

Supplementary Materials for

Bacterioboot—A novel tool to increase the half-life period of the orally administered drug

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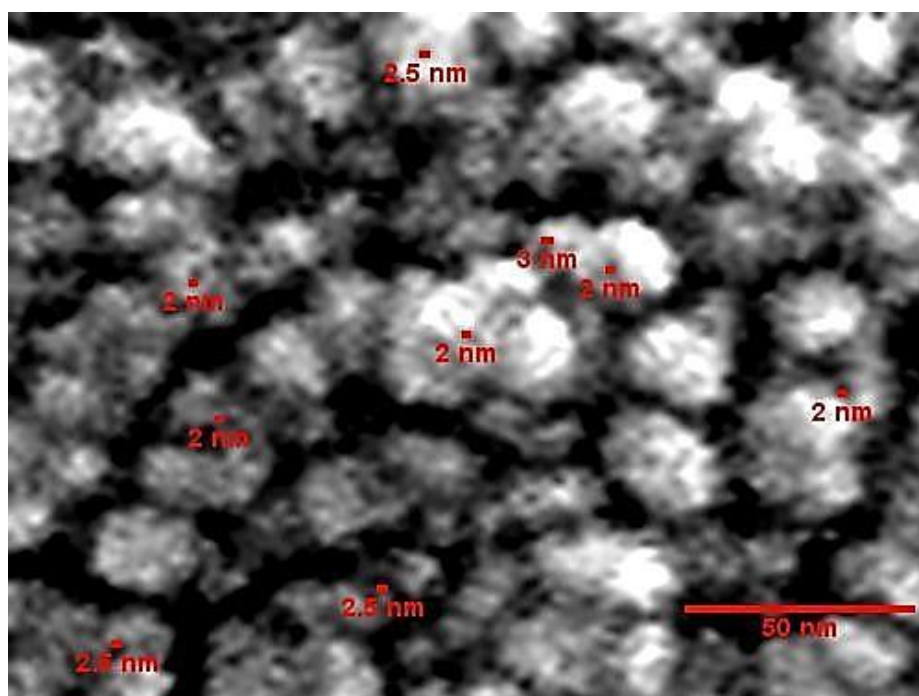


fig. S-1 FESEM image showing porous Bacterioblast surface with pore diameter 2-3 nm.

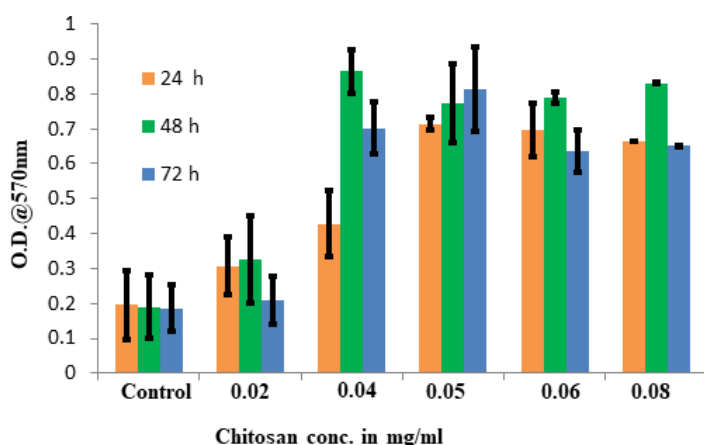


fig. S-2 Biofilm production at different chitosan concentrations: *L. reuteri* was treated with various concentrations of chitosan (0 to 0.8 mg/ml) for up to 72 h. And the production of the efficiency of biofilm by the cells was determined, as mentioned before. Orange, Green, and blue represents 24, 48, and 72 h biofilm production.

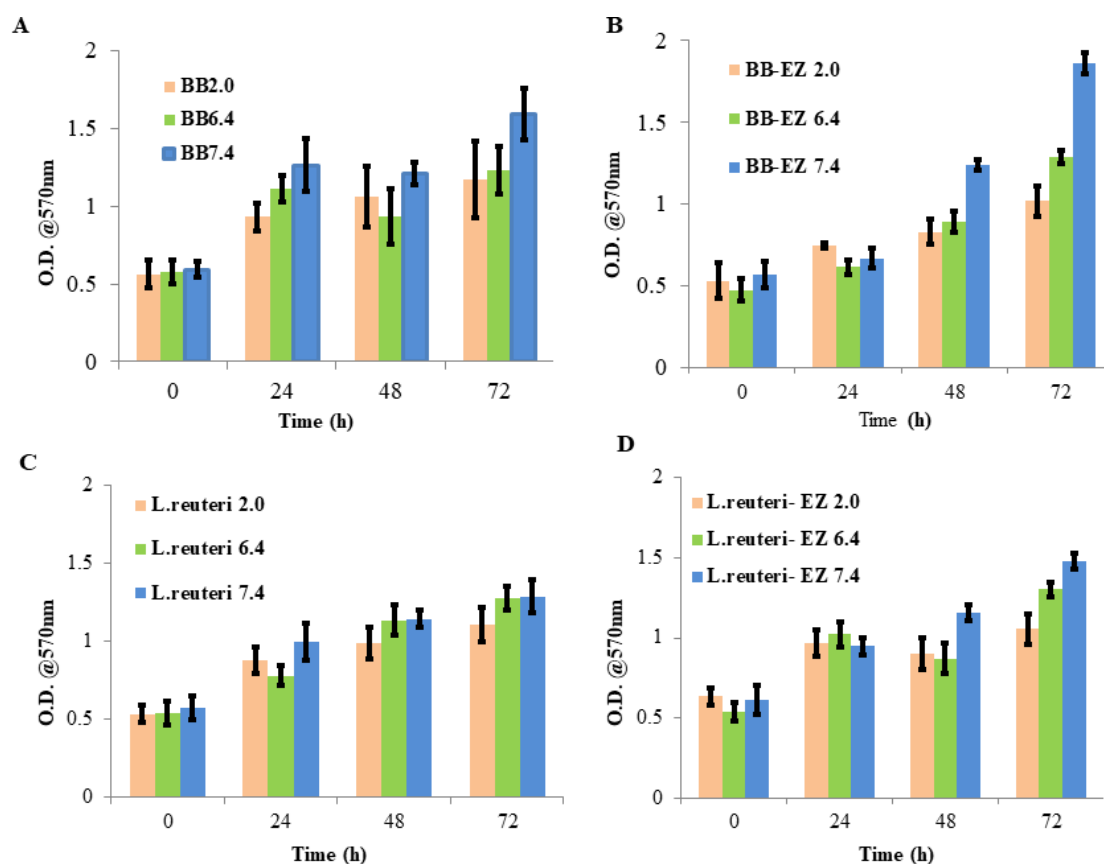


fig. S-3 Bio-film production at different pH for *L. reuteri* and BB (A) Biofilm production by BB in SGF and SIF without enzyme at pH 2.0, 6.4, 7.4 (B) Biofilm production by BB in SGF and SIF with the enzyme at pH 2.0, 6.4, 7.4 (C) Biofilm production by *L. reuteri* in SGF and SIF without enzyme at pH 2.0, 6.4, 7.4 (D) Biofilm production by *L. reuteri* in SGF and SIF with Enzyme at pH 2.0, 6.4, 7.4. Biofilm production was observed to be increasing with time in all the samples with and without enzymes.

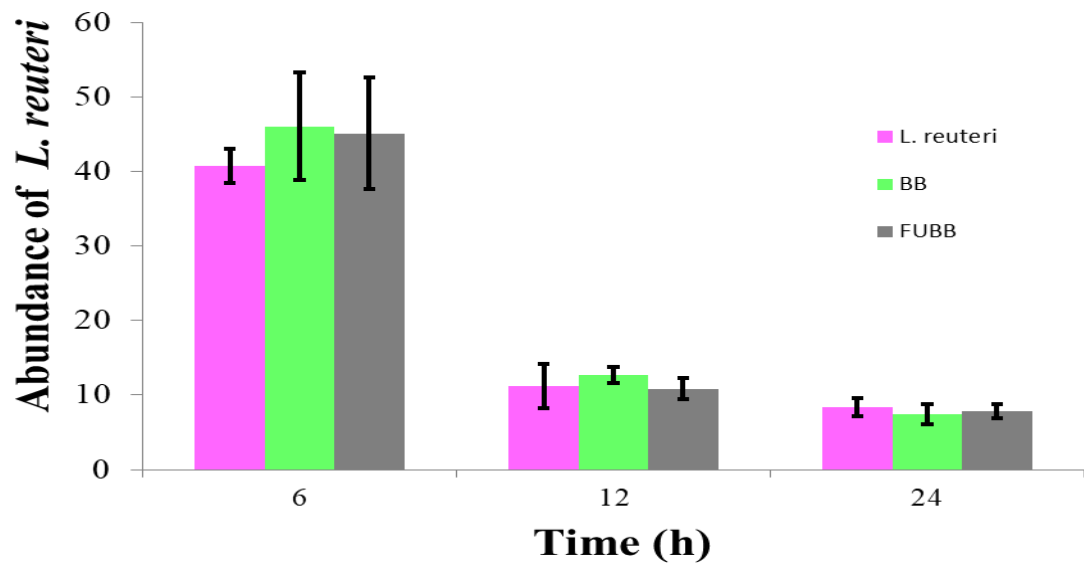


fig. S-4 Comparative expression of 16S rDNA of *L. reuteri* in the mice Feces of all three groups of mice at different time points (6h, 12h and 24 h) was collected to compare the 16S rDNA of *L. reuteri*.

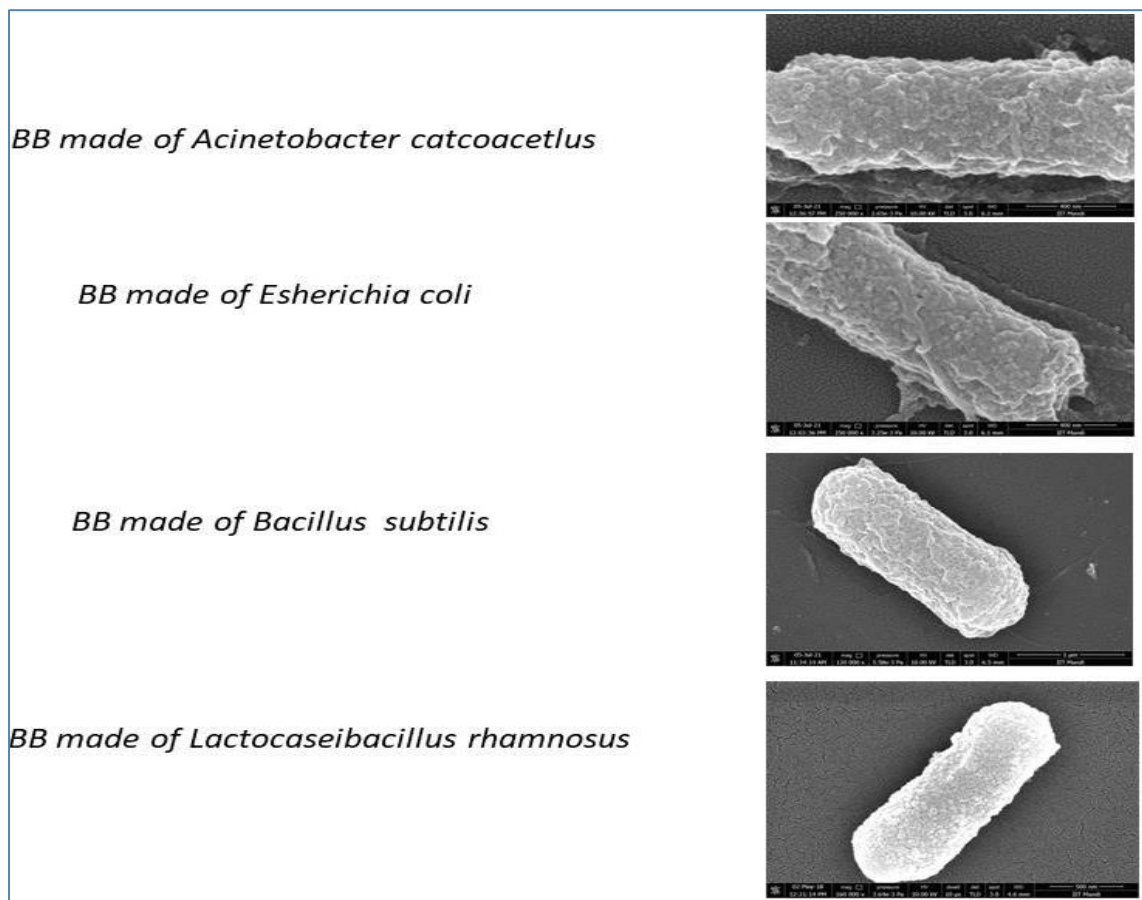


fig. S-5 Bacteriobioat formation with the different bacteria (*A. catcoacetlus*, *E. coli*, *B. subtilis*, and *L. rhamnosus*)

TS-1 Comparison of the growth curve by comparing the area ratio of *L. reuteri* with EBB and FUBB.

Time (h)	O.D. @600nm <i>L. reuteri</i>	O.D. @600nm EBB	O.D. @ 600nm FUBB	Area ratio EBB with <i>L. reuteri</i>	Area ratio FUBB with <i>L. reuteri</i>
0	3.168027	3.202721	3.282993	-0.03629	-3.62895
3	3.390476	3.390476	3.327211	0.01866	1.865971
6	3.460544	3.421769	3.434694	0.00747	0.747002
9	3.47619	3.506122	3.570748	-0.0272	-2.72016
12	3.52517	3.637415	3.50068	0.006947	0.694712
18	3.887075	3.595238	3.364626	0.134407	13.44067
24	4.253061	3.687075	3.336735	0.215451	21.54511
36	4.283673	3.932653	3.382993	0.210259	21.02589
48	5.641497	5.144898	5.314286	0.058001	5.800072
72	5.906122	5.22517	5.772109	0.022691	2.269062
96	6.211565	5.67415	5.808163	0.064944	6.49436

TS-2 Comparison of the percentage growth rate ratio of *L. reuteri* with EBB and FUBB

Time (h)	O.D. @ 600 <i>L. reuteri</i>	O.D. @600 EBB	O.D. @600 FUBB	%growth rate <i>L. reuteri</i> /EBB	%growth rate <i>L. reuteri</i> /FUBB
0	0.200333	0.231	0.200667	-15.3078	-0.00166
3	0.217333	0.213333	0.214667	1.840491	0.01227
6	0.267	0.229	0.251	14.23221	0.059925
9	0.415667	0.274667	0.235667	33.92141	0.433039
12	0.8675	0.6217	0.235	28.33429	0.729107
18	1.339333	1.028333	0.470333	23.22051	0.64883
24	1.670033	1.455667	0.6754	12.83607	0.595577
36	1.661633	1.584333	1.160933	4.652049	0.30133
48	1.678959	1.690933	1.558547	-0.71321	0.071718
72	1.696284	1.724633	1.649627	-1.67124	0.027505
96	1.701667	1.724667	1.659767	-1.35162	0.024623

TS-3 Tukey's Multiple Comparison Test for the significance of the Biofilm production at pH 2.0, 6.4, 7.4. within EBB without Enzyme

Tukey's multiple comparisons test EBB @570	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P-Value
Row 1					
EBB2.0 vs. EBB6.4	-0.0134	-0.3034 to 0.2766	No	ns	0.9927
EBB2.0 vs. EBB7.4	-0.03003	-0.3200 to 0.2600	No	ns	0.9639
EBB 6.4 vs. EBB7.4	-0.01663	-0.3066 to 0.2734	No	ns	0.9888
Row 2					
EBB 2.0 vs. EBB6.4	-0.1823	-0.4723 to 0.1077	No	ns	0.2776
EBB 2.0 vs. EBB7.4	-0.3317	-0.6216 to -0.04168	Yes	*	0.0228
EBB 6.4 vs. EBB7.4	-0.1493	-0.4393 to 0.1407	No	ns	0.4165
Row 3					
EBB 2.0 vs. EBB6.4	0.126	-0.1640 to 0.4160	No	ns	0.5322
EBB 2.0 vs. EBB7.4	-0.1482	-0.4382 to 0.1418	No	ns	0.4218
EBB 6.4 vs. EBB7.4	-0.2742	-0.5642 to 0.01578	No	ns	0.0663
Row 4					
EBB 2.0 vs. EBB6.4	-0.059	-0.3490 to 0.2310	No	ns	0.8681
EBB 2.0 vs. EBB7.4	-0.4197	-0.7096 to -0.1297	Yes	**	0.0038
EBB 6.4 vs. EBB7.4	-0.3607	-0.6506 to -0.07068	Yes	*	0.0129

TS-4 Tukey's Multiple Comparison Test for the significance of the biofilm production at pH 2.0, 6.4, 7.4. of EBB with enzyme

Tukey's multiple comparisons test BBE 570	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P-Value
Row 1					
EBB-EZ 2.0 vs. EBB-EZ 6.4	0.05933	-0.07655 to 0.1952	No	ns	0.529
EBB-EZ 2.0 vs. EBB-EZ 7.4	-0.03467	-0.1705 to 0.1012	No	ns	0.8012
EBB-EZ 6.4 vs. EBB-EZ 7.4	-0.094	-0.2299 to 0.04188	No	ns	0.2157
Row 2					
EBB-EZ 2.0 vs. EBB-EZ 6.4	0.131	-0.004879 to 0.2669	No	ns	0.0603
EBB-EZ 2.0 vs. EBB-EZ 7.4	0.077	-0.05888 to 0.2129	No	ns	0.3492
EBB-EZ 6.4 vs. EBB-EZ 7.4	-0.054	-0.1899 to 0.08188	No	ns	0.5886
Row 3					
EBB-EZ 2.0 vs. EBB-EZ 6.4	-0.0617	-0.1976 to 0.07418	No	ns	0.5031
EBB-EZ 2.0 vs. EBB-EZ 7.4	-0.4117	-0.5476 to -0.2758	Yes	****	<0.0001
EBB-EZ 6.4 vs. EBB-EZ 7.4	-0.35	-0.4859 to -0.2141	Yes	****	<0.0001
Row 4					
EBB-EZ 2.0 vs. EBB-EZ 6.4	-0.273	-0.4089 to -0.1371	Yes	***	0.0001
EBB-EZ 2.0 vs. EBB-EZ 7.4	-0.8423	-0.9782 to -0.7065	Yes	****	<0.0001
EBB-EZ 6.4 vs. EBB-EZ 7.4	-0.5693	-0.7052 to -0.4335	Yes	****	<0.0001

TS-5 Tukey's Multiple Comparison Test for the significance of the Biofilm production at pH 2.0 in EBB with *L. reuteri* with and without enzyme

Comparison of EBB with <i>L. reuteri</i> Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P-Value
Row 1					
<i>L. reuteri</i> 2.0 vs. <i>L. reuteri</i> - EZ 2.0	-0.0934	-0.3651 to 0.1783	No	ns	0.7793
<i>L. reuteri</i> 2.0 vs. EBB 2.0	-0.05737	-0.3290 to 0.2143	No	ns	0.9364
<i>L. reuteri</i> 2.0 vs. EBB-EZ 2.0	0.1316	-0.1400 to 0.4033	No	ns	0.5495
<i>L. reuteri</i> - EZ 2.0 vs. EBB 2.0	0.03603	-0.2356 to 0.3077	No	ns	0.9829
<i>L. reuteri</i> - EZ 2.0 vs. EBB-EZ 2.0	0.225	-0.04662 to 0.4967	No	ns	0.1297
EBB 2.0 vs. EBB-EZ 2.0	0.189	-0.08265 to 0.4607	No	ns	0.2468
Row 2					
<i>L. reuteri</i> 2.0 vs. <i>L. reuteri</i> - EZ 2.0	0.0857	-0.1860 to 0.3574	No	ns	0.82
<i>L. reuteri</i> 2.0 vs. EBB 2.0	-0.073	-0.3447 to 0.1987	No	ns	0.8794
<i>L. reuteri</i> 2.0 vs. EBB-EZ 2.0	0.164	-0.1077 to 0.4357	No	ns	0.3631
<i>L. reuteri</i> - EZ 2.0 vs. EBB 2.0	-0.1587	-0.4304 to 0.1130	No	ns	0.3913
<i>L. reuteri</i> - EZ 2.0 vs. EBB-EZ 2.0	0.0783	-0.1934 to 0.3500	No	ns	0.8559
EBB 2.0 vs. EBB-EZ 2.0	0.237	-0.03465 to 0.5087	No	ns	0.1028
Row 3					
<i>L. reuteri</i> 2.0 vs. <i>L. reuteri</i> - EZ 2.0	0.05	-0.2217 to 0.3217	No	ns	0.9564
<i>L. reuteri</i> 2.0 vs. EBB 2.0	-0.06634	-0.3380 to 0.2053	No	ns	0.906
<i>L. reuteri</i> 2.0 vs. EBB-EZ 2.0	0.08933	-0.1823 to 0.3610	No	ns	0.8012
<i>L. reuteri</i> - EZ 2.0 vs. EBB 2.0	-0.1163	-0.3880 to 0.1553	No	ns	0.644
<i>L. reuteri</i> - EZ 2.0 vs. EBB-EZ 2.0	0.03933	-0.2323 to 0.3110	No	ns	0.9779
EBB 2.0 vs. EBB-EZ 2.0	0.1557	-0.1160 to 0.4273	No	ns	0.4079

TS-6 Tukey's Multiple Comparison Test for the significance of the biofilm production at pH 6.4 in EBB with *L. reuteri* with and without enzyme

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P-Value
Row 1					
<i>L. reuteri</i> 6.4 vs. <i>L. reuteri</i> - EZ 6.4	-0.2463	-0.4577 to -0.03496	Yes	*	0.0181
<i>L. reuteri</i> 6.4 vs. EBB 6.4	-0.337	-0.5484 to -0.1256	Yes	**	0.001
<i>L. reuteri</i> 6.4 vs. EBB-EZ 6.4	0.1653	-0.04604 to 0.3767	No	ns	0.164
<i>L. reuteri</i> - EZ 6.4 vs. EBB 6.4	-0.09067	-0.3020 to 0.1207	No	ns	0.6429
<i>L. reuteri</i> - EZ 6.4 vs. EBB-EZ 6.4	0.4117	0.2003 to 0.6230	Yes	****	<0.0001
EBB 6.4 vs. EBB-EZ 6.4	0.5023	0.2910 to 0.7137	Yes	****	<0.0001
Row 2					
<i>L. reuteri</i> 6.4 vs. <i>L. reuteri</i> - EZ 6.4	0.2623	0.05096 to 0.4737	Yes	*	0.0111
<i>L. reuteri</i> 6.4 vs. EBB 6.4	0.197	-0.01437 to 0.4084	No	ns	0.074
<i>L. reuteri</i> 6.4 vs. EBB-EZ 6.4	0.2463	0.03493 to 0.4577	Yes	*	0.0182
<i>L. reuteri</i> - EZ 6.4 vs. EBB 6.4	-0.06533	-0.2767 to 0.1460	No	ns	0.8288
<i>L. reuteri</i> - EZ 6.4 vs. EBB-EZ 6.4	-0.01603	-0.2274 to 0.1953	No	ns	0.9967
EBB 6.4 vs. EBB-EZ 6.4	0.0493	-0.1621 to 0.2607	No	ns	0.9168
Row 3					
<i>L. reuteri</i> 6.4 vs. <i>L. reuteri</i> - EZ 6.4	-0.0283	-0.2397 to 0.1831	No	ns	0.9824
<i>L. reuteri</i> 6.4 vs. EBB 6.4	0.04703	-0.1643 to 0.2584	No	ns	0.9267
<i>L. reuteri</i> 6.4 vs. EBB-EZ 6.4	-0.0113	-0.2227 to 0.2001	No	ns	0.9988
<i>L. reuteri</i> - EZ 6.4 vs. EBB 6.4	0.07533	-0.1360 to 0.2867	No	ns	0.7602
<i>L. reuteri</i> - EZ 6.4 vs. EBB-EZ 6.4	0.017	-0.1944 to 0.2284	No	ns	0.996
EBB 6.4 vs. EBB-EZ 6.4	-0.05833	-0.2697 to 0.1530	No	ns	0.8709

TS-7 Tukey's Multiple Comparison Test for the significance of the biofilm production at pH 7.4 in EBB with *L. reuteri* with and without enzyme

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P-Value
Row 1					
<i>L. reuteri</i> 7.4 vs. <i>L. reuteri</i> - EZ 7.4	0.0497	-0.1607 to 0.2601	No	ns	0.9139
<i>L. reuteri</i> 7.4 vs. EBB 7.4	-0.2656	-0.4760 to -0.05525	Yes	**	0.0097
<i>L. reuteri</i> 7.4 vs. EBB-EZ 7.4	0.332	0.1216 to 0.5424	Yes	**	0.0012
<i>L. reuteri</i> - EZ 7.4 vs. EBB 7.4	-0.3153	-0.5257 to -0.1049	Yes	**	0.002
<i>L. reuteri</i> - EZ 7.4 vs. EBB-EZ 7.4	0.2823	0.07195 to 0.4927	Yes	**	0.0057
EBB 7.4 vs. EBB-EZ 7.4	0.5977	0.3873 to 0.8080	Yes	****	<0.0001
Row 2					
<i>L. reuteri</i> 7.4 vs. <i>L. reuteri</i> - EZ 7.4	-0.0134	-0.2238 to 0.1970	No	ns	0.998
<i>L. reuteri</i> 7.4 vs. EBB 7.4	-0.0669	-0.2773 to 0.1435	No	ns	0.8165
<i>L. reuteri</i> 7.4 vs. EBB-EZ 7.4	-0.0934	-0.3038 to 0.1170	No	ns	0.6177
<i>L. reuteri</i> - EZ 7.4 vs. EBB 7.4	-0.0535	-0.2639 to 0.1569	No	ns	0.8955
<i>L. reuteri</i> - EZ 7.4 vs. EBB-EZ 7.4	-0.08	-0.2904 to 0.1304	No	ns	0.7228
EBB 7.4 vs. EBB-EZ 7.4	-0.0265	-0.2369 to 0.1839	No	ns	0.9852
Row 3					
<i>L. reuteri</i> 7.4 vs. <i>L. reuteri</i> - EZ 7.4	-0.1903	-0.4007 to 0.02005	No	ns	0.0862
<i>L. reuteri</i> 7.4 vs. EBB 7.4	-0.3033	-0.5137 to -0.09295	Yes	**	0.0029
<i>L. reuteri</i> 7.4 vs. EBB-EZ 7.4	-0.5703	-0.7807 to -0.3599	Yes	****	<0.0001
<i>L. reuteri</i> - EZ 7.4 vs. EBB 7.4	-0.113	-0.3234 to 0.09738	No	ns	0.4636
<i>L. reuteri</i> - EZ 7.4 vs. EBB-EZ 7.4	-0.38	-0.5904 to -0.1696	Yes	***	0.0002
EBB 7.4 vs. EBB-EZ 7.4	-0.267	-0.4774 to -0.05662	Yes	**	0.0093

TS-8 Tukey's Multiple Comparison Test for the significance of Fluorescent Intensity Graph

Tukey's multiple comparisons test Fluorescent Intensity Graph	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P-Value
Intestine vs. <i>L. reuteri</i>	47	-1667 to 1761	No	ns	>0.9999
Intestine vs. EBB	34.67	-1679 to 1748	No	ns	>0.9999
Intestine vs. Rh-BB on intestine	-22182	-23896 to -20469	Yes	****	<0.0001
Intestine vs. Rh on Intestine	-624.7	-2338 to 1089	No	ns	0.8651
Intestine vs. <i>L. reuteri</i> -Rh on intestine	-60.66	-1774 to 1653	No	ns	>0.9999
Intestine vs. Rh-BB	-8443	-10156 to -6729	Yes	****	<0.0001
<i>L. reuteri</i> vs. EBB	-12.33	-1726 to 1701	No	ns	>0.9999
<i>L. reuteri</i> vs. Rh-BB on intestine	-22229	-23943 to -20516	Yes	****	<0.0001
<i>L. reuteri</i> vs. Rh on Intestine	-671.7	-2385 to 1042	No	ns	0.8235
<i>L. reuteri</i> vs. <i>L. reuteri</i> -Rh on intestine	-107.7	-1821 to 1606	No	ns	>0.9999
<i>L. reuteri</i> vs. Rh-BB	-8490	-10203 to -6776	Yes	****	<0.0001
EBB vs. Rh-BB on intestine	-22217	-23931 to -20503	Yes	****	<0.0001
EBB vs. Rh on Intestine	-659.3	-2373 to 1054	No	ns	0.8349
EBB vs. <i>L. reuteri</i> -Rh on the intestine	-95.33	-1809 to 1618	No	ns	>0.9999
EBB vs. Rh-BB	-8477	-10191 to -6764	Yes	****	<0.0001
Rh-BB on intestine vs. Rh on Intestine	21558	19844 to 23271	Yes	****	<0.0001
Rh-BB on intestine vs. <i>L. reuteri</i> -Rh on intestine	22122	20408 to 23835	Yes	****	<0.0001
Rh-BB on intestine vs. Rh-BB	13740	12026 to 15453	Yes	****	<0.0001
Rh on Intestine vs. <i>L. reuteri</i> -Rh on intestine	564	-1150 to 2278	No	ns	0.9103
Rh on Intestine vs. Rh-BB	-7818	-9532 to -6104	Yes	****	<0.0001
<i>L. reuteri</i> -Rh on intestine vs. Rh-BB	-8382	-10096 to -6668	Yes	****	<0.0001

TS -9 Sidak's Multiple Comparison Test for the significance of the LC-ESI-MS comparison of FU-50 and FUBB-50.

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P Value
FU-50 VS FUBB-50					
Row 2	67.33	-1270 to 1404	No	ns	>0.9999
Row 3	1860	523.2 to 3197	Yes	**	0.0027
Row 4	-370.0	-1707 to 966.8	No	ns	0.9805
Row 5	-2413	-3750 to -1076	Yes	***	0.0001
Row 6	-717.3	-2054 to 619.5	No	ns	0.6281
Row 7	-740.7	-2078 to 596.2	No	ns	0.5918
Row 8	-484.0	-1821 to 852.8	No	ns	0.9207

TS-10 Tukey's Multiple Comparison Test for the significance of RTV on 29th day

Tukey's Multiple Comparison Test RTV 29th day	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
con vs EBB	1.14	3.447	No	ns	-0.4312 to 2.711
con vs FU-25	6.53	19.75	Yes	***	4.959 to 8.101
con vs FUBB-25	8.48	25.64	Yes	***	6.909 to 10.05
con vs FU-50	8.88	26.85	Yes	***	7.309 to 10.45
con vs FUBB-50	10.32	31.21	Yes	***	8.749 to 11.89
EBB vs FU-25	5.39	16.3	Yes	***	3.819 to 6.961
EBB vs FUBB-25	7.34	22.19	Yes	***	5.769 to 8.911
EBB vs FU-50	7.74	23.4	Yes	***	6.169 to 9.311
EBB vs FUBB-50	9.18	27.76	Yes	***	7.609 to 10.75
FU-25 vs FUBB-25	1.95	5.896	Yes	*	0.3788 to 3.521

FU-25 vs FU-50	2.35	7.106	Yes	**	0.7788 to 3.921
FU-25 vs FUBB-50	3.79	11.46	Yes	***	2.219 to 5.361
FUBB-25 vs FU-50	0.4	1.21	No	ns	-1.171 to 1.971
FUBB-25 vs FUBB-50	1.84	5.564	Yes	*	0.2688 to 3.411
FU-50 vs FUBB-50	1.44	4.354	No	ns	-0.1312 to 3.011

TS-11 Comparative changes in biochemical parameters in mice for system toxicity

Serum component (unit)	Control	EBB	FU-25	FUBB-25	FU-50	FUBB-50
SGOT (IU/ml)	25±3.1	26±4.0	32±3.6	28±3.4	38±4.1	33±3.8
SGPT (IU/ml)	30±3.8	31±4.3	37±3.2	33±3.6	43±3.5	38±3.7
Albumin (mg/dL)	6.12±0.52	6.24±0.61	7.04±0.55	6.78±0.63	7.98±0.51	7.14±0.65
Bilirubin (mg/dL)	0.58±0.06	0.6±0.07	0.73±0.06	0.65±0.06	0.83±0.05	0.75±0.04
Cholesterol (mg/dL)	92.25±11.52	94.38±10.12	104.52±9.63	96.84±9.83	115.53±9.15	107.63±10.01
Triglyceride (mg/dL)	106.8±15.3	105.3±13.2	120.6±14.3	109.6±15.1	128.6±13.3	121.3±14.1
Globulin (mg/dL)	4.1±0.5	4.3±0.4	5.1±0.45	4.6±0.42	6.2±0.41	5.2±0.48
Creatinine (mg/dL)	0.73±0.04	0.75±0.04	0.81±0.05	0.76±0.04	0.89±0.05	0.84±0.04
Alkaline phosphatase (mg/dL)	21.05±4.15	19.65±5.01	28.35±5.23	22.15±4.73	30.62±4.98	29.25±5.11

TS-12 Tukey's Multiple Comparison Test for the significance of biochemical parameters for SGOT

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
SGOT					
Control vs. EBB	-1	-15.11 to 13.11	No	ns	>0.9999
Control vs. FU-25	-7	-21.11 to 7.109	No	ns	0.7045
Control vs. FUBB-25	-3	-17.11 to 11.11	No	ns	0.9897
Control vs. FU-50	-13	-27.11 to 1.109	No	ns	0.0893
Control vs. FUBB-50	-8	-22.11 to 6.109	No	ns	0.5724
EBB vs. FU-25	-6	-20.11 to 8.109	No	ns	0.8205
EBB vs. FUBB-25	-2	-16.11 to 12.11	No	ns	0.9985
EBB vs. FU-50	-12	-26.11 to 2.109	No	ns	0.1435
EBB vs. FUBB-50	-7	-21.11 to 7.109	No	ns	0.7045
FU-25 vs. FUBB-25	4	-10.11 to 18.11	No	ns	0.9631
FU-25 vs. FU-50	-6	-20.11 to 8.109	No	ns	0.8205
FU-25 vs. FUBB-50	-1	-15.11 to 13.11	No	ns	>0.9999
FUBB-25 vs. FU-50	-10	-24.11 to 4.109	No	ns	0.3193
FUBB-25 vs. FUBB-50	-5	-19.11 to 9.109	No	ns	0.9082
FU-50 vs. FUBB-50	5	-9.109 to 19.11	No	ns	0.9082

TS-13 Tukey's Multiple Comparison Test for the significance of biochemical parameters for SGPT

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P Value
SGPT					
Control vs. EBB	-1	-15.11 to 13.11	No	ns	>0.9999
Control vs. FU-25	-7	-21.11 to 7.109	No	ns	0.7045
Control vs. FUBB-25	-3	-17.11 to 11.11	No	ns	0.9897
Control vs. FU-50	-13	-27.11 to 1.109	No	ns	0.0893
Control vs. FUBB-50	-8	-22.11 to 6.109	No	ns	0.5724
EBB vs. FU-25	-6	-20.11 to 8.109	No	ns	0.8205
EBB vs. FUBB-25	-2	-16.11 to 12.11	No	ns	0.9985
EBB vs. FU-50	-12	-26.11 to 2.109	No	ns	0.1435
EBB vs. FUBB-50	-7	-21.11 to 7.109	No	ns	0.7045
FU-25 vs. FUBB-25	4	-10.11 to 18.11	No	ns	0.9631

FU-25 vs. FU-50	-6	-20.11 to 8.109	No	ns	0.8205
FU-25 vs. FUBB-50	-1	-15.11 to 13.11	No	ns	>0.9999
FUBB-25 vs. FU-50	-10	-24.11 to 4.109	No	ns	0.3193
FUBB-25 vs. FUBB-50	-5	-19.11 to 9.109	No	ns	0.9082
FU-50 vs. FUBB-50	5	-9.109 to 19.11	No	ns	0.9082

TS-14 Tukey's Multiple Comparison Test for the significance of biochemical parameters for Albumin

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P Value
Albumin					
Control vs. EBB	-0.22	-14.33 to 13.89	No	ns	>0.9999
Control vs. FU-25	-0.92	-15.03 to 13.19	No	ns	>0.9999
Control vs. FUBB-25	-0.66	-14.77 to 13.45	No	ns	>0.9999
Control vs. FU-50	-1.86	-15.97 to 12.25	No	ns	0.9989
Control vs. FUBB-50	-1.02	-15.13 to 13.09	No	ns	>0.9999
EBB vs. FU-25	-0.7	-14.81 to 13.41	No	ns	>0.9999
EBB vs. FUBB-25	-0.44	-14.55 to 13.67	No	ns	>0.9999
EBB vs. FU-50	-1.64	-15.75 to 12.47	No	ns	0.9994
EBB vs. FUBB-50	-0.8	-14.91 to 13.31	No	ns	>0.9999
FU-25 vs. FUBB-25	0.26	-13.85 to 14.37	No	ns	>0.9999
FU-25 vs. FU-50	-0.94	-15.05 to 13.17	No	ns	>0.9999
FU-25 vs. FUBB-50	-0.1	-14.21 to 14.01	No	ns	>0.9999
FUBB-25 vs. FU-50	-1.2	-15.31 to 12.91	No	ns	0.9999
FUBB-25 vs. FUBB-50	-0.36	-14.47 to 13.75	No	ns	>0.9999
FU-50 vs. FUBB-50	0.84	-13.27 to 14.95	No	ns	>0.9999

TS-15 Tukey's Multiple Comparison Test for the significance of biochemical parameters for Alkaline phosphatase

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P Value
Alkaline Phosphatase					
Control vs. EBB	1.4	-12.71 to 15.51	No	ns	0.9997
Control vs. FU-25	-7.3	-21.41 to 6.809	No	ns	0.6659
Control vs. FUBB-25	-1.1	-15.21 to 13.01	No	ns	>0.9999
Control vs. FU-50	-9.57	-23.68 to 4.539	No	ns	0.3688
Control vs. FUBB-50	-8.2	-22.31 to 5.909	No	ns	0.5455
EBB vs. FU-25	-8.7	-22.81 to 5.409	No	ns	0.4787
EBB vs. FUBB-25	-2.5	-16.61 to 11.61	No	ns	0.9956
EBB vs. FU-50	-10.97	-25.08 to 3.139	No	ns	0.2223
EBB vs. FUBB-50	-9.6	-23.71 to 4.509	No	ns	0.3653
FU-25 vs. FUBB-25	6.2	-7.909 to 20.31	No	ns	0.7992
FU-25 vs. FU-50	-2.27	-16.38 to 11.84	No	ns	0.9972
FU-25 vs. FUBB-50	-0.9	-15.01 to 13.21	No	ns	>0.9999
FUBB-25 vs. FU-50	-8.47	-22.58 to 5.639	No	ns	0.5092
FUBB-25 vs. FUBB-50	-7.1	-21.21 to 7.009	No	ns	0.6917
FU-50 vs. FUBB-50	1.37	-12.74 to 15.48	No	ns	0.9998

TS-16 Tukey's Multiple Comparison Test for the significance of biochemical parameters for Bilirubin

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P Value
Bilirubin					
Control vs. EBB	-0.02	-14.13 to 14.09	No	ns	>0.9999
Control vs. FU-25	-0.15	-14.26 to 13.96	No	ns	>0.9999
Control vs. FUBB-25	-0.07	-14.18 to 14.04	No	ns	>0.9999
Control vs. FU-50	-0.25	-14.36 to 13.86	No	ns	>0.9999
Control vs. FUBB-50	-0.17	-14.28 to 13.94	No	ns	>0.9999
EBB vs. FU-25	-0.13	-14.24 to 13.98	No	ns	>0.9999
EBB vs. FUBB-25	-0.05	-14.16 to 14.06	No	ns	>0.9999
EBB vs. FU-50	-0.23	-14.34 to 13.88	No	ns	>0.9999
EBB vs. FUBB-50	-0.15	-14.26 to 13.96	No	ns	>0.9999
FU-25 vs. FUBB-25	0.08	-14.03 to 14.19	No	ns	>0.9999
FU-25 vs. FU-50	-0.1	-14.21 to 14.01	No	ns	>0.9999
FU-25 vs. FUBB-50	-0.02	-14.13 to 14.09	No	ns	>0.9999
FUBB-25 vs. FU-50	-0.18	-14.29 to 13.93	No	ns	>0.9999
FUBB-25 vs. FUBB-50	-0.1	-14.21 to 14.01	No	ns	>0.9999
FU-50 vs. FUBB-50	0.08	-14.03 to 14.19	No	ns	>0.9999

TS-17 Tukey's Multiple Comparison Test for the significance of biochemical parameters for Cholesterol levels

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P Value
Cholesterol					
Control vs. EBB	-2.13	-16.24 to 11.98	No	ns	0.9979
Control vs. FU-25	-12.27	-26.38 to 1.839	No	ns	0.1269
Control vs. FUBB-25	-4.59	-18.70 to 9.519	No	ns	0.9346
Control vs. FU-50	-23.28	-37.39 to -9.171	Yes	****	<0.0001
Control vs. FUBB-50	-15.38	-29.49 to -1.271	Yes	*	0.0241
EBB vs. FU-25	-10.14	-24.25 to 3.969	No	ns	0.304
EBB vs. FUBB-25	-2.46	-16.57 to 11.65	No	ns	0.9959
EBB vs. FU-50	-21.15	-35.26 to -7.041	Yes	***	0.0004
EBB vs. FUBB-50	-13.25	-27.36 to 0.8590	No	ns	0.0787
FU-25 vs. FUBB-25	7.68	-6.429 to 21.79	No	ns	0.6155
FU-25 vs. FU-50	-11.01	-25.12 to 3.099	No	ns	0.2188
FU-25 vs. FUBB-50	-3.11	-17.22 to 11.00	No	ns	0.9879
FUBB-25 vs. FU-50	-18.69	-32.80 to -4.581	Yes	**	0.0027
FUBB-25 vs. FUBB-50	-10.79	-24.90 to 3.319	No	ns	0.2387
FU-50 vs. FUBB-50	7.9	-6.209 to 22.01	No	ns	0.5859

TS-18 Tukey's Multiple Comparison Test for the significance of biochemical parameters for Triglycerides

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Triglycerides					
Control vs. EBB	1.5	-12.61 to 15.61	No	ns	0.9996
Control vs. FU-25	-13.8	-27.91 to 0.3090	No	ns	0.0591
Control vs. FUBB-25	-2.8	-16.91 to 11.31	No	ns	0.9925
Control vs. FU-50	-21.8	-35.91 to -7.691	Yes	***	0.0002
Control vs. FUBB-50	-14.5	-28.61 to -0.3910	Yes	*	0.0402
EBB vs. FU-25	-15.3	-29.41 to -1.191	Yes	*	0.0253
EBB vs. FUBB-25	-4.3	-18.41 to 9.809	No	ns	0.95

EBB vs. FU-50	-23.3	-37.41 to -9.191	Yes	****	<0.0001
EBB vs. FUBB-50	-16	-30.11 to -1.891	Yes	*	0.0165
FU-25 vs. FUBB-25	11	-3.109 to 25.11	No	ns	0.2197
FU-25 vs. FU-50	-8	-22.11 to 6.109	No	ns	0.5724
FU-25 vs. FUBB-50	-0.7	-14.81 to 13.41	No	ns	>0.9999
FUBB-25 vs. FU-50	-19	-33.11 to -4.891	Yes	**	0.0022
FUBB-25 vs. FUBB-50	-11.7	-25.81 to 2.409	No	ns	0.1639
FU-50 vs. FUBB-50	7.3	-6.809 to 21.41	No	ns	0.6659

TS-19 Tukey's Multiple Comparison Test for the significance of biochemical parameters for Globulin

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P Value
Globulin					
Control vs. EBB	-0.2	-14.31 to 13.91	No	ns	>0.9999
Control vs. FU-25	-1	-15.11 to 13.11	No	ns	>0.9999
Control vs. FUBB-25	-0.5	-14.61 to 13.61	No	ns	>0.9999
Control vs. FU-50	-2.1	-16.21 to 12.01	No	ns	0.9981
Control vs. FUBB-50	-1.1	-15.21 to 13.01	No	ns	>0.9999
EBB vs. FU-25	-0.8	-14.91 to 13.31	No	ns	>0.9999
EBB vs. FUBB-25	-0.3	-14.41 to 13.81	No	ns	>0.9999
EBB vs. FU-50	-1.9	-16.01 to 12.21	No	ns	0.9988
EBB vs. FUBB-50	-0.9	-15.01 to 13.21	No	ns	>0.9999
FU-25 vs. FUBB-25	0.5	-13.61 to 14.61	No	ns	>0.9999
FU-25 vs. FU-50	-1.1	-15.21 to 13.01	No	ns	>0.9999
FU-25 vs. FUBB-50	-0.1	-14.21 to 14.01	No	ns	>0.9999
FUBB-25 vs. FU-50	-1.6	-15.71 to 12.51	No	ns	0.9995
FUBB-25 vs. FUBB-50	-0.6	-14.71 to 13.51	No	ns	>0.9999
FU-50 vs. FUBB-50	1	-13.11 to 15.11	No	ns	>0.9999

TS -20 Tukey's Multiple Comparison Test for the significance of biochemical parameters for Creatinine

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Creatinine					
Control vs. EBB	-0.02	-14.13 to 14.09	No	ns	>0.9999
Control vs. FU-25	-0.08	-14.19 to 14.03	No	ns	>0.9999
Control vs. FUBB-25	-0.03	-14.14 to 14.08	No	ns	>0.9999
Control vs. FU-50	-0.16	-14.27 to 13.95	No	ns	>0.9999
Control vs. FUBB-50	-0.11	-14.22 to 14.00	No	ns	>0.9999
EBB vs. FU-25	-0.06	-14.17 to 14.05	No	ns	>0.9999
EBB vs. FUBB-25	-0.01	-14.12 to 14.10	No	ns	>0.9999
EBB vs. FU-50	-0.14	-14.25 to 13.97	No	ns	>0.9999
EBB vs. FUBB-50	-0.09	-14.20 to 14.02	No	ns	>0.9999
FU-25 vs. FUBB-25	0.05	-14.06 to 14.16	No	ns	>0.9999
FU-25 vs. FU-50	-0.08	-14.19 to 14.03	No	ns	>0.9999
FU-25 vs. FUBB-50	-0.03	-14.14 to 14.08	No	ns	>0.9999
FUBB-25 vs. FU-50	-0.13	-14.24 to 13.98	No	ns	>0.9999
FUBB-25 vs. FUBB-50	-0.08	-14.19 to 14.03	No	ns	>0.9999
FU-50 vs. FUBB-50	0.05	-14.06 to 14.16	No	ns	>0.9999

TS-21 Tukey's Multiple Comparison Test for the significance of biochemical parameters for liver weight

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P Value
Liver weight					
Control vs. EBB	-0.1	-14.21 to 14.01	No	ns	>0.9999
Control vs. FU-25	-0.8	-14.91 to 13.31	No	ns	>0.9999
Control vs. FUBB-25	-0.2	-14.31 to 13.91	No	ns	>0.9999
Control vs. FU-50	-1.3	-15.41 to 12.81	No	ns	0.9998
Control vs. FUBB-50	-0.9	-15.01 to 13.21	No	ns	>0.9999
EBB vs. FU-25	-0.7	-14.81 to 13.41	No	ns	>0.9999
EBB vs. FUBB-25	-0.1	-14.21 to 14.01	No	ns	>0.9999
EBB vs. FU-50	-1.2	-15.31 to 12.91	No	ns	0.9999
EBB vs. FUBB-50	-0.8	-14.91 to 13.31	No	ns	>0.9999
FU-25 vs. FUBB-25	0.6	-13.51 to 14.71	No	ns	>0.9999
FU-25 vs. FU-50	-0.5	-14.61 to 13.61	No	ns	>0.9999
FU-25 vs. FUBB-50	-0.1	-14.21 to 14.01	No	ns	>0.9999
FUBB-25 vs. FU-50	-1.1	-15.21 to 13.01	No	ns	>0.9999
FUBB-25 vs. FUBB-50	-0.7	-14.81 to 13.41	No	ns	>0.9999
FU-50 vs. FUBB-50	0.4	-13.71 to 14.51	No	ns	>0.9999

TS- 22 Dunnett's Multiple Comparison Test for the significance Biofilm with different chitosan concentrations

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P Value
24 hrs					
0.0 vs. 0.02	-0.111	-0.2243 to 0.002369	No	ns	0.0571
0.0 vs. 0.04	-0.232	-0.3453 to -0.1186	Yes	****	<0.0001
0.0 vs.0.05	-0.5191	-0.6325 to -0.4058	Yes	****	<0.0001
0.0 vs. 0.06	-0.5001	-0.6135 to -0.3868	Yes	****	<0.0001
0.0 vs. 0.08	-0.4697	-0.5830 to -0.3563	Yes	****	<0.0001
48 hrs					
0.0 vs. 0.02	-0.1363	-0.2496 to -0.02295	Yes	*	0.0123
0.0 vs. 0.04	-0.6739	-0.7873 to -0.5606	Yes	****	<0.0001
0.0 vs.0.05	-0.5818	-0.6951 to -0.4685	Yes	****	<0.0001
0.0 vs. 0.06	-0.6003	-0.7136 to -0.4869	Yes	****	<0.0001
0.0 vs. 0.08	-0.641	-0.7543 to -0.5276	Yes	****	<0.0001
72 hrs					
0.0 vs. 0.02	-0.02293	-0.1363 to 0.09041	No	ns	0.9801
0.0 vs. 0.04	-0.5167	-0.6300 to -0.4033	Yes	****	<0.0001
0.0 vs.0.05	-0.6272	-0.7406 to -0.5139	Yes	****	<0.0001
0.0 vs. 0.06	-0.4497	-0.5630 to -0.3364	Yes	****	<0.0001
0.0 vs. 0.08	-0.4654	-0.5787 to -0.3520	Yes	****	<0.0001