	3.36E+04
45			0	0	0	0	0	0
69			Sacrificed		0	0	0	0
93					Sacrificed		5.00E+02	5.00E+02
96							Sacrificed	

³ BHIC(10) – BHI agar + 10 µg/ml chloramphenicol

⁴ BIHC(5)E(50) - BHI agar + 5 µg/ml chloramphenicol + 50 µg/ml erythromycin

⁵ NS – No faecal sample produced

Figure 19: Distribution of Antibiotic Resistant Bacteria (5 mg/ml Cm + 50 mg/ml Em) in Rat Gut Compartments of Rats inoculated with pKPSPgfpCm^R

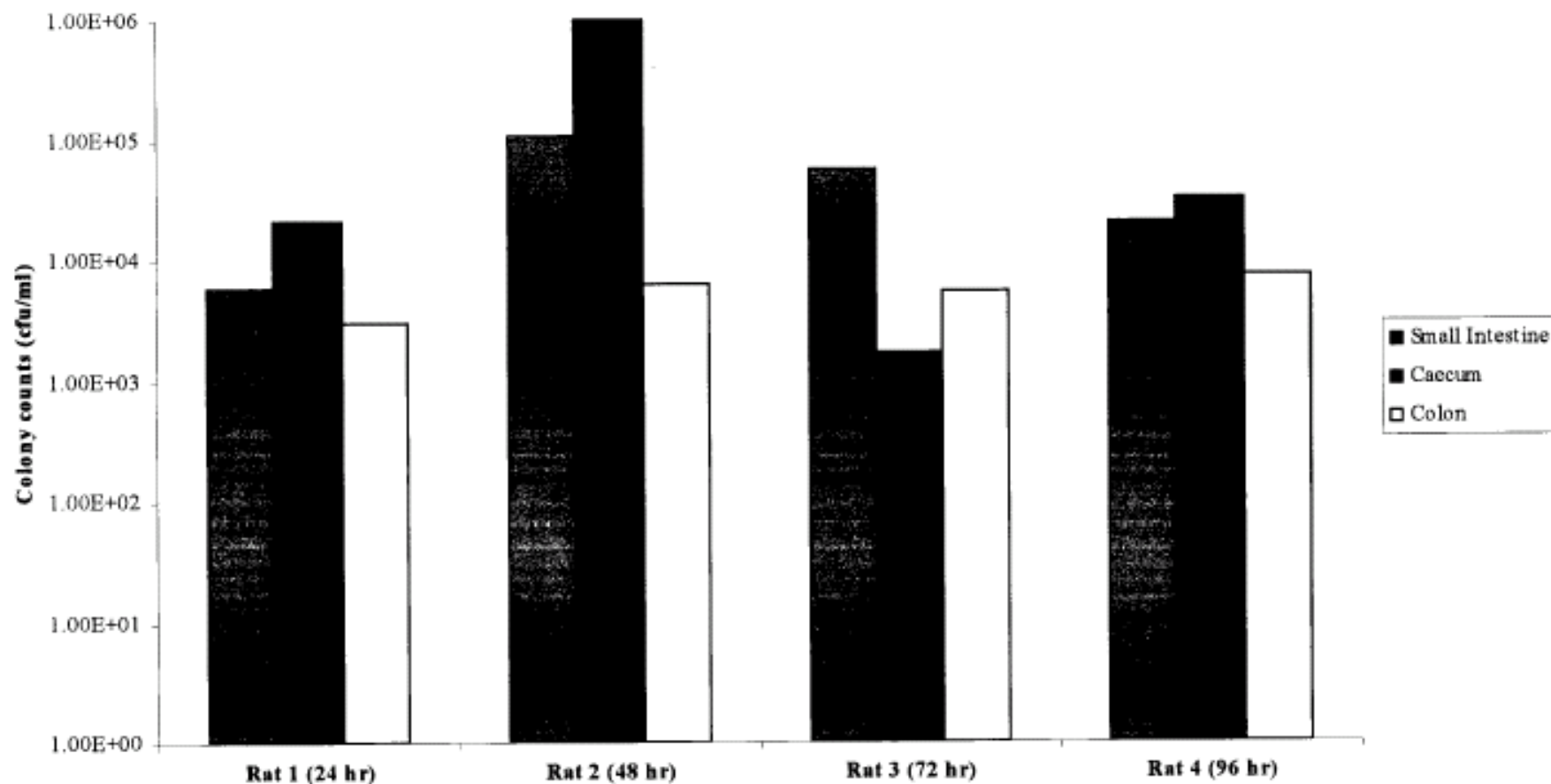
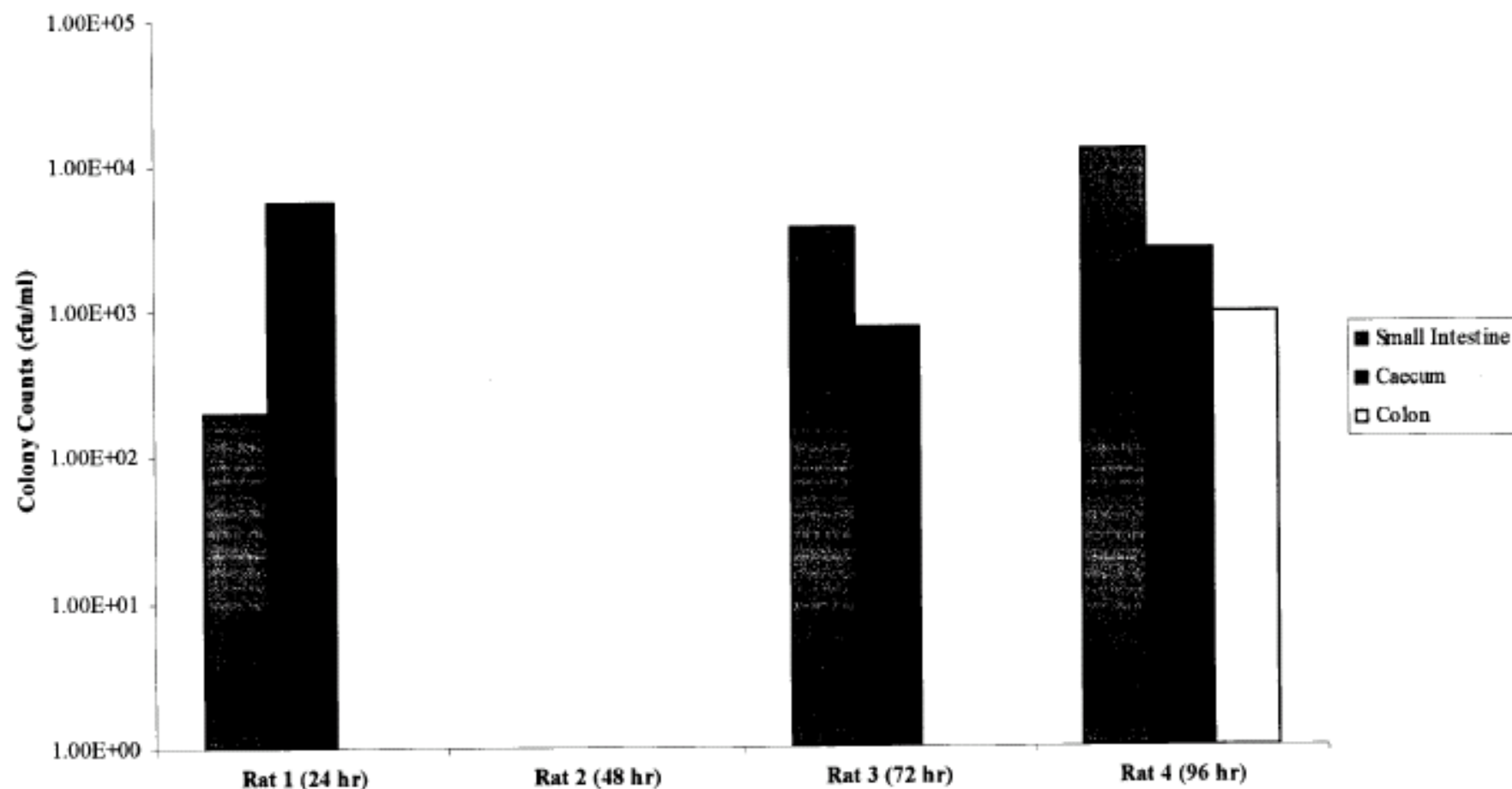


Figure 20: Distribution of Antibiotic Resistant Bacteria ($10 \mu\text{g/ml}$ Cm) in Rat Gut Compartments of Rats inoculated with $\text{pKPSPgfpCm}^{\text{R}}$



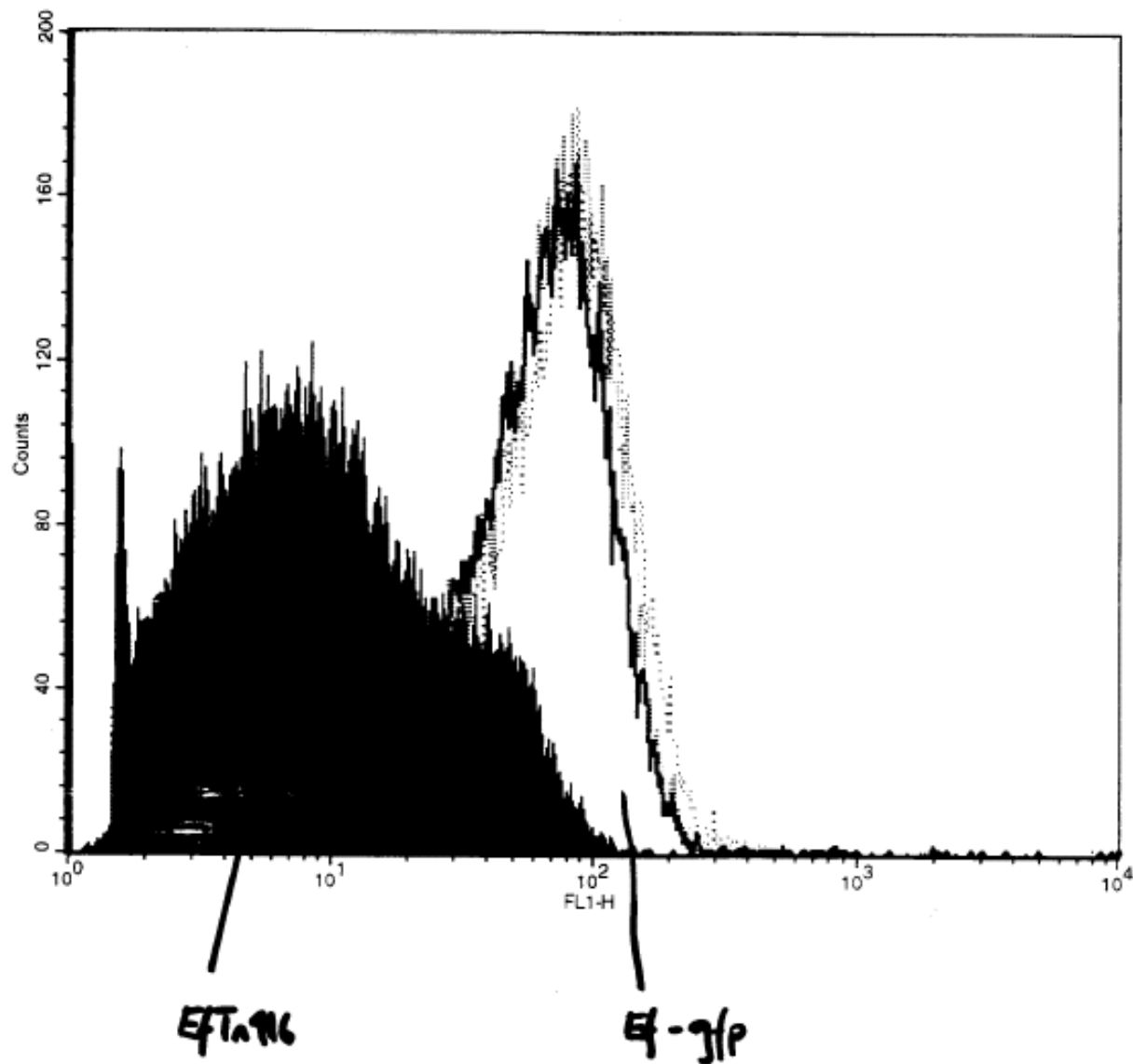


Figure 21. FACS analysis of a 1:1 mixed population of *E. faecalis* Tn916. One fraction is fluorescent due to expression of the green fluorescent protein, whereas the other fraction is non-fluorescent