AN INVESTIGATION ON THE ALLEVIATION AND REPAIR EFFECTS OF *BACILLUS PUMILUS* LV-149 SOLUTIONS AT DIFFERENT CONCENTRATIONS ON CELLULAR ULCERATIVE COLITIS IN MICE COLON CELLS OVER DSS SOLUTION - INDUCED TREATMENT

ANDY MA
School of Molecular and Cellular Biology, University of Illinois Urbana-Champaign

ABSTRACT
Bacillus pumilus (*B. pumilus*) is a facultative, gram-positive probiotic bacterium with immunomodulatory, anti-inflammatory, and antimicrobial properties. Previous studies have demonstrated its ability to inhibit pathogenic bacteria, promote intestinal health, and serve as a biological control agent. However, its potential role in alleviating and repairing inflammatory damage in ulcerative colitis (UC) remains under-explored. UC is a chronic inflammatory bowel disease characterized by disruption of the intestinal barrier, increased intestinal permeability, excessive production of pro-inflammatory cytokines, and decreased levels of tight-junction proteins. This study investigates the effects of different concentrations of *B. pumilus* LV-149 (1×10^8 CFU/mL and 1×10^9 CFU/mL) on UC-induced mice models treated with 3% DSS solution. Key inflammatory markers, including pro-inflammatory cytokines (IL-6, IL-1β), anti-inflammatory cytokine (IL-10), and tight-junction protein claudin-1, are measured at the gene expression level using real-time fluorescence quantitative polymerase chain reaction (qRT-PCR). The study aims to determine whether *B. pumilus* can mitigate intestinal inflammation by regulating cytokine production and restoring tight-junction integrity. By evaluating the impact of *B. pumilus* on the intestinal barrier and inflammatory response, this research provides new insights into its potential as a probiotic therapeutic for UC treatment. Findings from this study may contribute to the development of novel probiotic-based interventions for inflammatory bowel diseases, offering a safe and effective alternative to conventional treatments.

BACKGROUND

THE INTESTINAL BARRIER AND ULCERATIVE COLITIS

Mechanical barriers are the first line of defense for substances harmful to human health, effectively inhibiting the entry of pathogenic substances into the bloodstream (Fahey et al., 2018). The chemical barrier is mainly composed of antibacterial substances secreted by intestinal microorganisms, mucins and antimicrobial peptides secreted by intestinal epithelial cells, which can prevent intestinal contents from damaging the upper skin cells (Johansson et al., 2012). The intestinal permeability increases the severity of colitis by interacting with the inflammatory response (Kitajima et al.). The intestinal barrier in UC patients is destroyed, causing immune response and releasing a large number of inflammatory factors, triggering intestinal inflammation (Vancamelbeke et al., 2017). The goblet cells responsible for the mucus secretion in the intestine of UC patients are reduced, resulting in thinning or even disappearance of the mucus layer, and antigens invade exposed cells, exacerbating intestinal inflammation (Vindigni et al., 2016).

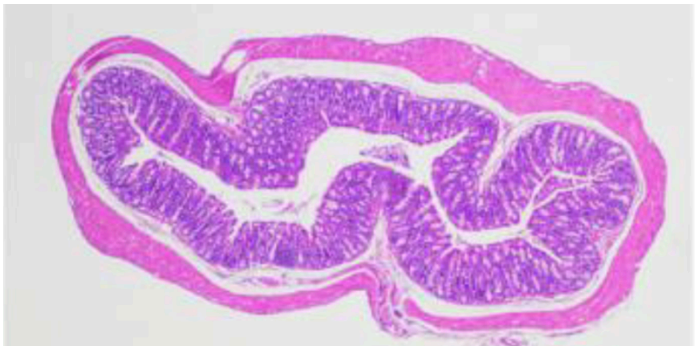

Figure 1: Mouse colon slice (Shao et al., 2021)

BACILLUS PUMILUS

B.pumilus is a short rod-shaped, facultative, gram-positive bacterium belonging to the *Bacillus* family genus, and is considered a probiotic. Probiotics produce molecules with immunomodulatory and anti-inflammatory functions, which regulate the immune system by stimulating epithelial, lymphocytes, or dendritic cells. (D'Amelio et al., 2018).

Studies show that including probiotics in shrimp feed could effectively inhibit intestinal pathogen growth, reduce the occurrence of vibrio disease, and be possessed with high biological safety (Luo & Jiang et al., 2013). Zeng et al. found that *B. pumilus* isolated from the intestines of yaks exhibited strong antioxidant, colonization, tolerance (acid, bile salt, and heat), as well as the ability to inhibit the growth of pathogenic bacteria. Dai et al. found that *B. pumilus* is a biological control bacterium, and the protein contained in the fermentation broth of the strain can resist fungal substances. Saggese et al. found that the SF214 strain of *Bacillus pumilus* isolated from the ocean can produce two different antibiotics, which have specific antibacterial effects against *Staphylococcus aureus* and *Listeria monocytogenes*. *B. pumilus* acts against pathogenic bacteria and is safe for biological usage. However, the optimal concentration of *B. pumilus* solution for the process and repair of inflammatory tissue has not yet been explored. As a probiotic with extremely high medicinal potential, *B. pumilus* could be utilized as an effective method for treating human ulcerative colitis.

COLONIC PRO AND ANTI-INFLAMMATORY CYTOKINES IN ULCERATIVE COLITIS

Although the etiology of UC has not been defined, numerous studies have shown that loss of anti-inflammatory factors or excessive production of pro-inflammatory factors induce the intestinal barrier structure. IL-6 is an environment-dependent multifunctional cytokine, which cannot only promote normal immunity and tissue regeneration, but can also regulate the proliferation of T cells and promote the production of other inflammatory factors (Hunter & Jones et al, 2015). IL-6 was found to play an important role in UC development, in addition to intensifying tissue inflammation by inhibiting T cell apoptosis, and also plays a repair function at the intestinal barrier (Friedrich et al, 2019). IL-1β is a typical pro-inflammatory cytokine that is crucial for the host's defense against infection and injury. IL-1β is expressed in macrophages of non-lymphoid organs such as lungs, digestive tract, and liver. Interleukin-10 (IL-10) is a multifunctional cytokine that can exert immunostimulatory effects in various types of cells. IL-10 can downregulate the expression of major histocompatibility antigen II on the surface of monocytes, and reduce its antigen presentation effect.

TIGHT JUNCTION PROTEINS IN ULCERATIVE COLITIS

Tight junction proteins (occludin, claudin, ZOs, etc.) are important components of the intestinal barrier. The content of claudins affects the formation of tight junction bands and the ion-selective permeability of intestinal epithelium. These proteins are connected to the epithelial cytoskeleton by actin and myosin and play key roles in tissue differentiation, maintaining intestinal permeability and homeostasis, and preventing invasion of enteric pathogens and toxins into the colonic barrier (Chen & Zhang et al., 2019). A study by Kitajima et al. states that, in DSS induced UC mice, the colonic mucosa did not show inflammatory cells initially, the colonic epithelial cells were shed later, and the expression of tight junction protein was reduced, leading to bacteria entering the intestinal mucosa and eventually an inflammatory response. Tan et al. found that abnormal expression of tight junction proteins caused intestinal mucosa to heal, and had a higher degree of mucosal healing. Therefore, the expression of the tight junction proteins may be an important indicator to evaluate the mucosal healing in UC.

DISUCCINIMIDYL SUBERATE INDUCED UC COLON CELLS

Antibodies were cross-linked to Protein A or G agarose beads using disuccinimidyl suberate (DSS, Fig. 2), a bifunctional cross-linker that reacts with two amine groups to form stable amide bonds (DeCaprio et al., 2019). A 3% DSS solution was also used to induce an inflammatory response and simulate ulcerative colitis in pre-cultured mouse colon cells.

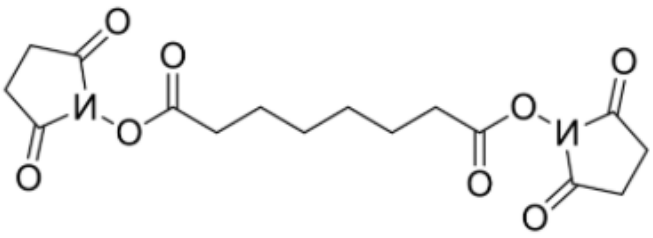

Figure 2: Chemical structure of disuccinimidyl suberate (Cayman Chemical, 2025)

HYPOTHESIS

ALTERNATIVE HYPOTHESIS

There will be a significant difference in the expression level of inflammatory factor genes (IL-1β, IL-6 and IL-10) and a tight junction protein gene (Claudin-1) between colon cells receiving high and low concentrations of LV149 probiotic solutions.

NULL HYPOTHESIS

There will be no significant difference in the expression level of inflammatory factors genes (IL-1β, IL-6 and IL-10) and a tight junction protein gene (Claudin-1) between colon cells receiving high and low concentrations of LV149 probiotic solutions.

EXPERIMENTAL DESIGN

INDEPENDENT AND DEPENDENT VARIABLES

The independent variable in this study was the concentration of *B. pumilus* LV-149 probiotic strain solutions, which were set at 1×10^8 CFU/mL and 1×10^9 CFU/mL. These concentrations were chosen to ensure optimal therapeutic effects on colon cells. Higher concentrations of the probiotic solution were hypothesized to have a stronger ability to alleviate and repair damaged UC colon cells compared to lower concentrations. The curative effects of the probiotic treatments were measured through gene expression levels. The dependent variable was the gene expression level of inflammatory factors and tight-junction proteins. The primary factors used to evaluate the therapeutic effects of LV-149 were pro-inflammatory cytokines IL-6 and IL-1β, the anti-inflammatory cytokine IL-10, and the tight-junction protein Claudin-1. It was expected that probiotic-treated samples would exhibit increased expression levels of IL-10 and Claudin-1 while reducing IL-6 and IL-1β, thereby indicating improved intestinal barrier function and reduced inflammation.

CONTROLLED VARIABLES

Several controlled variables were maintained throughout the experiment to ensure accuracy and consistency in results. Temperature control was essential as high temperatures could lead to the inactivation of proteases and fluorescent enzymes, potentially causing experimental failure. Repeated freezing and thawing inhibited enzyme activity and could affect the stability of the cDNA structure, ultimately influencing qPCR data. To mitigate this, solutions were prepared and RNA was extracted on a porous metal plate stored at 4°C, and reagents were frozen immediately after use. DSS processing time

was another critical factor since variations in exposure duration could lead to inconsistent inflammatory responses in the colon cells. To maintain consistency, all samples were subjected to DSS treatment for a strictly controlled period.

Similarly, the processing time of *B. pumilus* LV-149 was regulated, as prolonged exposure could influence cytokine secretion and immune protein production. The immersion period of cells in bacterial solutions was carefully controlled to ensure standardization across all experimental groups. RNA concentration consistency was also ensured, as variations in nucleic acid concentrations could lead to errors during amplification, affecting qPCR data reliability. RNA concentrations were meticulously measured using a UV-Vis spectrophotometer, and cDNA samples were thoroughly mixed with system buffers before spotting. Finally, oscillation and vortex duration were precisely controlled. Excessive vortexing could rupture cell membranes, leading to invalid qPCR amplification. To prevent this, oscillation was performed within a predetermined operational window to minimize the risk of excessive cell wall fragmentation.

MATERIALS

Table 1: Materials and apparatus in this study

Measurement Apparatus	General Apparatus	Materials
RT-qPCR instrument	Pipette gun	Trizol reagent
Invert microscope	Clean bench	Isopropanol
Analytic balance	Fume hood	RevertAidTM First Strand cDNA synthesis kit
Thermostat water bath	Ultra-low temp storage	oligo(dT)18 primer
Nucleic acid protein concentration analyzer	High-pressure steam Sterilization pot	Mice colon cells (bought from Shanghai Biotechnology Co., Ltd.)
PCR instrument	Ultra-pure water system	Chloroform
Volumetric flask	Electric blast drying oven	DEPC water
Measuring cylinder	Cell culture incubator	Cell culture medium
	Cell culture plate	Ethanol (anhydrous/75%)
	Vortex oscillator	RNA free enzyme water
		PCR tube Cooling Chamber

Table 2: Genetic sequence for Interleukin and reference gene primers

Genetic Sequence Used (F/R Primers)		
Primers		**Sequence**
IL-1β	**F**	GCAACTGTTCCTGAACTCAACT
	R	ATCTTTTGGGGTCCGTCAACT
IL-6	**F**	TAGTCCTTCCTACCCCAATTTCC
	R	TTGGTCCTTAGCCACTCCTTC
IL-10	**F**	GCTCTTACTGACTGGCATGAG
	R	CGCAGCTCTAGGAGCATGTG
Claudin-1	**F**	TATCATCCTGGCCGTGCTA
	R	CATCATCCACGCAGTTGGT
GAPDH	**F**	AGGTCGGTGTGAACGGATTT
	R	TGTAGACCATGTAGTTGAGGTCA

SAFETY, ETHICAL, AND ENVIRONMENTAL CONSIDERATIONS

Table 3: Safety, ethical, and environmental considerations

	Description	Methods taken
Safety	The experiment requires the usage of multiple hazardous chemicals and biological agents, which could lead to severe health issues, like skin and eye irritation, if some agents were accidentally intaken.	(1) Wear protective eye goggles and nitrile gloves. (2) Experimental procedures should be finished within the fume hood to prevent volatile liquid escape. (3) Follow standardized procedures.
Ethical considerations	Mice colon cells are used during the experiment and treated with reagents.	The mice colon cells have been previously extracted from experimental mice and target cells have been cultivated at the biological instrument company.
Environmental	The LV149 probiotic fluids, chemicals and different kinds of enzymes during the process of reverse transcription and extraction of total RNA and RT-qPCR could pollute the environment.	The chemicals and reagents used were strictly regulated. All chemicals should be collected using a waste tank instead of pouring into the sink directly. The wasted reagents will then be treated using professional methods.

PROCEDURES

Initially, pre-cultured mice colon cells were prepared. These cells were randomly separated into four groups. Afterwards, the colon cells were treated with DSS solution with the concentration of 3%, while the cells were immersed into the DSS solution for 18 hours to successfully induce the UC inflammatory response. Cells were then placed in a constant temperature shaker to ensure uniform mixing of reagents with each cell, reducing the possibility of experimental errors and failures. Cells, after being treated with 3% DSS solution, were rinsed three times with 0.9% physiological saline solution. All cell suspensions were loaded into EP tubes and

centrifuged at 12000 r/min for 10 minutes. The supernatant was retained and repackaged. One portion of the retained supernatant was used to conduct the experiment, and the rest were kept at -80 °C for future usage.

PREPARATION OF LIVE *B. PUMILUS* LV-149 SUSPENSION

B. pumilus LV-149, stored at -80°C, was inoculated into LB medium and incubated at 37°C for 12 hours to generate seed cultures. Seed culture (5% v/v) was transferred into fresh LB liquid medium and incubated at 37°C for an additional 5 hours. Bacterial cells were harvested by centrifugation at 6500g for 5 minutes at 4°C. The pellet was washed twice with 0.9% physiological saline solution (PSS) and re-suspended in PSS to achieve a final concentrations of 1×10^8 CFU/mL and 1×10^9 CFU/mL.

DSS-INDUCED UC MODEL

Mice colon cells were pre-cultured under standard conditions. Cells were randomly divided into four experimental groups. The UC model was induced by exposing cells to a 3% DSS solution for 18 hours, ensuring an adequate inflammatory response. The cells were kept in a constant temperature shaker to maintain homogeneous reagent mixing. After DSS treatment, cells were washed three times with 0.9% physiological saline solution to remove residual DSS. The cells were then harvested, suspended in EP tubes, and centrifuged at 12,000 r/min for 10 minutes. The supernatant was retained for further experiments, while remaining samples were stored at -80°C for future use.

RNA EXTRACTION AND RT-qPCR

Total RNA was extracted using Trizol reagent. Briefly, 100 mg of treated cells was transferred into an EP tube, followed by the addition of 1 mL Trizol. The mixture was vortexed thoroughly, and 250 µL of chloroform was added. After mixing and resting at room temperature for 3 minutes, samples were centrifuged, and the aqueous phase was transferred to a new tube. RNA was precipitated by adding 0.8× volume of isopropanol, followed by centrifugation. The pellet was washed with ethanol, dried, and re-suspended in 100 µL RNase-free water. The RNA concentration was determined using a UV-Vis spectrophotometer and standardized to 100 ng/µL before storage at -80°C.

Reverse transcription to cDNA was performed using the RevertAidTM First Strand cDNA Synthesis Kit. cDNA synthesis was carried out at 42°C for 1 hour, followed by enzyme inactivation at 70°C for 5 minutes. For qRT-PCR, reactions were prepared in a 96-well plate using SYBR Green Master Mix, gene-specific primers, and cDNA template. GAPDH was used as an internal reference gene. Each reaction was performed in triplicate. The 96-well plate was sealed with thermoplastic film, placed in a qPCR instrument, and subjected to thermal cycling and fluorescence detection.

Figure 3: Plate setup of 96-well plate containing GAPDH & inflammatory cytokine genes

DATA COLLECTION

QUALITATIVE DATA

There is no significant qualitative data presented, since the investigations are mainly based on micro-aspects of genes and protein levels of cells.

QUANTITATIVE DATA

Due to the extensively large sample data, one qPCR data of Interleukin and its Ct value calculation will be retained in the article, while the rest will be presented in the Appendix section at the end of the article. The Ct (cycle threshold) value refers to the number of PCR cycles required for the fluorescence signal in each reaction tube to exceed a predefined threshold, indicating the point at which the target nucleic acid becomes detectable above background levels. Ct values correspond to initial quantities of DNA/RNA, making Ct useful for expressing relative gene expression.

Table 4: Ct value of Interleukin-1β in mice colon cells collect from qPCR instrument under the treatment of DSS solution, high, and low dose of LV-149 probiotic strain solution

Interleukin-1β RT-qPCR Ct Value Data											
Group	GAP	IL-1β	Group	GAP	IL-1β	Group	GAP	IL-1β	Group	GAP	IL-1β
C_1	18.97	29.40	D_1	22.13	27.41	H_1	20.33	27.95	L_1	23.91	31.17
	18.49	28.82		22.43	27.21		20.53	27.96		24.53	30.8
	18.7	29.75		22.34	27.11		20.27	27.95		24.48	31.42
C_2	19.78	31.12	D_2	22.05	27.04	H_2	21.17	28.2	L_2	23.07	29.68
	19.48	30.65		22.13	27.01		20.29	28.07		22.77	29.53
	19.74	30.63		22.16	27.06		20.14	28.12		22.35	29.55
C_3	19.29	30.77	D_3	22.95	27.87	H_3	19.23	26.51	L_3	23.00	29.73
	19.63	30.86		22.84	28.1		19.08	26.77		23.19	29.82
	19.66	30.58		23.89	28.6		19.32	26.97		22.87	29.91
C_4	19.54	30.62	D_4	23.09	28.01	H_4	18.97	26.40	L_4	18.38	25.26
	19.94	30.89		23.26	28.01		18.44	26.37		18.52	25.59
	19.89	31.6		23.55	28.87		19.27	26.59		18.62	25.19

DATA PROCESSING

The mean value of the number of the Ct values corresponding to every inflammatory indicator will be calculated for four categories: the control, DSS-intervenue, high, and low groups.

$$Average(\overline{X}) = \frac{Total\ value\ of\ all\ trials}{Number\ of\ trials}$$

Taking the data of the Interleukin-10 group as an example:

Table 5: Calculation of mean Ct values

Ct	C_1			C_2			C_3			C_4		
IL-10	32.69	32.05	32.02	32.79	32.95	33.8	32.74	32.52	32.79	31.59	31.73	31.41
$Mean_1$	32.25			33.18			32.68			31.58		
GAP	18.33	18.12	18.09	19.55	19.25	19.51	18.61	19.02	18.87	17.23	17.44	17.44
$Mean_2$	18.18			19.44			18.83			17.37		
M_1-M_2	32.25 - 18.18 = 14.07			33.18 - 19.44 = 13.74			32.68 - 18.83 = 13.85			31.58 - 17.37 = 14.21		
$Mean_3$	Average (C) = $\frac{14.07 + 13.74 + 13.85 + 14.21}{4}$ = 13.97											

Standard deviation was also calculated for the Ct values corresponding to every indicator, and the value of target genes was compared with GAPDH. Taking the data for the Ct values of GAPDH in the IL-10 control group as an example:

Table 6: Calculation of standard deviation of Ct values using data collected from the IL-10 control group

GAP	Average($\overline{X}$)	$(X - \overline{X})$	$(X - \overline{X})^2$	$\sigma = \sqrt{\frac{(X-\overline{X})^2}{n-1}}$
18.33	$\overline{X} = \frac{18.33+18.12+18.09}{3}$ = 18.18	-0.15	0.023	$\sigma = \sqrt{\frac{0.023+0.004+0.008}{3-1}}$ = $\sqrt{0.0175}$ = 0.132
18.12		0.06	0.004	
18.09		0.09	0.008	

After obtaining the Ct mean values of the internal reference gene GAPDH and the inflammatory factor gene, the average subtraction values between the two sets of data was calculated using biological replicates.

Taking the Ct of GAPDH and IL-10 as an example:

$$\overline{\overline{\Delta Ct}} = \frac{\overline{\Delta Ct}_{C1} + \overline{\Delta Ct}_{C2} + \overline{\Delta Ct}_{C3}}{number\ of\ biological\ replicates}$$

The relative quantification was also calculated:

$$RQ = 2^{-\overline{\overline{\Delta\Delta Ct}}}$$

The purpose of relative quantification is to determine the relative proportion of the content of the target gene in two or more samples, without the need to know their copy number in each sample.

DATA ANALYSIS

GRAPHICAL ANALYSIS

The relative quantification obtained through the calculation of the Ct values of the target gene and the housekeeping gene is presented in a bar chart in figures 4-7, showing the general trend of the data. Relative quantification is used to determine the difference in the amount of target genes in two or more different samples, and the obtained data is the relative proportion of the content of target genes in each sample.

The Ct values of target genes in the experimental sample will be compared with those in the control sample. The data obtained from relative quantification reflect the fold change between the starting amount of the target gene and the amount detected after amplification. It is usually used in the data analysis of fluorescence real-time quantitative PCR to determine the changes of the target gene before and after treatment.

In this study, a single factor analysis of variance (ANOVA) test was conducted to collect statistical models between the average values under the influence of a specific independent variable at different levels. If the difference between the control group, DSS induced inflammation group and high-dose group among the four genes is statistically significant in the experimental group, it can fully prove the alternative hypothesis. A p value less than 0.05 indicates statistical significance, suggesting that the likelihood of the observed differences occuring by chance is very low, and thus the null hypothesis can be rejected.

A. **Relative Quantitation of target gene IL-1β**

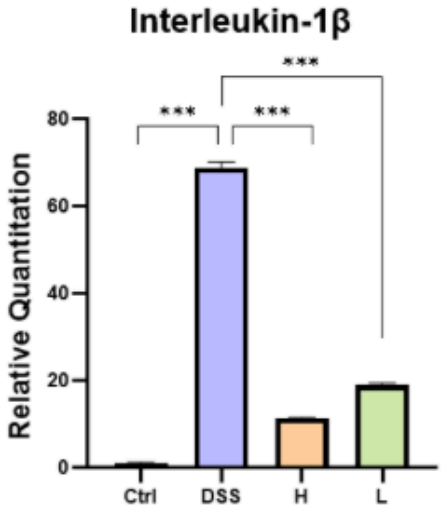

Figure 4: Relative quantitation of target gene IL-1β. The values are expressed as means + SE of three parallel trials (n=3). The number of asterisks (*) next to the bars represents level of significant difference ***P ≤ 0.00l by single-factor ANOVA analysis using PRISM (8.0.2)

B. **Relative Quantitation of target gene IL-6**

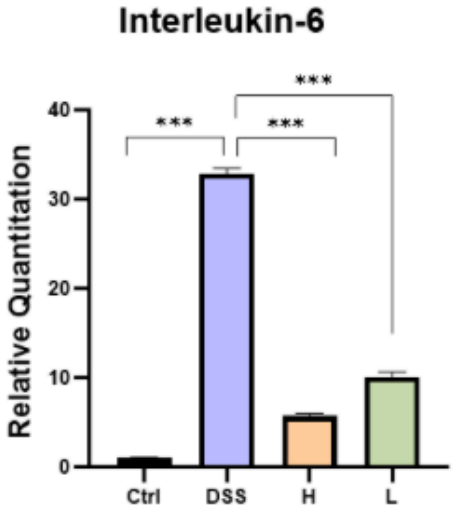

Figure 5: Relative quantitation of target gene IL-6. The values are expressed as means + SE of three parallel trials (n=3). The number of asterisks (*) next to the bars represents level of significant difference ***P ≤ 0.00l by single-factor ANOVA analysis using PRISM (8.0.2)

C. **Relative Quantitation of target gene IL-10**

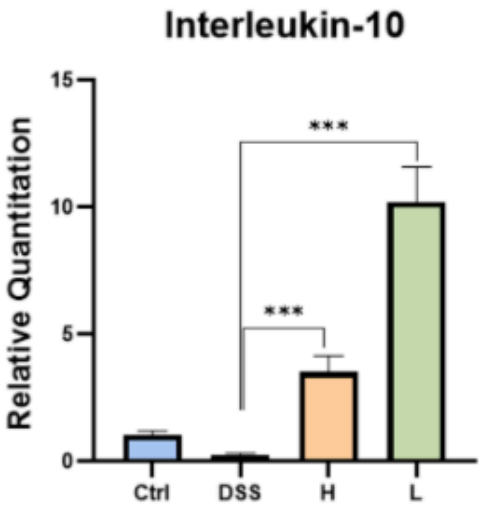

Figure 6: Relative quantitation of target gene IL-10. The values are expressed as means + SE of three parallel trials (n=3). The number of asterisks (*) next to the bars represents level of significant difference ***P ≤ 0.00l by single-factor ANOVA analysis using PRISM (8.0.2)

D. **Relative Quantitation of target gene Claudin-1**

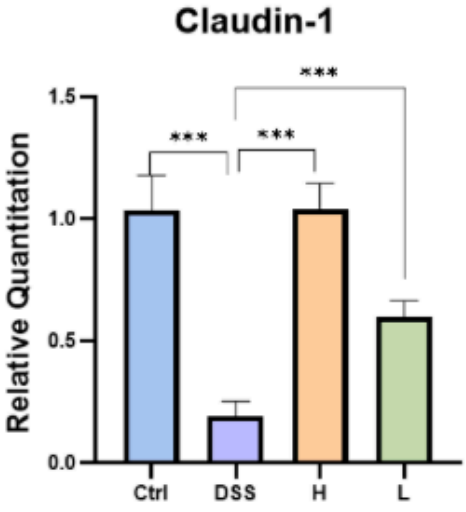

Figure 7: Relative quantitation of target gene Claudin-1. The values are expressed as means + SE of three parallel trials (n=3). The number of asterisks (*) next to the bars represents level of significant difference ***P ≤ 0.00l by single-factor ANOVA analysis using PRISM (8.0.2)

RESULT ANALYSIS

Both alternative hypotheses have been fully demonstrated in the experiment. There will be a significant difference in the expression level of inflammatory factor genes (IL-1β, IL-6 and IL-10) and a tight junction protein gene (Claudin-1) between colon cells receiving high and low concentrations of LV149 probiotic solutions.

INTERLEUKIN-1β

It is observed from the graph that the DSS group has the greatest value of relative quantification: up to 68.87. The high-dose group leads to an decrement of relative quantification level to 11.35, and the low-dose group exhibits a relative quantification level of 18.96. Data collected fully proves the expression of the proinflammatory factor gene in the four experimental groups.

INTERLEUKIN-6

As a pro-inflammatory and anti-inflammatory factor similar to IL-1β, IL-6 showed a significant increase in expression in the DSS treatment group. In the treatment of high-dose *B. pumilus* solution, IL-6 showed a relatively lower expression level, reaching a relative quantification of 5.72, and a relative quantification of 10.09 under low-dose treatment with probiotics. The results reflect the therapeutic effect of IL-6 on probiotics, especially in high concentration solutions with sensitive reactions.

INTERLEUKIN-10

IL-10 is an anti-inflammatory factor gene. In the DSS-induced group, the relative expression of the IL-10 gene decreased to 0.274 compared to the control group. In the high-dose group, the IL-10 gene showed a relative quantitative value of 3.52, which was far less than the relative quantitative value of 10.19 in the low-dose probiotic group.

CLAUDIN-1

Claudin-1 is an indicator reflecting the permeability of intestinal epithelial cell barrier. According to the chart, the DSS induced inflammation group showed a lower expression compared to the control group, reaching a relative quantification value of 0.192. In the high and low dose groups, the relative quantitative values were 1.04 and 0.59, respectively.

DISCUSSION

In the preliminary experiment, using an interleukin gene as the target, it was ultimately determined that the expression level of the inflammation related gene was significantly increased in the bacterial solution at two concentrations of 1×10^8 CFU/mL and 1×109 CFU/mL within the range of 1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9 and 1×10^{10} CFU/mL. Therefore, within the scope of this article's exploration, the changes in the expression levels of three interleukin genes (IL-1β, IL-6 and IL-10) and a tight junction protein gene (Claudin-1) were investigated using the concentrations of these two bacterial solutions as variables.

AN EXPLANATION OF THE POSITIVE CORRELATION BETWEEN LV-149 AND THE ALLEVIATION OF UC

Further evidence suggests that the colonization of probiotics in the intestine helps to form an antibacterial environment and reduce the production of pro-inflammatory cytokines. There are reports that *B. pumilus* and its metabolites can activate the NF- κB signal NLRP3 inflammasome and inhibit IL-1β secretion (Ahn et al., 2011). This study has also yielded similar results, with supplementation of *B. pumilus* reducing IL-1β, IL-6, TNF-α and increasing the level of IL-10. One possible explanation for these findings is that activated PPAR-γ mediates the inhibition of pro-inflammatory cytokines such as TNF-α and IL-1β. Additionally, the production of IL-6 may contribute to anti-inflammatory effects. TGF-β plays a role in reducing intestinal mucosal damage by inhibiting the production of inflammatory factors from macrophages (Maheshwari et al., 2011). In this experiment, the addition of *Bacillus pumilus* increased IFN-γ levels. Although IFN-γ is an effective immune molecule against pathogenic microbes, its abnormal elevation is associated with autoimmune dysregulation and alterations in the gut microbiota. Excessive IFN-γ activation can also lead to tissue damage, necrosis, and inflammation (Sun & Guo et al., 2022). The intestinal mucosal layer is secreted by goblet cells distributed throughout the gastrointestinal tract. In this study, administration of *B. pumilus* appeared to increase the thickness of the muscular layer, possibly due to improvements

in gut microbiota composition. Notably, the expression of Claudin-1, a key tight junction protein, was elevated in the experimental group. This aligns with findings by Sheng et al., who reported that *B. pumilus* reduced epithelial barrier damage by upregulating tight junction protein expression. The observed changes in Claudin-1 expression in our study were consistent across the three treatment groups.

CONCLUSION

The analysis in this study considers the characteristics of each gene. IL-6 and IL-1 β are proinflammatory factor genes–that is, they are largely expressed in inflammatory conditions. The DSS group in the chart often has a higher relative quantitative value compared with the control group and the high-dose group, which also proves the correctness of this theory from the experimental level. IL-10 is an anti-inflammatory factor gene, and has a relatively sensitive response to low-dose probiotics–that is, the best concentration of efficacy. Claudin-1, to a certain extent, acts as a response to the permeability of intestinal epithelial cells–that is, its expression level is reduced in the DSS induced group due to the thinning of the intestinal barrier caused by inflammation. In the following high and low dose groups, there was a significant increase in the expression level of the Claudin-1 gene, with the high-dose group showing the most significant increase. This shows that the concentration of probiotic solution for the best therapeutic effect belongs to the high-dose group, which has the important function of improving intestinal mucosal permeability.

FUTURE DIRECTIONS

This experiment successfully evaluated the alleviating effect of *B. pumilus* LV149 on UC mice and the repairing effect of this bacteria on colon mucosal damage. Additionally, the study effectively provided theoretical guidance for the future relief and treatment of ulcerative colitis with live probiotics. While probiotics such as *Bacillus pumilus* have been explored for their potential in treating human diseases, there are currently no published studies specifically investigating the role of *B. pumilus* in alleviating clinical symptoms of UC or in repairing colonic mucosal damage

At present, the screening of probiotics is mainly determined based on their tolerance to gastrointestinal conditions (gastric acid and bile), ability to adhere to the gastrointestinal mucosa, and competitive rejection ability against pathogens. However, for probiotics to have a positive effect in the host's body, they must have a significant beneficial effect on the host, be non toxic, be non pathogenic, and have no obvious adverse effects. They also must be able to survive in the gastrointestinal tract with a sufficient number of cells and maintain probiotic properties under storage and processing conditions. Numerous studies have reported the potential clinical efficacy of probiotics and their formulations in the treatment of many diseases, such as functional dyspepsia.

Although modern molecular technology has become popular, the mechanisms involved are explored through changes in certain protein levels to explore possible pathways. Further studies may delve into the transcriptome gene differences and explore the role of *B. pumilus* in alleviating UC, which is a relatively novel and accurate biological method. Under the conditions of this experiment, *B. pumilus* can improve the morphology of colon tissue, regulate the content of inflammatory cytokines and related serum indicators, increase the content of colonic tight junction protein, and reduce tissue inflammatory cell infiltration.

REFERENCES

1. Ahn, Huijeong, et al. “Poly-Gamma-Glutamic Acid from Bacillus Subtilis Upregulates Pro-Inflammatory Cytokines While Inhibiting NLRP3, NLRC4 and AIM2 Inflammasome Activation.” *Cellular & Molecular Immunology*.

2. Cayman Chemical. *Disuccinimidyl Suberate (DSS)*.

3. Chen, Yun, et al. “A Role of Exopolysaccharide Produced by Streptococcus Thermophilus in the Intestinal Inflammation and Mucosal Barrier in Caco-2 Monolayer and Dextran Sulphate Sodium-Induced Experimental Murine Colitis.” *Molecules*, vol. 24, no. 3, 31 Jan. 2019, p. 513

4. Dai, Yun, et al. “Medium Optimization to Analyze the Protein Composition of Bacillus Pumilus HR10 Antagonizing Sphaeropsis.” *AMB Express.*

5. D'Amelio, P., and F. Sassi. "Gut Microbiota, Immune System, and Bone." *Calcified Tissue International*, vol. 102, no. 4, 30 Sept. 2017, pp. 415–425.

6. DeCaprio, James, and Thomas O. Kohl. "Cross-Linking Antibodies to Beads with Disuccinimidyl Suberate (DSS)." *Cold Spring Harbor Protocols*, vol. 2019, no. 2, Feb. 2019, p. pdb.prot098632.

7. Fahey, James K., et al. "The Intercellular Tight Junction and Spontaneous Coronary Artery Dissection." *Journal of the American College of Cardiology*, vol. 72, no. 14, 1 Oct. 2018, pp. 1752–1753.

8. Friedrich, Matthias, et al. "Cytokine Networks in the Pathophysiology of Inflammatory Bowel Disease." *Immunity*, vol. 50, no. 4, Apr. 2019, pp. 992–1006.

9. Hunter, Christopher A., and Simon A. Jones. "IL-6 as a Keystone Cytokine in Health and Disease." *Nature Immunology*, vol. 16, no. 5, 21 Apr. 2015, pp. 448–457.

10. Johansson, Malin E. V. "Fast Renewal of the Distal Colonic Mucus Layers by the Surface Goblet Cells as Measured by in Vivo Labeling of Mucin Glycoproteins." *PLoS ONE*, vol. 7, no. 7, 17 July 2012, p. e41009.

11. KITAJIMA, Shuji, et al. "Changes in Colonic Mucosal Permeability in Mouse Colitis Induced with Dextran Sulfate Sodium." *Experimental Animals*, vol. 48, no. 3, 1999, pp. 137–143.

12. Luo, Peng, et al. *Preparation of Bacillus Short Bacteria and Probiotics and Its Preparation Method and Application*. 19 June 2013.

13. Maheshwari, Akhil, et al. "TGF-β2 Suppresses Macrophage Cytokine Production and Mucosal Inflammatory Responses in the Developing Intestine." *Gastroenterology*, vol. 140, no. 1, 1 Jan. 2011.

14. Merga, Yvette, et al. "Mucosal Barrier, Bacteria and Inflammatory Bowel Disease: Possibilities for Therapy." *Digestive Diseases*, vol. 32, no. 4, 2014, pp. 475–483,

15. Saggese, Anella, et al. "An Antimicrobial Peptide Specifically Active against Listeria Monocytogenes Is Secreted by Bacillus Pumilus SF214." *BMC Microbiology*, vol. 22, no. 1, 3 Jan. 2022.

16. Shao, Ying, et al. "The Role of Intestinal Microbiota in Inflammatory Bowel Disease." *Antibiotics*, vol. 10, no. 6, 2021, p. 643.

17. Sheng, Kangliang, et al. "Probiotic Bacillus Cereus Alleviates Dextran Sulfate Sodium-Induced Colitis in Mice through Improvement of the Intestinal Barrier Function, Anti-Inflammation, and Gut Microbiota Modulation." *Journal of Agricultural and Food Chemistry*, vol. 69, no. 49, 22 Oct. 2021, pp. 14810–14823.

18. Sun, Lei, et al. "Quercetin Reduces Inflammation and Protects Gut Microbiota in Broilers." *Molecules*, vol. 27, no. 10, 19 May 2022, p. 3269.

19. Tan, Yue, et al. "Correlation of Intestinal Mucosal Healing and Tight Junction Protein Expression in Ulcerative Colitis Patients." *The American Journal of the Medical Sciences*, vol. 357, no. 3, 1 Mar. 2019, pp. 195–204.

20. Vancamelbeke, Maaike, et al. "Genetic and Transcriptomic Basis of Intestinal Epithelial Barrier Dysfunction in Inflammatory Bowel Disease." *Inflammatory Bowel Diseases*, vol. 23, no. 10, 1 Oct. 2017, pp. 1718–1729.

21. Vindigni, Stephen M., et al. "The Intestinal Microbiome, Barrier Function, and Immune System in Inflammatory Bowel

22. Zeng, Zhibo, et al. "Probiotic Potential of Bacillus Licheniformis and Bacillus Pumilus Isolated from Tibetan Yaks, China." *Probiotics and Antimicrobial Proteins*, 20 Apr. 2022, pp. 579–594.

Table 7: Calculation of Standard deviations of Ct values of IL-1β, IL-6, IL-10 and Claudin

Standard Deviation of Ct values				
	IL-1β	**IL-6**	**IL-10**	**Claudin-1**
$C_{1\ STD}$	0.106	0.038	0.068	0.117
$C_{2\ STD}$	0.062	0.095	0.143	0.204
$C_{3\ STD}$	0.076	0.031	0.087	0.041
$C_{4\ STD}$	0.111	0.077	0.057	0.178
$D_{1\ STD}$	0.079	0.060	0.106	0.071
$D_{2\ STD}$	0.037	0.019	0.055	0.125
$D_{3\ STD}$	0.029	0.063	0.024	0.127
$D_{4\ STD}$	0.079	0.136	0.039	0.246
$H_{1\ STD}$	0.137	0.056	0.107	0.062
$H_{2\ STD}$	0.049	0.050	0.115	0.099
$H_{3\ STD}$	0.062	0.064	0.057	0.031
$H_{4\ STD}$	0.015	0.107	0.094	0.0391
$L_{1\ STD}$	0.137	0.054	0.058	0.034
$L_{2\ STD}$	0.056	0.086	0.226	0.163
$L_{3\ STD}$	0.068	0.023	0.027	0.132
$L_{4\ STD}$	0.055	0.138	0.104	0.128

Table 8: IL-1β data Ct processing table

IL-1β Data Ct processing table				
Control	C_{M1}	C_{M2}	C_{M3}	C_{M4}
GAP	18.72	19.67	19.53	18.79
IL-1β	29.32	30.8	30.74	31.037
D-value	10.60	11.13	11.21	12.25
Mean $_{\text{D-value}}$	$\text{Average}_{Ct}\,(C) = \frac{10.60 + 11.13 + 11.21 + 12.25}{4} = 11.29$			
DSS	D_{M1}	D_{M2}	D_{M3}	D_{M4}
GAP	20.3	22.81	23.36	21.3
IL-1β	29.24	27.19	28.19	28.29
D-value	8.94	4.37	4.83	6.99
Mean $_{\text{D-value}}$	$\text{Average}_{Ct}\,(D) = \frac{8.94 + 4.37 + 4.83 + 6.99}{4} = 6.29$			
High	H_{M1}	H_{M2}	H_{M3}	H_{M4}
GAP	21.69	21.57	19.21	18.46
IL-1β	27.79	27.03	24.75	26.45
D-value	6.09	5.47	5.54	7.99
Mean $_{\text{D-value}}$	$\text{Average}_{Ct}\,(H) = \frac{6.09 + 5.47 + 5.54 + 7.99}{4} = 6.27$			
Low	L_{M1}	L_{M2}	L_{M3}	L_{M4}
GAP	24.31	22.16	23.25	18.51
IL-1β	31.13	27.59	28.62	25.61
D-value	6.82	5.42	5.37	7.11
Mean $_{\text{D-value}}$	$\text{Average}_{Ct}\,(L) = \frac{6.82 + 5.42 + 5.37 + 7.11}{4} = 6.18$			

Table 9: Relative quantification value of four genes calculated using the logarithm method.

Relative quantification of qPCR data				
	$C_{D\text{-value}}$	$D_{D\text{-value}}$	$H_{D\text{-value}}$	$L_{D\text{-value}}$
IL-1β	1	-5.013	-5.026	-5.118
RQ ($2^{-\overline{\overline{\Delta\Delta Ct}}}$)		32.278	32.578	34.735
IL-6	1	-4.894	-7.941	-5.683
RQ ($2^{-\overline{\overline{\Delta\Delta Ct}}}$)		29.737	245.714	51.357
IL-10	1	-2.904	-2.690	-3.097
RQ ($2^{-\overline{\overline{\Delta\Delta Ct}}}$)		7.486	6.453	8.554
Claudin-1	1	0.752	0.510	0.278
RQ ($2^{-\overline{\overline{\Delta\Delta Ct}}}$)		0.594	0.702	0.825

Table 10: Ct value of Interleukin-6 in mice colon cells collected from qPCR instrument under the treatment of DSS solution, high, and low dose of LV-149 probiotic strain solution

Interleukin-6 RT-qPCR Ct Value Data										
group	GAP	IL-6	group	GAP	IL-6	group	GAP	IL-6	group	GAP	IL-6
C_1	20.37	35.32	D_1	21.44	30.79	H_1	21.01	33.22	L_1	21.72	33.12
	20.28	35.19		20.95	31.19		20.85	33.38		21.59	33.04
	20.34	35.51		20.7	31.14		20.82	33.44		21.51	33.27
C_2	20.01	35.01	D_2	21.58	31.53	H_2	20.21	32.19	L_2	21.56	33.49
	20.07	35.26		21.63	31.64		20.04	32.88		21.43	33.24
	20.19	35.25		21.66	31.53		20.07	32.56		21.95	33.31
C_3	20.38	35.92	D_3	21.1	31.44	H_3	20.11	32.42	L_3	21.68	33.13
	20.21	34.94		21.32	31.43		20.01	32.34		21.68	33.31
	20.28	35.02		21.14	30.53		19.82	32.4		21.56	33.35
C_4	20.34	34.97	D_4	20.97	30.54	H_4	18.82	31.69	L_4	20.32	31.59
	20.57	35.14		21.12	30.91		19.31	31.46		19.59	31.32
	20.25	35.34		20.07	30.8		18.97	31.75		19.37	31.65

APPENDIX - 5

Table 11: Ct value of Interleukin-10 in mice colon cells collected from qPCR instrument under the treatment of DSS solution, high, and low dose of LV-149 probiotic strain solution

Interleukin-10 RT-qPCR Ct Value Data											
group	GAP	IL-10	group	GAP	IL-10	group	GAP	IL-10	group	GAP	IL-10
C_1	18.33	32.69	D_1	18.14	34.65	H_1	21.47	33.98	L_1	19.73	30.08
	18.12	32.05		18.08	34.19		21.47	33.71		19.8	30.25
	18.09	32.02		18.53	34.26		21.65	33.78		19.43	30.19
C_2	19.55	32.79	D_2	18.32	34.41	H_2	22.18	34.31	L_2	22.09	31.91
	19.25	32.95		18.58	34.32		22.59	34.04		22.25	33.73
	19.51	33.8		18.42	34.51		22.61	34.97		22.55	33.1
C_3	18.61	32.74	D_3	18.98	34.52	H_3	22.95	35.14	L_3	22.34	33.28
	19.02	32.52		18.96	34.46		23.18	35.19		22.24	33.26
	18.87	32.79		18.23	34.6		23.05	34.82		22.54	33.36
C_4	17.23	31.59	D_4	18.18	33.71	H_4	22.43	35.3	L_4	23.17	33.54
	17.44	31.73		18.08	33.85		23	35.18		23.2	34.03
	17.44	31.41		18.22	33.71		22.95	35.45		23.01	33.71

Table 12: Ct value of Claudin-1 in mice colon cells collected from qPCR instrument under the treatment of DSS solution, high, and low dose of LV-149 probiotic strain solution

Claudin-1 RT-qPCR Ct Value Data											
group	GAP	Claudin-1	group	GAP	Claudin-1	group	GAP	Claudin-1	group	GAP	Claudin-1
C_1	19.35	29.91	D_1	20.49	33.07	H_1	23.92	33.76	L_1	20.18	31.35
	19.92	29.94		20.49	33.05		23.94	34.06		19.96	31.28
	19.41	29.39		20.01	33.13		23.79	34.08		20.37	31.44
C_2	19.18	28.48	D_2	20.59	33.05	H_2	22.98	32.88	L_2	22.89	33.69
	19.23	29.48		20.53	33.21		22.97	33.59		22.73	33.62
	19.18	29.96		20.38	33.72		22.82	33.45		22.95	33.89
C_3	19.87	30.2	D_3	20.24	33.38	H_3	23.69	33.85	L_3	22.77	33.29
	19.89	30.24		20.43	33.15		23.38	33.82		22.74	33.53
	19.78	30.38		20.1	33.9		23.43	33.82		23.11	34.57
C_4	19.23	29.77	D_4	22.54	34.46	H_4	23.21	33.37	L_4	22.53	33.53
	19.37	29.55		22.26	34.4		23.36	33.3		22.39	33.21
	19.26	29.53		22.41	34.73		23.16	33.37		22.65	34.36

Table 13: IL-6 Ct data processing table

IL-6 Data Ct processing table				
Control	**C_{M1}**	**C_{M2}**	**C_{M3}**	**C_{M4}**
GAP	20.33	20.09	20.29	20.39
IL-6	34.34	35.41	35.96	35.15
D-value	14.01	15.32	15.67	14.76
Mean $_{D\text{-value}}$	**$Average_{Ct}$ (C)** = $\frac{14.01 + 15.32 + 15.67 + 14.76}{4}$ = 14.94			
DSS	**D_{M1}**	**D_{M2}**	**D_{M3}**	**D_{M4}**
GAP	21.03	21.62	21.19	20.72
IL-6	31.53	31.57	30.77	30.88
D-value	10.49	9.94	9.58	10.16
Mean $_{D\text{-value}}$	**$Average_{Ct}$ (D)** = $\frac{10.49 + 9.94 + 9.58 + 10.16}{4}$ = 10.05			
High	**H_{M1}**	**H_{M2}**	**H_{M3}**	**H_{M4}**
GAP	23.89	22.07	20.05	19.0
IL-6	30.81	26.21	26.39	29.63
D-value	6.92	4.14	6.34	10.6
Mean $_{D\text{-value}}$	**$Average_{Ct}$ (H)** = $\frac{6.92 + 4.14 + 6.34 + 10.6}{4}$ = 6.99			
Low	**L_{M1}**	**L_{M2}**	**L_{M3}**	**L_{M4}**
GAP	21.61	21.65	21.64	18.76
IL-6	33.57	30.21	29.36	27.52
D-value	11.98	8.57	7.72	8.76
Mean $_{D\text{-value}}$	**$Average_{Ct}$ (L)** = $\frac{11.98 + 8.57 + 7.72 + 8.76}{4}$ = 9.26			

Text

Table 14: IL-10 Ct data processing table

IL-10 Data Ct processing table				
Control	C_{M1}	C_{M2}	C_{M3}	C_{M4}
GAP	18.18	19.44	18.83	17.37
IL-10	32.25	33.18	32.68	31.58
D-value	14.07	13.74	13.85	14.21
Mean $_{\text{D-value}}$	$\text{Average}_{Ct}\,(C) = \frac{14.07 + 13.74 + 13.85 + 14.21}{4} = 13.97$			
DSS	D_{M1}	D_{M2}	D_{M3}	D_{M4}
GAP	20.25	22.44	21.06	22.06
IL-10	32.37	32.41	31.53	33.76
D-value	12.12	9.97	10.47	11.69
Mean $_{\text{D-value}}$	$\text{Average}_{Ct}\,(D) = \frac{12.12 + 9.97 + 10.47 + 11.69}{4} = 11.06$			
High	H_{M1}	H_{M2}	H_{M3}	H_{M4}
GAP	23.53	22.46	23.06	22.79
IL-10	32.49	33.11	35.05	36.31
D-value	8.96	10.65	11.99	13.52
Mean $_{\text{D-value}}$	$\text{Average}_{Ct}\,(H) = \frac{8.96 + 10.65 + 11.99 + 13.52}{4} = 11.28$			
Low	L_{M1}	L_{M2}	L_{M3}	L_{M4}
GAP	19.65	22.29	22.37	22.34
IL-10	30.17	32.91	33.3	33.76
D-value	10.52	10.62	10.93	11.42
Mean $_{\text{D-value}}$	$\text{Average}_{Ct}\,(L) = \frac{10.52 + 10.62 + 10.93 + 11.42}{4} = 10.87$			

Table 15: Claudin-1 data Ct processing table

Claudin-1 Ct Data processing table				
Control	**C_{M1}**	**C_{M2}**	**C_{M3}**	**C_{M4}**
GAP	19.56	19.19	19.85	19.29
Claudin-1	28.11	29.31	30.27	31.28
D-value	8.55	10.11	10.43	11.99
Mean $_{D\text{-value}}$	**$Average_{Ct}$ (C)** = $\frac{8.55 + 10.11 + 10.43 + 11.99}{4}$ = 10.27			
DSS	**D_{M1}**	**D_{M2}**	**D_{M3}**	**D_{M4}**
GAP	20.33	20.5	20.26	22.40
Claudin-1	31.75	33.33	29.98	32.53
D-value	11.42	12.83	9.72	10.13
Mean $_{D\text{-value}}$	**$Average_{Ct}$ (D)** = $\frac{11.42 + 12.83 + 9.72 + 10.13}{4}$ = 11.02			
High	**H_{M1}**	**H_{M2}**	**H_{M3}**	**H_{M4}**
GAP	23.88	22.92	23.5	23.24
Claudin-1	33.97	33.17	35.19	34.35
D-value	10.08	10.25	11.69	11.10
Mean $_{D\text{-value}}$	**$Average_{Ct}$ (H)** = $\frac{10.08 + 10.25 + 11.69 + 11.10}{4}$ = 10.78			
Low	**L_{M1}**	**L_{M2}**	**L_{M3}**	**L_{M4}**
GAP	20.17	22.86	22.87	22.52
Claudin-1	30.36	32.77	33.79	33.70
D-value	10.19	9.91	10.92	11.18
Mean $_{D\text{-value}}$	**$Average_{Ct}$ (L)** = $\frac{10.19 + 9.91 + 10.92 + 11.18}{4}$ = 10.55			