

Adherence of *Bacillus subtilis* (DE111[®]) on Caco-2 cells

Objective:

To determine if *Bacillus subtilis* adhere to the surface of Caco-2 cells at various periods of time.

Methods:

Caco-2 cells were grown to confluency in multiwall chambers at 37C and 5% CO₂. *Bacillus subtilis* was provided by Deerland Enzymes and grown as recommended by the provider.

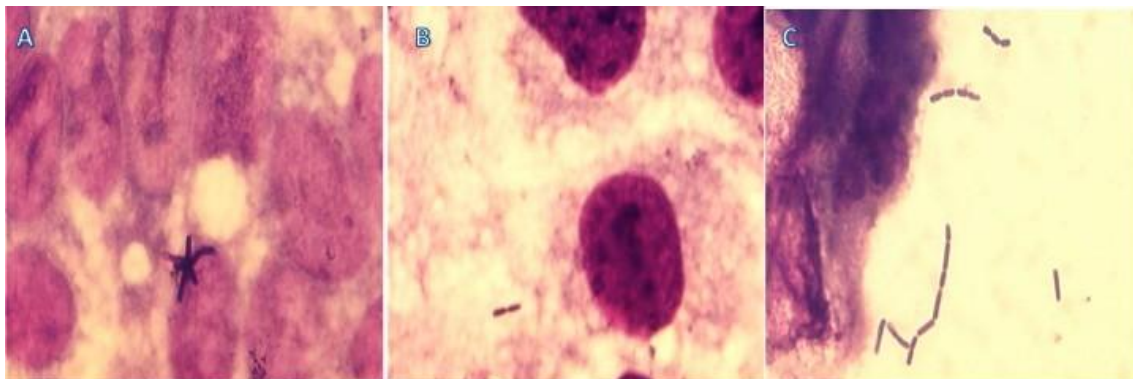
One treatment was evaluated in the present study. Bacilli at various concentrations (4, 5, and 6 logs) were exposed to tissue culture media or to pepsine/HCl pH 4 for 5 minutes. After that process, the reaction was neutralized and added to the cells.

Cells were examined after 30 min, 1 hour, and 2 hours post exposure. One hundred cells per treatment were counted.

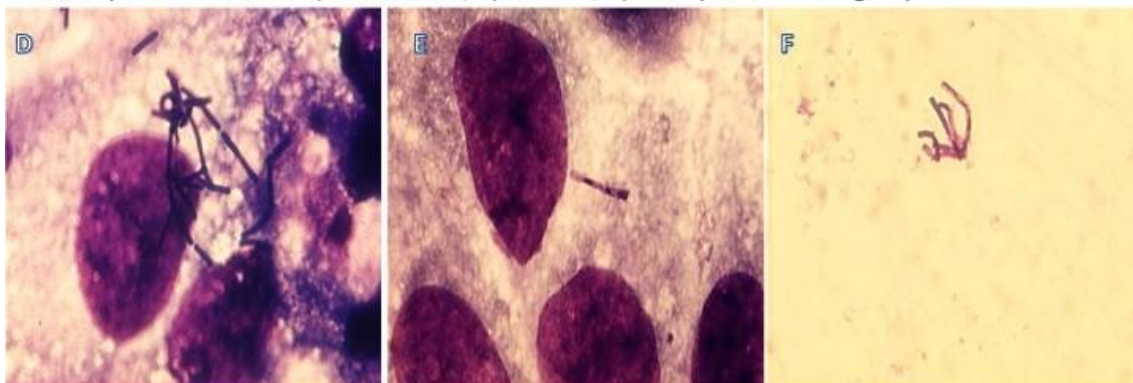
Results:

The HCl /pepsine negatively affected the adherence of the Bacilli on the surface of the Caco-2 cells. Adherence was more noticeable at 2 hours than at 30 minutes.

No cytotoxicity was noted by microscopy.



30 min post-treatment: A) not treated, B) treated, C) non specific binding to plastic



2 h post-treatment: D) not treated, E) treated, F) non specific binding to plastic

Presence of bacteria on the surface of Caco-2 cells at various periods of time post treatment:

Number of cells with *Bacillus subtilis*

	no treatment (%)	with treatment(%)
30 min		
6 LOG	18	0
5 LOG	0	1
4 LOG	0	0
60 min		
6 LOG	10	2
5 LOG	2	5
4 LOG	0	0
2 H		
6 LOG	16	4
5 LOG	4	2
4 LOG	1	0

The number of cells per cell increased with time, however *Bacillus* distribution among cells was not homogenous.

Number of bacteria present in Caco-2 cells with *Bacillus subtilis*:

	<i>Bacillus</i> (N) no treatment	<i>Bacillus</i> (N) treatment	range of <i>Bacillus</i> /cell no treatment	range of <i>Bacillus</i> /cell treatment	average of <i>Bacillus</i> /cell no treatment	average of <i>Bacillus</i> /cell treatment
30 min						
6 LOG	34	0	1-5	0	1.9	0
5 LOG	0	1	0	1	0	0
4 LOG	0	0	0	0	0	0
60 min						
6 LOG	26	3	2-5	1-2	2.6	1.5
5 LOG	3	9	1-2	1-5	1.5	1.8
4 LOG	0	0		0	0	0
2 H						
6 LOG	100	8	1-26	1-3	6.25	2
5 LOG	9	11	1-4	5-6	2.25	5.5
4 LOG	2	0	2	0	2	0

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