

Probiotics as alternative antimicrobial agents

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"TRANSLATIONAL RESEARCH IN BIOMEDICINE" Molecular diagnostics, biomarkers & targeted therapies



Alexandroupolis, 2024

Probiotics: from ancient murals to contemporary applications

"Live microorganisms that when consumed in adequate amounts confer health benefits to the host"



Probiotics: from ancient murals to contemporary applications



- Lactobacilli
- Bifidobacteria
- Escherichia coli Nissle 1917
- Streptomyces cerevisiae
- Bacillus spp.
- Enterococcus spp.
- Leuconostoc spp., Pediococcus spp.



Criteria for probiotic selection



Introduction

Intestinal and extraintestinal activity of probiotics



- Neurodegenerative disorders
 - Autoimmune skin disorders
 - Cholesterol metabolism
 - TMAO metabolism
 - Functional disorders
 - Endocrine disorders

- ✓ Strain-specific
- ✓ Host-specific
- ✓ Diet-specific
- ✓ Disease-specific

Probiotics in Extraintestinal Diseases: Current Trends and New Directions

Despoina E. Kiousi¹, Athanasios Karapetsas^{1,†}, Kyriaki Karolidou¹, Mihalis I. Panayiotidis², Aglaia Pappa¹ and Alex Galanis^{1,*}

Strain-specificity

99.9% genomic similarity \rightarrow functional differences



The mechanism behind the phenotype



The human microbiota

In every surface and crevice!

The human microbiota is affected by both genetic and environmental factors **Host Genetics** в Geographical Exercise location Stress Mode of delivery Antibiotics Diet **Gastric secretion** Antimicrobial peptides & Gastric motility IgA



The mechanism behind the phenotype

Distal effects

THE MICROBIOTA-GUT-BRAIN AXIS

John F. Cryan, Kenneth J. O'Riordan, Caitlin S. M. Cowan, Kiran V. Sandhu, Thomaz F. S. Bastiaanssen, Marcus Boehme, Martin G. Codagnone, Sofia Cussotto, Christine Fulling, Anna V. Golubeva, Katherine E. Guzzetta, Minal Jaggar, Caitriona M. Long-Smith, Joshua M. Lyte, Jason A. Martin, Alicia Molinero-Perez, Gerard Moloney, Emanuela Morelli, Enrique Morillas, Rory O'Connor, Joana S. Cruz-Pereira, Veronica L. Peterson, Kieran Rea, Nathaniel L. Ritz, Eoin Sherwin, Simon Spichak, Emily M. Teichman, Marcel van de Wouw, Ana Paula Ventura-Silva, Shauna E. Wallace-Fitzsimons, Niall Hyland, Gerard Clarke, and Timothy G. Dinan

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- Gut-immune-brain
- Vagus nerve
- Blood circulation
- Neurotransmitter production



Introduction

Practical application?



EN	=
English	Men



Home

Scientific Opinion on the substantiation of health claims related to live yoghurt cultures and improved lactose digestion (ID 1143, 2976) pursuant to Article 13(1) of Regulation (EC) No 1924/2006

Published: 19 October 2010 | Adopted: 9 July 2010 | Updated: 11 January 2011



Contents

Meta data Abstract

Related topic

- Yoghurt with ≥ 10⁸ CFU of *L. delbrueckii* subsp. bulgaricus and Streptococcus thermophilus
- "For improved digestion of lactose in populations with lactose maldigestion"

Construction of a database for microorganisms with *in vitro* probiotic traits

- Available literature increases at an **exponential rate**
- Isolation of **several novel** strains in each study
- Lack of a tool to classify and comprehensively present available data



Probio-ichnos is a manually curated literature-based database



🛆 Home	≡ Brows	e	Data Statistics	22 About us		🗉 Help		
Browse Search for:		B Select Properties				Easy navigation of available		
search by species or stra Sort by: N	ain name	 Resistant Resistant Adhesion 	t to acid t to bile n/Attachment		(Comparative anal Selection of reference 		
Search by: Species		Antimicr Immuno Antiproli	obial modulation ferative			· · PMID		
Descending		Antioxidant				Links to PubMed and NCBI Genome db		
Species	Strain	Resistant to acid	Resistant to bile	, N	PMID	=, Explore		
Lacticaseibacillus rhamnosus	GG=ATCC 53103	~	1	270	11409159 115800	9.		
Escherichia coli	Nissle 1917	Ø	Ø	59	12014725 115183	2.		
Lacticaseibacillus paracasei	Shirota	1	?	42	10196761 104369	2		

[Tsifintaris M*, Kiousi DE* et al., 2024]



- **11,202** strains
- 470 species
- **143** genera
- 789 WGS

Top entries:

- Genus: Lactiplantibacillus spp.
- Species: Lp. plantarum
- Strain: LGG

Most investigated properties:

- Resistance to bile (5873)
- Resistance to acid (5950)
- Antimicrobial (6110)

[Tsifintaris M*, Kiousi DE* et al., 2024]



"Probiotics possess antimicrobial activity"



"Probiotics possess antimicrobial activity"





17

Introduction

Lactobacilli

- Gram-positive
- Non-motile
- Non-spore forming
- Oxygen tolerant or anaerobic
- Optimal temperature: 2-53°C
- Nutrient-dense environments
- ~2-4 Mb



[Zheng et al., 2020]



Species	Strain	Isolation Source	Probiotic attributes	Biotechnological application	Available WGS
Lc. paracasei	SP5	Kefir grains ^[1]	Yes	Yes	Yes ^[2]
Lp. plantarum	L125	Fermented sausage ^[3]	Yes	No	Yes ^[4]
Lp. pentosus	L33	Fermented sausage ^[3]	Yes	No	Yes ^[5]
Lc. rhamnosus	GG*	Human gut microbiota	Yes	Yes	Yes
Lc. casei	ATCC 393*	Dairy product	Yes	Yes	Yes

* Commercially available strains

[1] Mantzourani et al., 2019
[2] Kiousi et al., 2022
[3] Pavli et al., 2016
[4] Tegopoulos et al., 2021
[5] Stergiou et al., 2021

19

- Accurate taxonomic classification
- Evaluation of the safety profile of the SOI
- Evaluation of the probiotic properties of the SOI



Average Nucleotide Identity (ANI)

Phylogenomic analysis



Introduction

- Accurate taxonomic classification
- Evaluation of the safety profile of the SOI
- Evaluation of the probiotic properties of the SOI



Fig. 1. Cumulative number of species described in the genera *Pediococcus* and *Lactobacillus* until January 2020. The species description in January 2020 is included in the 2010–2019 count.



Introduction

[Zheng et al., 2020]

- Accurate taxonomic classification
- Evaluation of the safety profile of the SOI
- Evaluation of the **probiotic properties** of the SOI



- Accurate taxonomic classification
- Evaluation of the safety profile of the SOI
- Evaluation of the probiotic properties of the SOI



- Accurate taxonomic classification
- Evaluation of the safety profile of the SOI
- Evaluation of the probiotic properties of the SOI

As of 2021, EFSA requires WGS for strains intended for use in the food chain



Niche adaptation

Genome size and GC content Biosynthetic capacity Metabolic activity

Niche adaptation = targeted application?



Lacticaseibacillus paracasei SP5

- SP5 can withstand simulated GI tract conditions^[1]
- SP5 is **susceptible** to common antibiotics^[1]
- SP5 exerts anti-proliferative activity in vitro [1]
- SP5 has been incorporated in **both dairy**^[2] and non-dairy^[3-5] products



Genomic features



Genome Characteristics	Value
Length	2,958,982 bp
GC content	46.3 %
Total genes	2,920
CDSs	2,870
rRNAs	5
tRNAs	42
ncRNAs	3
Pseudogenes	105
No. of CRISPR Arrays	4
IS elements	122
Phages	
Intact	1
Incomplete	2
Questionable	2
Plasmids	0

Genome stability

Results

[Kiousi et al., 2022]

SP5 clusters with other members of the species

Genome alignment was performed with progressiveMauve





[Kiousi et al., 2022]

Average nucleotide identity (ANI)



Species threshold (>96%)

Strain	Isolation source	ANI (%) with
	Isolation source	SP5
FAM18172	Milk	99.41
1001216st1_B6_D55t1_190419	Homo sapiens	99.39
1001216st1_C7_1001216I_160404	Homo sapiens	99.39
1001216st1_H7_1001713B170131_170501	Homo sapiens	99.39
1001216st1_D9_D55t1_190419	Homo sapiens	99.39
844_LCAS	Homo sapiens	99.39
1001713B170131_170501_F11	Homo sapiens	99.39
EG9	Cheese	99.35
FAM18123	Cheese	99.31
FAM18110	Cheese	99.27
BIOML-A2	Homo sapiens	99.18
BIOML-A3	Homo sapiens	99.17
BIOML-A4	Homo sapiens	99.17
ATCC 334	Cheese	99.14
KL1-Liu	Kefir	99.03
ATCC 25302	Homo sapiens	99.02
KL1	Milk	99.02
FAM8407	Cheese	99.01
LC2W	Dairy product	99.01

29





Pangenome analysis

COG Class	Pangenome	Unique
C, Energy production and conversion	299 (3.17%)	1 (2.3%)
D, Cell cycle control and mitosis	134 (1.42%)	0 (0%)
E, Amino Acid metabolism and transport	539 (5.71%)	1 (2.38%)
F, Nucleotide metabolism and transport	237 (2.51%)	0 (0%)
G, Carbohydrate metabolism and transport	879 (9.32%)	7 (16.67%)
H, Coenzyme metabolism	164 (1.74%)	2 (4.76%)
I, Lipid metabolism	134 (1.42%)	0 (0%)
J, Translation	306 (3.24%)	0 (0%)
K, Transcription	632 (6.70%)	6 (14.29%)
L, Replication and repair	1521 (16.11%)	5 (11.90%)
M, Cell wall/membrane/envelop biogenesis	598 (6.34%)	4 (9.52%)
N, Cell motility	29 (0.31%)	2 (4.76%)
O, Post-translational modification, protein turnover,	0 (0%)	
chaperone functions		
P, Inorganic ion transport and metabolism	450 (4.77%)	1 (2.38%)
Q, Secondary Structure	72 (0.76%)	1 (2.38%)
T, Signal Transduction	155 (1.64%)	1 (2.38%)
U, Intracellular trafficking and secretion	144 (1.53%)	2 (4.76%)
V, Defense mechanisms	365 (3.87%)	1 (2.38%)
S, Function Unknown	1614 (17.11%)	8 (19.05%)
No category, General function prediction only	1019 (10.81%)	5 (11.9%)
Total	9430 (100%)	42 (100%)

[Kiousi et al., 2022]

Results

Functional annotation of SP5

	Organismal systems		
	Xenobiotics biodegradation and metabolism		
	Metabolism of terpenoids and polyketides		
ories	Cellular processes	COG Class	SP5
ateg	Unclassified: signaling and cellular processes Glucan biosynthesis and metabolism	C. Energy production and conversion	111 (4 81%)
3	Energy metabolism		
ona	Lipid metabolism	D, Cell cycle control and mitosis	38 (1.62%)
G	Unclassified: Genetic Information Processing	E, Amino Acid metabolism and transport	184 (7.83%)
fu	Metabolism of cofactors and vitamins	E Nucleotide metabolism and transport	111 (4 72%)
99	Nucleotide metabolism		111 (4.7270)
KEC	Unclassified	G, Carbohydrate metabolism and transport	231 (9.83%)
	Amino acid metabolism Unclassified: metabolism	H, Coenzyme metabolism	66 (2.81%)
Ρ	rotein families: signaling and cellular processes	I, Lipid metabolism	57 (2.43%)
	Genetic Information Processing	J. Translation	166 (7.06%)
Р	rotein families: genetic information processing	K Transprintion	211 (9 0904)
	Carbohydrate metabolism	K, hanschption	211 (0.96%)
	Drug resistance: antineoplastic	L, Replication and repair	157 (6.68%)
	Cell motility	M. Cell wall/membrane/envelop biogenesis	125 (5.32%)
	Cellular community - prokaryotes	N. Coll motility	Q (0 2406)
	Transport and catabolism	N, Cett Houtity	8 (0.34%)
	Signaling molecules and interaction	O, Post-translational modification, protein turnover, chaperone	EQ (2 2104)
S	Signal transduction	functions	52 (2.21%)
va)	Replication and repair	D Inorgania ion transport and matcheliam	122 (5 6204)
ath	Folding, sorting and degradation	P, inolganic for transport and metabolism	132 (5.62%)
dg	Translation	Q, Secondary Structure	26 (1.11%)
(EG(Transcription Xenobiotics biodegradation and metabolism	T, Signal Transduction	52 (2.21%)
-	Biosynthesis of other secondary metabolites Metabolism of terpenoids and polyketides	U, Intracellular trafficking and secretion	45 (1.91%)
	Metabolism of cofactors and vitamins	V. Defense mechanisms	74 (3,15%)
	Glycan biosynthesis and metabolism		
	Metabolism of other amino acids Amino acid metabolism	S, Function Onknown	502 (21.36%)
	Nucleotide metabolism	No category, General function prediction only	269 (11.45%)
	Lipid metabolism	Total	2350 (100%)
	Carbohydrate metabolism		

No of Genes

Kiousi et al., 2022]

Biosynthesis of other secondary metabolites

Human diseases

Nomadic features of Lc. paracasei SP5



- Capacity to metabolize a broad range of carbohydrates
- Limited amino acid biosynthetic capacity (3/20 aa)
- Proteolytic systems

[Kiousi et al., 2022]

Phenotypic prediction



[Kiousi et al., 2022]

33

[Healy et al., 2000]

Safety profile

- No virulence factors
- No transferable antibiotic resistance genes
- Prophages without transposition capacity
- Intrinsic resistance to vancomycin
- Multidrug resistance proteins (SugE, LmrA)



Resistance to host-related stressors

- Acid tolerance (atpABCDEFG) ٠
- Bile salt tolerance (linear amide C-N hydrolase) •
- Oxidative stress (NADH peroxidases, mtsCBA) .
- Osmotic stress (opuAC, opuCC, choS, gbuAB) .
- Chaperones and chaperonines •
- Heat/Cold shock proteins ٠



[Kiousi et al., 2022]

Results

Niche adaptation

Lc. paracasei strains do not cluster based on isolation source

0

Isolation source:

- Fermented dairy
- Fermented vegetables
- Host-associated
- Fermented beverages
- \bigcirc No isolation source disclosed



Resul

Conclusions

- ✓ Lc. paracasei SP5 carries a genome of 2,958,982 bp with a GC content of 46.3% and 2,920 predicted genes.
- ✓ WGS confirmed the classification of the strain to the *Lc. paracasei* species.
- The strains lacks plasmids, transposable and mobile elements, and does not code for acquired antibiotic resistance genes.
- ✓ The strain exhibits genomic features characteristic to nomadic lactobacilli.
- SP5 codes for several proteins that facilitate growth in diverse environments and under several stressors.
- ✓ SP5 may be able to **ferment different carbohydrates**, supporting its use in several fermented products.


Evaluation of the antimicrobial and antibiofilm activity of selected lactobacilli with probiotic activity

Bacterial warfare



Introduction



Introduction

The three novel lactobacilli code for bacteriocins



B

Regulation

Cleavage

40

The three novel lactobacilli code for bacteriocins



[Kiousi et al., 2023a]

The three novel lactobacilli code for bacteriocins



Pathogens



antibiotics

٠

•

٠

Live lactobacilli limit pathogen growth





В

	<i>S. aureus</i> (cm)	<i>S. enterica</i> (cm)	<i>E. coli</i> (cm)
Lp. pentosus L33	0.38 ± 0.17	0.33 ± 0.16	0.46 ± 0.16
Lp. pentosus L125	0.36 ± 0.2	0.22 ± 0.06	0.46 ± 0.15
Lc. paracasei SP5	0.35 ± 0.17	0.28 ± 0.11	0.56 ± 0.15
<i>Lc. rhamnosus</i> GG	0.3 ± 0.15	0.36 ± 0.08	0.53 ± 0.13
Lc. casei ATCC 393	0.35 ± 0.13	0.36 ± 0.15	0.54 ± 0.11

Live lactobacilli affect planktonic pathogen growth in a strain-specific basis





Live lactobacilli affect planktonic pathogen growth in a strain-specific basis





Results

[Kiousi et al., 2023a]

46

Lactobacilli CFCS limit the viability of planktonic pathogens in a dose- and pH-dependent manner



[Kiousi et al., 2023a]

Pathogen biofilms

Biofilms: a complex (poly)microbial community embedded in a matrix comprised by EPS, proteins and DNA

- Increased antibiotic resistance
- Immune evasion
- Contamination of surfaces
- During dispersion they can enter the bloodstream and cause systemic infections



Lactobacilli CFCS limit biofilm viability and mass

50% v/v CFCS, pH 6





[Kiousi et al., 2023a]

50



Results

[Kiousi et al., 2023a]

Effect of lactobacilli CFCS on the expression levels of biofilm-related genes

Staphylococcus aureus





Effect of lactobacilli CFCS on the expression levels of biofilm-related genes

Salmonella enteritidis





csgD

2.5

cxpR

2.5

[Kiousi et al., 2023a]

Effect of lactobacilli CFCS on the expression levels of biofilm-related genes

Escherichia coli





[Kiousi et al., 2023a]



Conclusions

- ✓ L33, L125 and SP5 code for **bacteriocins** and **small molecules** with antimicrobial activity.
- ✓ L125 also codes for a AHL lactonase, possibly involved in degradation of QS signals.
- ✓ LGG and Lc393 do not code for functional bacteriocins.
- CFCS derived from the five lactobacilli exhibited strain-, pathogen-, pH- and dose-dependent antimicrobial and antibiofilm activity.
- ✓ Lactobacilli CFCS also modulated the expression of **key genes involved in biofilm formation**.





Evaluation of the cytoprotective effects of lactobacilli against pathogen-induced cytotoxicity

The host-pathogen interface



Lactobacilli-pathogen-host interactions



S. aureus and E. coli limit HT-29 cell viability in a time-dependent manner



Pretreatment with L125 reduces pathogen-induced cell death





[Kiousi et al., 2024]

Direct contact of L125 with HT-29 cells prevents pathogen-induced cell death

Α





Direct contact of L125 with HT-29 cells prevents pathogen-induced cell death



E. coli

[Kiousi et al., 2024]

L125 inhibits pathogen invasion in HT-29 cells

Α

Results



[Kiousi et al., 2024]

⁶³

LGG limits S. aureus-induced cytotoxicity in keratinocytes

Multiple Proteins of Lacticaseibacillus rhamnosus GG Are Involved in the Protection of Keratinocytes From the Toxic Effects of Staphylococcus aureus

Cecile El-Chami^{1†}, Rawshan Choudhury^{1†}, Walaa Mohammedsaeed¹, Andrew J. McBain², Veera Kainulainen³, Sarah Lebeer⁴, Reetta Satokari³ and Catherine A. O'Neill^{1*}

(El-Chami et al., *Frontiers Microb* 2022)

- **Moonlighting proteins** (enolase, triosephosphate isomerase)
- **SpaC** (*spaCBA*) cluster

Lacticaseibacillus rhamnosus GG inhibits infection of human keratinocytes by Staphylococcus aureus through mechanisms involving cell surface molecules and pH reduction

I. Spacova¹, C. O'Neill² and S. Lebeer^{1*}

(Spacova et al., Benef Microbes 2020)

L125 codes for several putative adhesins

81 putative adhesins were annotated in the genome of L125:

- 28 are predicted to be localized in the cell wall/be extracellular
- Cell-wall associated domains: WxL, LysM, SH3 domains, LPxTG or LPxTG-like motifs, SLH or SLAP domains (S-layer proteins)
- Host-interacting domains: MucBPs, Fn, Cna_B, Big_3/_5 (Ig-like), LRRs



L125 codes for proteins that participate in microbe-host interactions









[Kiousi et al., 2024]

Dual RNA-seq



Transcriptomic profile of L125 in co-incubation with HT-29

Α



Category	L125 transcripts	
C, Energy production and conversion	4 (4.21%)	
D, Cell cycle control and mitosis	1 (1.05%)	
E, Amino Acid metabolism and transport	1 (1.05%)	
F, Nucleotide metabolism and transport	0 (0%)	
G, Carbohydrate metabolism and transport	4 (4.21%)	
H, Coenzyme metabolism	2 (2.11%)	
I, Lipid metabolism	3 (3.16%)	
J, Translation	22 (23.16%)	
K, Transcription	7 (7.37%)	
L, Replication and repair	5 (5.26%)	
M, Cell wall/membrane/envelop biogenesis	6 (6.32%)	
N, Cell motility	0 (0%)	
O, Post-translational modification, protein turnover,	C (C 2204)	
chaperone functions	0 (0.32%)	
P, Inorganic ion transport and metabolism	4 (4.21%)	
Q, Secondary Structure	0 (0%)	
T, Signal Transduction	3 (3.16%)	
U, Intracellular trafficking and secretion	1 (1.05%)	
V, Defense mechanisms	0 (0%)	
S, Function Unknown	12 (12.63%)	
No category, General function prediction only	14 (14.73%)	
Total	95 (100%)	

[Kiousi et al., 2024]

Lactobacilli-host co-incubation induces the production of adhesins in L125

	Locus tag	Protein annotation	TDM	
	(L125_)		IPM	
	RS10075	SpaA isopeptide-forming pilin-related protein	15.44	
	RS10525	peptide ABC transporter substrate-binding protein	13.45	
	RS11095	LPXTG cell wall anchor domain-containing protein	18.12	
	RS11130	WxL domain-containing protein	10.45	
	RS11335	MBG domain-containing protein	11.73	
	RS11350	BspA family leucine-rich repeat surface protein	9.57	
	RS11385	MucBP domain-containing protein	12.82	
	RS11935	LPXTG cell wall anchor domain-containing protein	0	
	RS12380	WxL domain-containing protein	32.59	
	RS01150	MucBP domain-containing protein	1.33	
	RS01490	SpaA isopeptide-forming pilin-related protein	1.75	
	RS02875	bacterial Ig-like domain-containing protein	12.78	
	RS13800	LamG-like jellyroll fold domain-containing protein	8.5	
	RS02315	CAP domain-containing protein	25.8	
	RS02875	bacterial Ig-like domain-containing protein	10.83	
	RS04395	WxL domain-containing protein	20.64	
	RS06780	KxYKxGKxW signal peptide domain-containing protein	9.29	
	RS07055	SEC10/PgrA surface exclusion domain-containing protein	12.42	
	RS07170	MucBP domain-containing protein	15.28	
	RS08445	WxL domain-containing protein	0.27	
	RS09470	LPXTG cell wall anchor domain-containing protein	17.45	
	RS09530	collagen-binding domain-containing protein	13.26	
	D010040	Extracellular protein, NlpC/P60 family,gamma-D-glutamate-	211.56	
	RS13040	meso-diaminopimelate muropeptidase		
_	RS01700	SH3 domain-containing protein	641.4	
	DC14000	extracellular protein, gamma-D-glutamate-meso-	1001.15	
	K514880	diaminopimelate muropeptidase	1661.45	
	RS05575	N-acetylmuramoyl-L-alanine amidase	171.39	
	RS08480	C40 family peptidase	963.72	
	RS09840	LysM domain-containing protein	1171.0	

69

Results

[Kiousi et al., 2024]

Lactobacilli-host co-incubation induces the production of adhesins in L125

Locus tag (L125_)	Protein annotation	TPM	
RS10155	Maltose phosphorylase mapA1	14.95	
RS06445	Maltose phosphorylase mapA2	1.4	
RS02920	Triosephosphate isomerase	621.45	
RS08655	Elongation factor Tu	8859.1	
RS02930	Glyceraldehyde-3-phosphate dehydrogenase	4938.06	
RS07515	Elongation factor G	488.97	
RS03220	10 kDa chaperonin	817.33	
RS03215	60 kDa chaperonin	1806.17	
RS05940	ATP-dependent 6-phosphofructokinase	217.86	
RS05465	Chaperone protein DnaK	447.11	
RS02915	Enolase	954.83	
RS01190	Glucose-6-phosphate isomerase	389.4	
RS05000	Glutamine synthetase	195.69	
RS10650	Ornithine carbamoyltransferase, catabolic	0	
RS02925	Phosphoglycerate kinase	486.27	
RS06220	Phosphoglycerate mutase GpmB	8.8	

[Kiousi et al., 2024]

L125 treatments alter the transcriptomic profile of HT-29 cells



L125 treatments downregulate central cellular pathways

ID	GeneSet	NumGenes	avg.logfc.dir
hsa04520	Adherens junction	72/72	-3.19
hsa03040	Spliceosome	134/134	-3.29
hsa05220	Chronic myeloid leukemia	71/71	-2.74
hsa04919	Thyroid hormone signaling pathway	116/116	-3.11
hsa04330	Notch signaling pathway	48/48	-3.3
hsa05016	Huntington's disease	193/193	-2.98
hsa00190	Oxidative phosphorylation	133/133	-2.62
hsa00310	Lysine degradation	59/59	-3.6
hsa05100	Bacterial invasion of epithelial cells	76/76	-2.84
hsa05213	Endometrial cancer	50/50	-2.7
hsa03015	mRNA surveillance pathway	91/91	-2.74
hsa05212	Pancreatic cancer	64/64	-2.59
hsa04152	AMPK signaling pathway	120/121	-3.00
hsa05010	Alzheimer's disease	171/171	-2.86
hsa04140	Regulation of autophagy	127/127	-2.08
hsa04144	Endocytosis	260/260	-2.65
hsa05205	Proteoglycans in cancer	203/203	-3.12
hsa04810	Regulation of actin cytoskeleton	211/212	-3.41
hsa05221	Acute myeloid leukemia	55/55	-2.99
hsa04120	Ubiquitin mediated proteolysis	137/137	-2.92


Conclusions

- Pretreatment (4h) with L125 in direct contact with HT-29 cells limited pathogen-induced viability. These effects were attributed to the capacity of the strain to competitively exclude pathogens and modulate cellular pathways involved in pathogen endocytosis.
- ✓ **Dual RNA-seq** was used for the first time to provide a **snapshot** of the **L125-HT-29 interactions** in co-incubation.



Take home message



Searching for causality.....



Searching for causality.....





#Correlation is not #Causation

...





Thank you!