Response of *Lactobacillus sp.* in different physiological state to the oxidative stress induced by H_2O_2 and ionizing radiation

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Introduction

Ionizing radiation exposure leads to oxidative damages in organisms. Free radicals produced by oncology radiotherapy are often a source of serious side effects. Resistance of lactobacilli to oxidative stress is of great importance for their applicability as probiotics. This response is strongly dependent on the type of cell metabolism.

In contrast to heat, acid and detergent stresses, which respond to growth phase and adaptation, tolerance to oxidative stress implies completely unrelated mechanisms (Ricciardi et al., 2012).
Thus, on the one hand, a high resistance of lactobacilli to gamma irradiation is proved, on the other hand, serious disorders in the microbial (probably, also lactobacilli) ecosystem in the human organism are caused by ionizing radiation.

Due to the lack of fundamental knowledges about the functionality of probiotic cultures in irradiation-exposed organisms, additional experiments are needed to support this hypothesis.
This study was aimed at evaluating the response of *Lactobacillus plantarum* ATCC® 14917™ to 2 mM H_2O_2 after being exposed to different doses of ionising radiation.

Materials & Methods

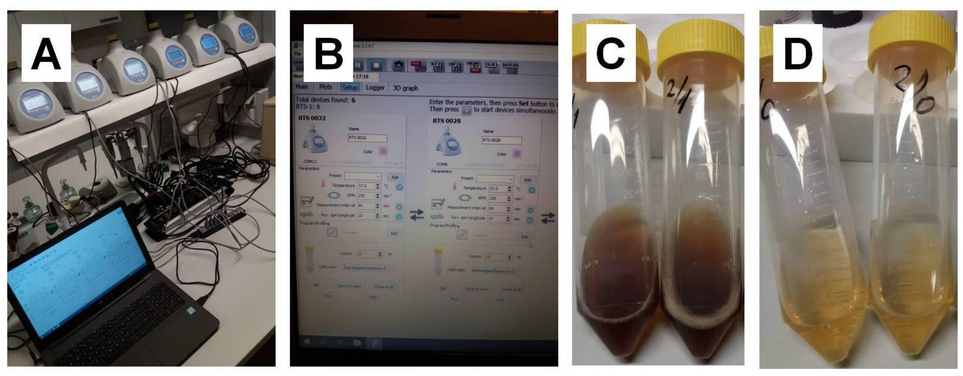

Figure 1. Cultivation of *L. plantarum* in the bioreactor RTS-1 (Biosan Ltd., Latvia). A – RTS-1 bioreactors connected with a software; B – parameters of cultivation; C – bacterial culture in MRS broth; D – bacterial culture in TSB broth.

Cultures of *L.plantarum* grown in MRS and TSB broth, have been compared by growth kinetic parameters. 24h old cultures were subjected to irradiation in microplates (Fig.2), afterwards the enzyme activity of the control and irradiated cultures was tested in the presence of 2 mM H_2O_2 and without it.

L. plantarum ATCC® 14917™ was cultivated in the bioreactor RTS-1, which utilizes patented Reverse-Spin® technology. Culture was grown aerobically in 50 ml falcon tubes with 10 mL De Man, Rogosa and Sharpe (MRS) or Tryptone Soya Broth (TSB), amended with 0.1% Tween 80, at 37 °C (Fig.1). Each variant in triplicate.
For culture irradiation clinical LINAC *trueBEAM* with photon energy 6 MeV was used (Fig.2). To distribute the dose 1.5 cm bolus (H2O equivalent) and 1.5 cm PMMA plate was used. A - two culture plates are stacked up and placed between the bolus and PMMA. B - Source surface distance (SSD) was set to 100 cm and measured with calibrated pointer, collimator of the LINAC was opened to create 15x15 cm field. C – dispaying the beam parameters during irradiation (Fig.2).

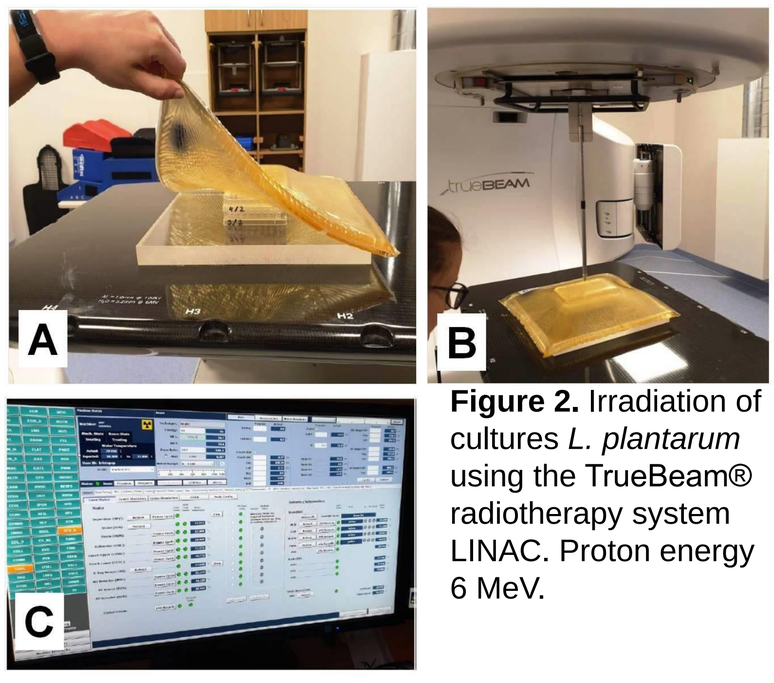

Figure 2. Irradiation of cultures *L. plantarum* using the TrueBeam® radiotherapy system LINAC. Proton energy 6 MeV.

Results

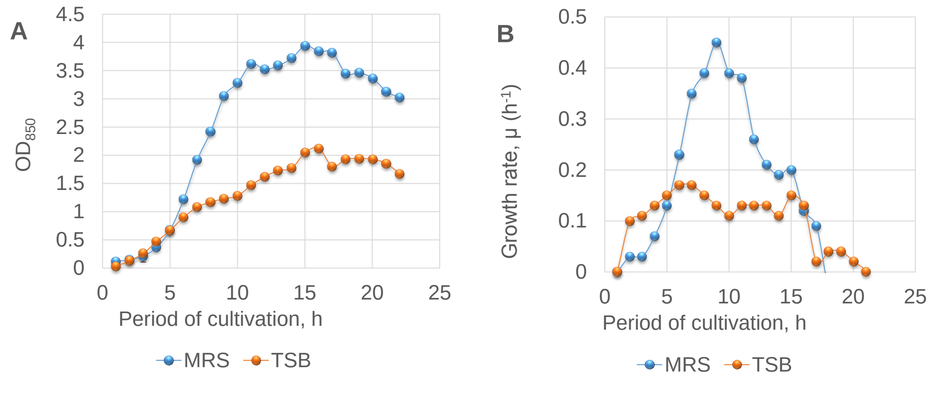

Figure 3. Growth of *L. plantarum* in the bioreactor RTS-1. A – culture turbidity, monitored by a near-infrared optical system; B – growth rate calculated automatically.

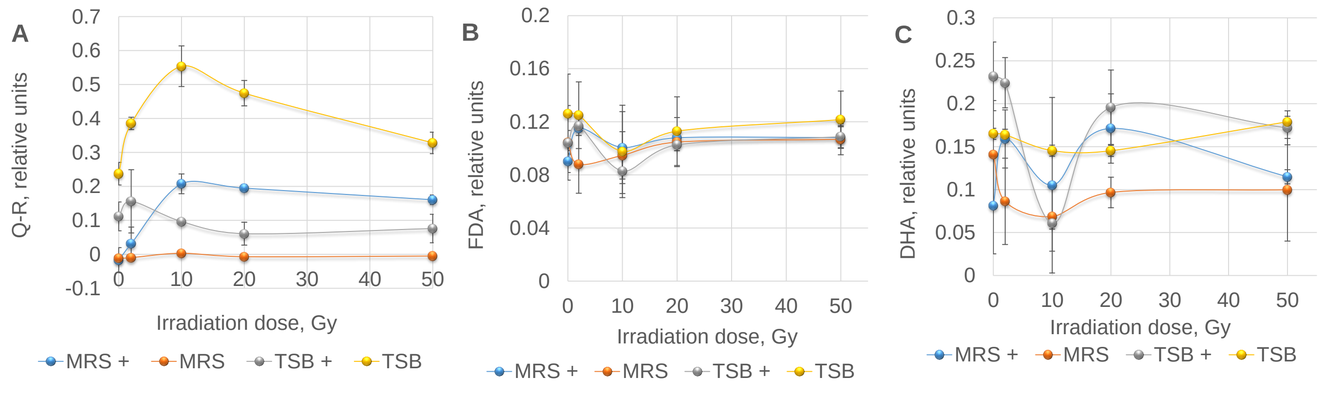

Figure 4. Enzyme activity of *L. plantarum* in the presence of H_2O_2 in dependence on irradiation dose. A – quinone-reductase activity; B – fluorescein acetate hydrolysis activity; C – dehydrogenase activity. «+» 2 mM H_2O_2.

Conclusions

1. Cultivation of *L. plantarum* in MRS after 24h resulted in a two-fold higher turbidity, as compared to TSB. Different growth kinetics evidences about contrasting physiological states of *L. plantarum* grown in MRS and TSB.
2. The irradiated culture of *L. plantarum* grown in TSB, showed an increasing quinone-reductase activity at irradiation dose from 2 to 50 Gy, with the maximum at 10 Gy, comparing with non-irradiated cells.
3. Effect of 2 mM H_2O_2 on the enzyme activity of irradiated *L. plantarum* differed in dependence on the enzyme and broth type. A quinone-reductase activity of the MRS-grown culture was significantly ($p<0.05$) increased in the presence of H_2O_2 for the cells subjected to 2-50 Gy, compared with control.
4. Some decrease of dehydrogenase activity of *L. plantarum* was detected for cells subjected to 10 Gy irradiation. This effect was especially pronounced in variants with H_2O_2 for TSB-grown cells.

Further studies will continue experiments on physiological response of probiotics to irradiation.

Acknowledgements

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