Effect of different sublethal stress treatments of bacteria on survival rates following freeze-drying (pilot study)

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For the experiment two microbes, *Escherichia coli* and *Lactobacillus plantarum* were used. Cell counts were determined on TGE (Tripton Glucose Extract) nutritive media (MERCK) and MRS agar (MERCK), respectively. Cell counts were determined before preparing the treatment groups, after pressure treatments, and after 96 hours of incubation at 37 ºC following freeze-drying (c.f.u.). Pressure treatments were executed with 9 pressurizing machines at the same time according to the following table:

Pressure \ Time	30 min	60 min	90 min
200 bar	Treatment group 1	Treatment group 2	Treatment group 3
400 bar	Treatment group 4	Treatment group 5	Treatment group 6
600 bar	Treatment group 7	Treatment group 8	Treatment group 9

Samples were filled into 0.5 ml sterile artificial straws (IMV, L'Aigle, France), and were sealed with sterile iron ball. Treatment was accomplished with computer controlled pressurizing device (Cryo-Innovation Inc., Budapest, Hungary www.cryo-innovation.com) Freeze-drying was made in Edwards freeze-drying equipment. Experiments were replicated two times. Results are presented in the following tables.

Table 5.: Number of living *Lactobacillus plantarum* cells (c.f.u./0.4ml) before and after freeze-drying (two repetitions). (Initial cell count (c.f.u./ml) before treatment: 9.0×10^6 - 1.1×10^7)

	Number of living cells \ 0.4 ml		
Treatment group No.	Before freeze-drying	After freeze-drying I.	After freeze- drying II.
1	1.30×10^7	2.29×10^6	2.56×10^6
2	7.80×10^6	3.02×10^6	2.54×10^6
3	8.80×10^6	2.01×10^6	2.05×10^6
4	5.70×10^6	4.25×10^6	4.19×10^6
5	6.38×10^6	1.99×10^6	2.10×10^6

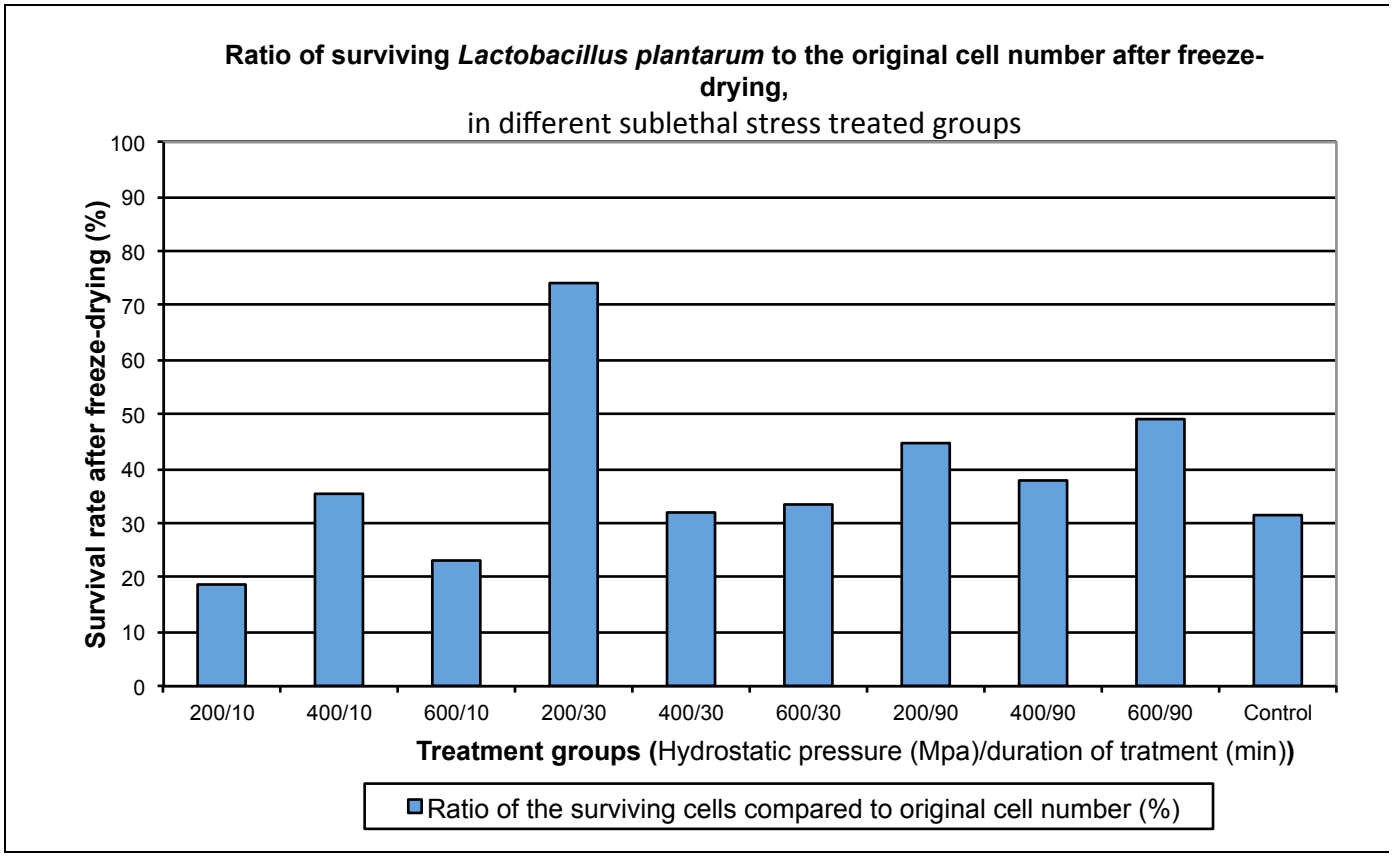
6	1.26×10^7	4.34×10^6	4.13×10^6
7	7.50×10^6	3.12×10^6	3.59×10^6
8	8.70×10^6	2.71×10^6	3.88×10^6
9	8.30×10^6	3.36×10^6	4.83×10^6
Control	1.05×10^7	2.56×10^6	4.08×10^6

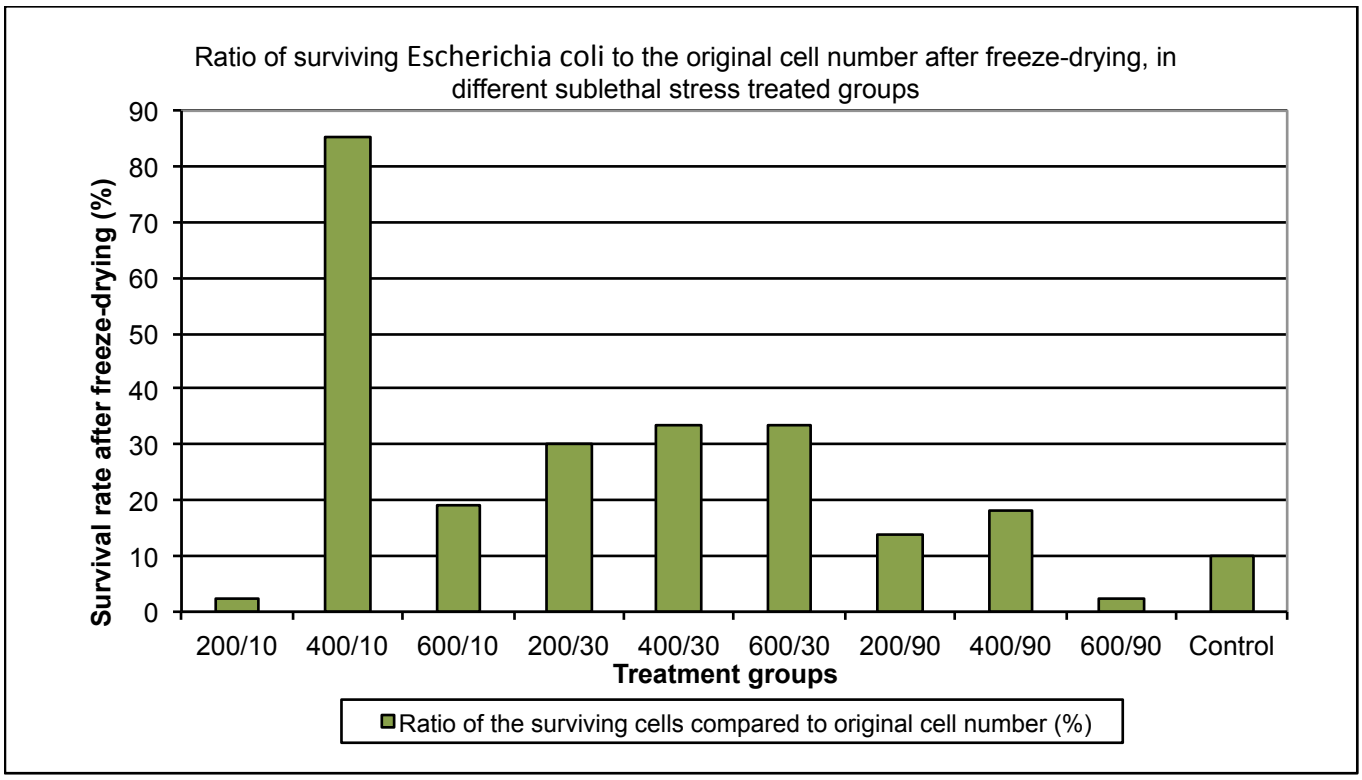
Table 6.: Number of living *Escherichia coli* cells (c.f.u./0.4ml) before and after freeze-drying (two repetitions). (Initial cell count (c.f.u./ml) before treatment: 4.08×10^8 - 4.10×10^8)

	Number of living cells \ 0.4 ml		
Treatment group No.	Before freeze-drying	After freeze-drying I.	After freeze-drying II.
1	6.10×10^8	1.55×10^7	1.65×10^7
2	3.20×10^8	2.56×10^8	2.90×10^8
3	4.80×10^8	9.30×10^7	9.0×10^7
4	3.90×10^8	1.36×10^8	1.00×10^8
5	5.90×10^8	2.14×10^8	1.80×10^8
6	5.95×10^8	2.27×10^8	1.70×10^8
7	6.10×10^8	7.50×10^7	9.50×10^7
8	6.60×10^8	1.65×10^8	7.80×10^7
9	6.20×10^8	1.52×10^7	1.21×10^7
Control	5.10×10^8	2.50×10^7	7.7×10^7

Treatment groups marked with bold numbers represent significantly higher cell survival rate compared to the control group. Amongst the treatment groups, groups No. 2 and No. 4 proved to be superior. It was concluded that a specific high hydrostatic pressure treatment before freeze-drying enhances significantly the cell survival rate after freeze-drying. Different optimal treatment parameters apply for different species. Treatment parameters shall further be optimized, regarding treatment temperature, equilibration period between the end of the treatment and initiation of freeze-drying.

The following figures represent the post-freeze drying survival of each treatment and control groups.


Ratio of surviving *Lactobacillus plantarum* to the original cell number after freeze-drying,
in different sublethal stress treated groups
Survival rate after freeze-drying (%)
100
90
80
70
60
50
40
30
20
10
0
200/10
400/10
600/10
200/30
400/30
600/30
200/90
400/90
600/90
Control
Treatment groups (Hydrostatic pressure (Mpa)/duration of tratment (min))
Ratio of the surviving cells compared to original cell number (%)


Ratio of surviving Escherichia coli to the original cell number after freeze-drying, in different sublethal stress treated groups
Survival rate after freeze-drying (%)
90
80
70
60
50
40
30
20
10
0
200/10
400/10
600/10
200/30
400/30
600/30
200/90
400/90
600/90
Control
Treatment groups
Ratio of the surviving cells compared to original cell number (%)