Cytogenetical Analysis of Oat Seedling Root Meristem Treated with Permafrost-Derived *Bacillus* spp.

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Material and Methods

The studied bacterial strains were cultivated at 5 °C and 25 °C in LB medium. Barley seeds (n = 30) were soaked in a bacterial suspension for 2 h, then they were placed in sterile Petri dishes (10 seeds per dish) on the surface of a wet filter paper in a checkerboard pattern with embryos in one direction. Petri dishes were incubated at 20 ± 2 °C for 3 days. The control seeds were soaked in distilled water. The root tips were fixed in Carnoy solution [(ethanol (99%): glacial acetic acid (3:1)] for 24 h and stained with acetocarmine dye [1]. Plant cells were microscopically evaluated, and the mitotic index, phase indices (I) of dividing cells were scored [2]. The mitotic index (%), was calculated according to formula (1)

$$MI = (\text{Number of dividing cells}/\text{Total number of cells}) \times 100 \quad (1)$$

Data were expressed as mean ± SD. Differences between control group and treatment groups were determined using Student's t-test. P-values of less than 0.05 were considered statistically significant.

Results

The cytotoxic effect of permafrost-derived *Bacillus* spp. was assessed by the mitotic activity of root meristems of seedlings *Avena sativa* L after the treatment with bacterial suspensions. Genotoxicity was assessed by determining the mitotic index.

Table S1. Mitotic index of oat seedling root meristem cells under the treatment *Bacillus* spp.

Strains	5 ˚C			22 ˚C		
	N	n	Mitotic index MI ± m	N	n	Mitotic index MI ± m
B.megaterium 312	1411	241	17,08 ± 0,56	815	166	20,36 ± 0,76
B. megaterium 629	3596	842	23,41 ± 0,44	3377	852	25,22 ± 0,45*
B. cereus 1257	2013	464	23,05 ± 0,59	3281	765	23,31 ± 0,49*
B. cereus 630	2169	572	26,36 ± 0,77*	3854	1033	26,80 ± 0,39*
B. cereus 875	2582	681	26,37 ± 0,43*	1256	264	21,01 ± 0,56
B. subtilius 948P	3134	820	26,16 ± 0,89*	3042	681	22,38 ± 0,39*
B. simplex 948P1	3201	830	25,93 ± 0,72*	3084	597	19,36 ± 0,42
Bacillus sp. B-10130	2452	159	6,49 ± 0,33*	2473	317	12,82 ± 0,51*
Control group	3454	351	10,16 ± 0,89	3454	351	10,16 ± 0,89

Note: N is the total number of scored cells; n is the number of investigated phases; MI ± m - mitotic index. * differences with control are significant at $P < 0.05$.

In general, the treatment of oat seeds with bacterial suspensions does not lead to suppression of cell`s division (Table S1). With the exception of one strain (*Bacillus* sp. B-10130), treatment with permafrost-derived *Bacillus* spp. leads to an intensification of cells division. Calculated MI was increased by two times comparing to water-treated control group (Table S1). Thus, a reliably positive effect on the mitotic activity of the cells of oat seedling roots was revealed during seed treatment with permafrost-derived *Bacillus* strains.

References

1. Ivanova, E., et al. "Somatostatic Effect of Heavy Metal Contaminated Waters In The Region of The Town Of Panagjurishte, Bulgaria." Journal of Environmental Protection 4.2 (2003): 284-287.
2. Oney-Birol, S. Exogenous L-Carnitine Promotes Plant Growth and Cell Division by Mitigating Genotoxic Damage of Salt Stress. *Sci Rep* **9,** 17229 (2019). https://doi.org/10.1038/s41598-019-53542-2.