IMMOBILIZATION AND SURVIVAL OF PLANT GROWTH-PROMOTING BACTERIA *RHIZOBIUM LEGUMINOSARUM*, *STREPTOMYCES GRISEOVIRIDIS* AND *AZOTOBACTER* SP.

S. Žvagiņa, A. Bērziņa,
Z. Petriņa, V. Nikolajeva, A. Lielpētere

Department of Microbiology and Biotechnology
University of Latvia

LATVIJAS UNIVERSITATE
ANNO 1919

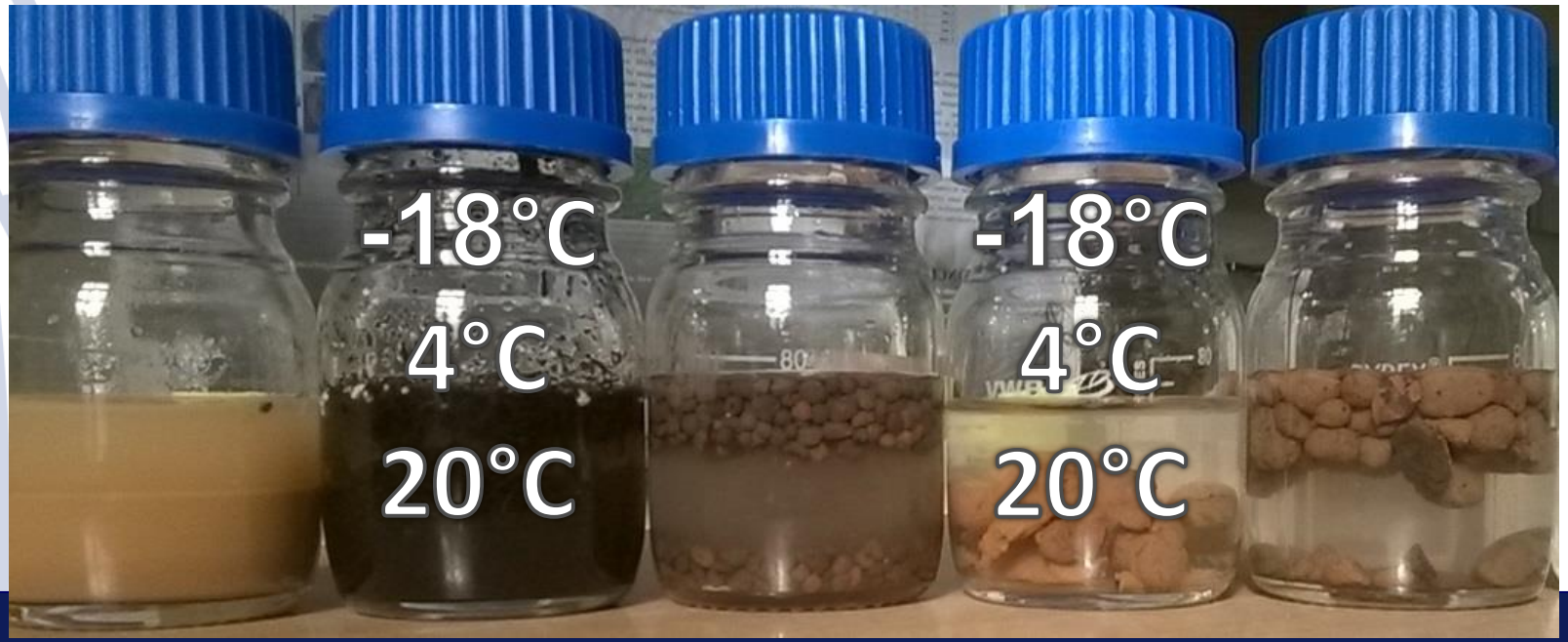
Introduction

- Plant growth-promoting bacteria represent a wide variety of soil bacteria which, when grown in association with a host plant, result in stimulation of growth of their host.
- There is a growing interest in the use of plant growth-promoting microorganisms also called biostimulants due to the demand for chemically uncontaminated food.
- A problem in the production of microbial preparations is the maintenance of bacterial viability. The period of validity depends on the storage temperature and carrier material of microorganisms.

LATVIJAS UNIVERSITATE
ANNO 1919

Objective

The aim was to immobilize *Rhizobium leguminosarum*, *Streptomyces griseoviridis* un *Azotobacter* sp. in the peat and ceramic granules and evaluate their viability during storage at 20 °C, 4 °C and -18 °C for continuous periods of time as well as to compare these parameters with bacterial viability in the liquid.



Materials and methods

- *Rhizobium leguminosarum* biovar *viciae* MSCL 802
- *Streptomyces griseoviridis* MSCL 369
- *Azotobacter* sp. MSCL 299

The strains were cultivated in R2A agar for 3 or 5 days at a temperature of 20 °C

Carriers:

- Peat
- Experimental cylindrical (on average 5 x 10 mm) ceramic granules with an apparent porosity of 17.8%, a specific surface area of 4.30 m^2/g and bulk density 1.58 g/cm^3

Materials and methods

- Sterile carriers were watered with fresh suspension of *R. leguminosarum*, *S. griseoviridis* or *Azotobacter* sp.
- Materials were stored at 20 °C and the excess liquid was decanted after 2.5 h
- A series of 1.5 ml eppendorf tubes with 0.4 ml of bacterial suspensions was prepared separately.
- The prepared samples were stored at a temperature of -18 °C, 4°C and 20 °C and sampled after various time periods.
- Peat and granules were scrubbed and ground in a sterile mortar with a pestle in sterile water to recover the adhered bacteria.
- The number of colony-forming units (CFU) in the initial suspension and in the final liquid of detached bacteria was determined by plating 10-fold serial dilutions on R2A agar plates using the spread plate method.

Results

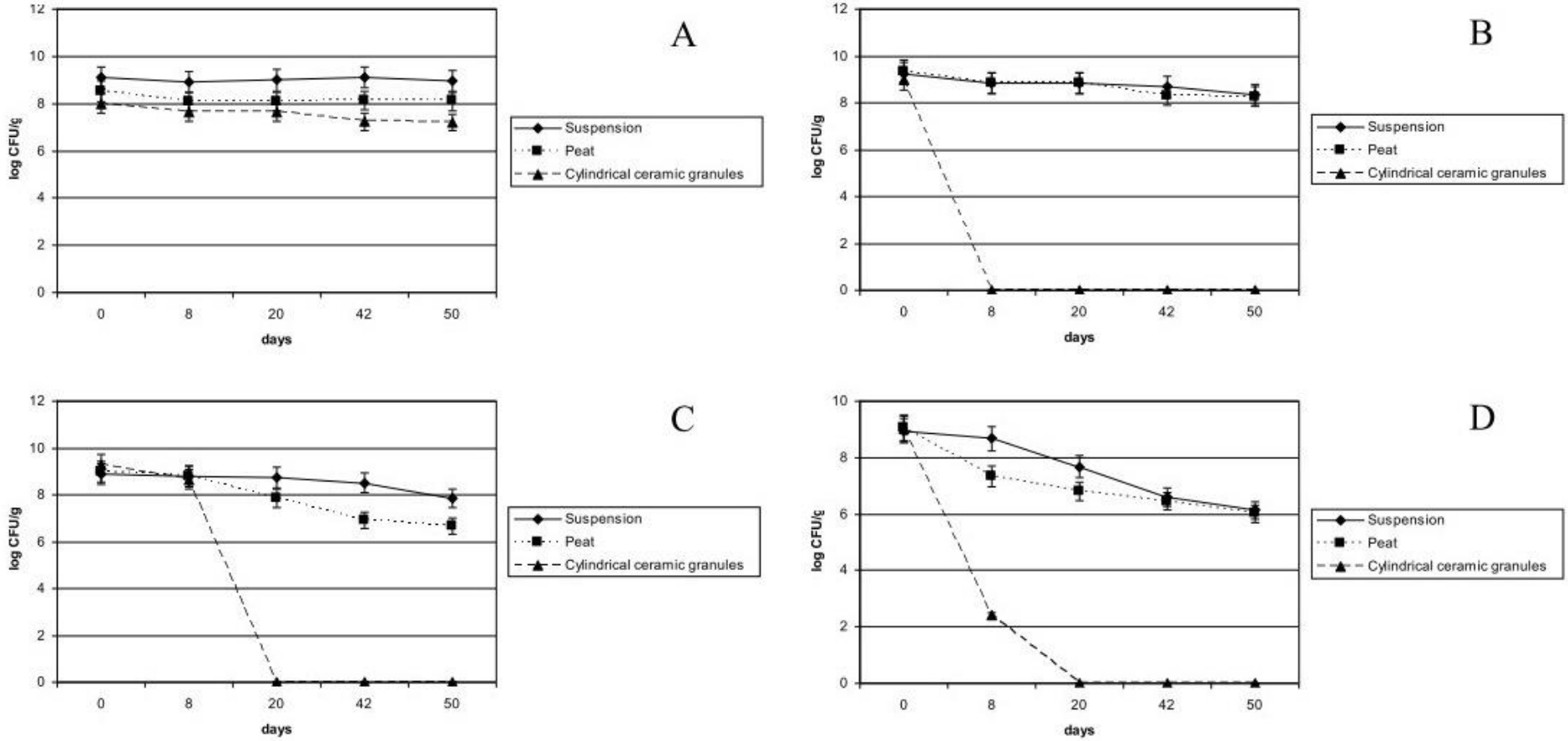


Fig. 1. Survival of *R. leguminosarum* in the suspension and carrier materials stored at −18 °C (A), 4 °C (B), 20 °C (C) and 30 °C (D). 1 g of peat and cylindrical ceramic granules was inoculated with 3 ml of *R. leguminosarum* suspension. The experiment was conducted in duplicate.

Results

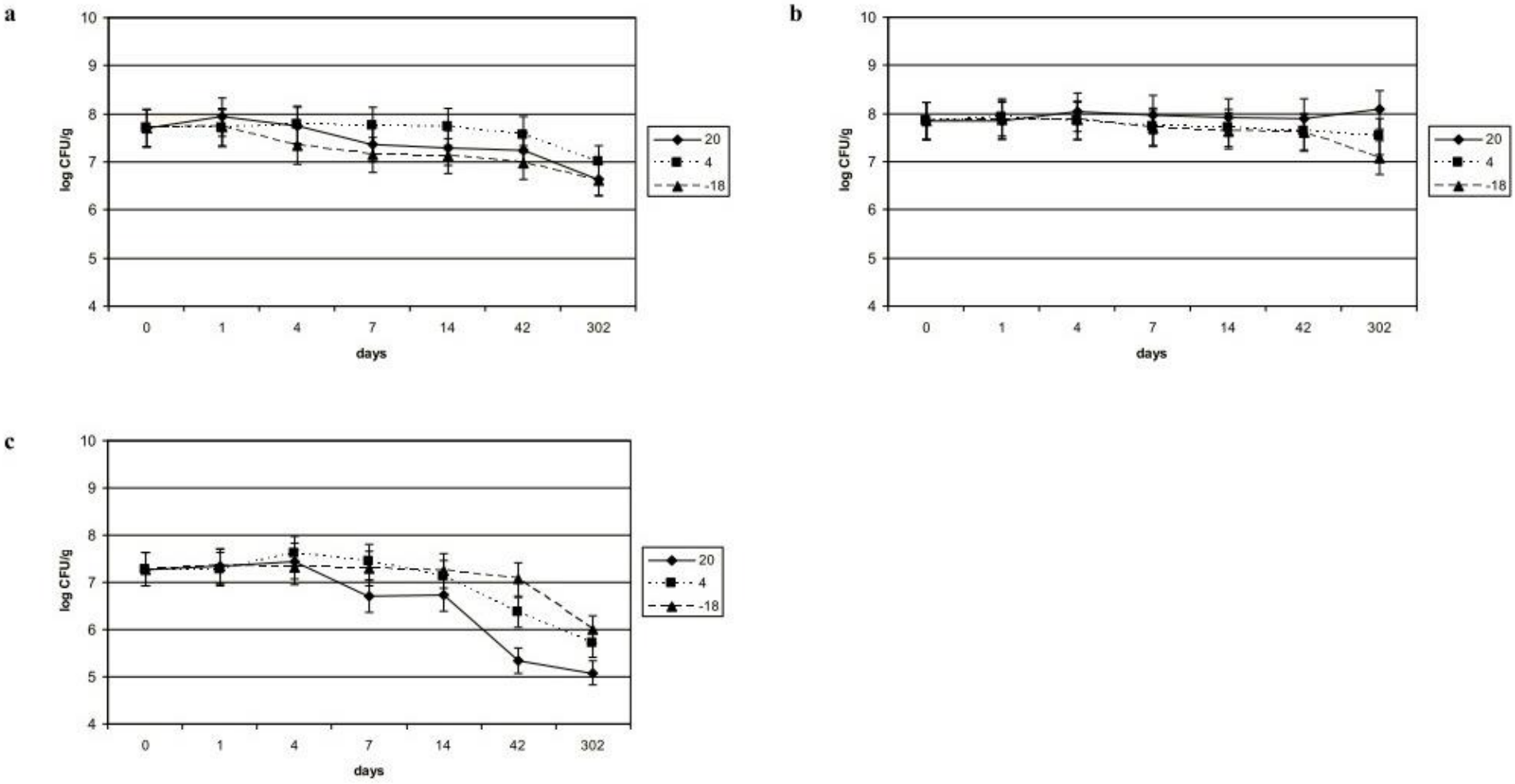


Figure 2: Viability of *Streptomyces griseoviridis* in the suspension (a) and in the peat (b) and ceramic granules (c) at different temperatures (20 °C, 4 °C, -18 °C).

Results

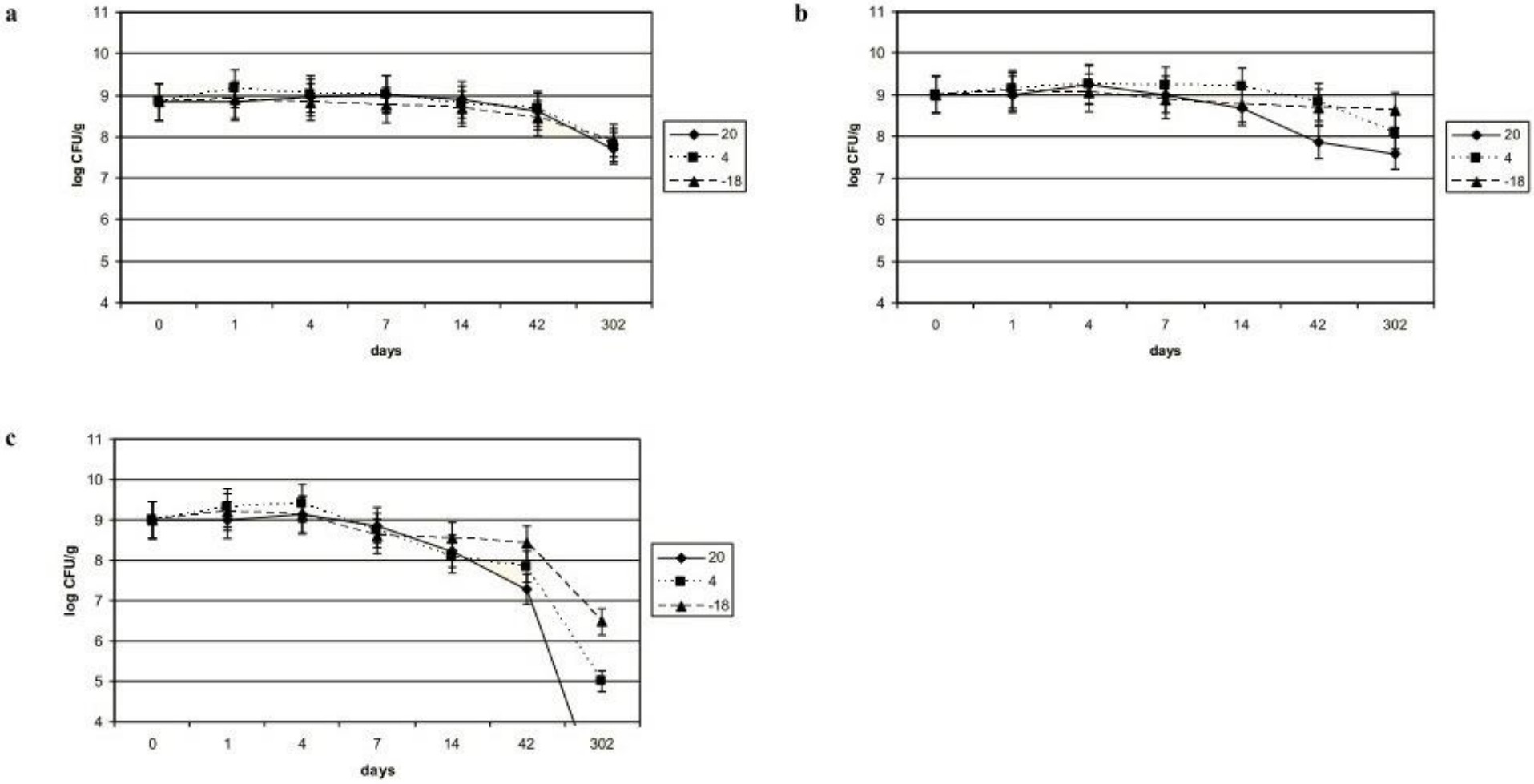


Figure 3: Viability of *Azotobacter* sp. in the suspension (a) and in the peat (b) and ceramic granules (c) at different temperatures (20 °C, 4 °C, -18 °C).

Conclusion

1. Immobilization of *Rhizobium leguminosarum*, *Streptomyces griseoviridis* and *Azotobacter* sp. took place in all the carriers, but more bacteria bound to the peat.
2. Both the carrier material and storage temperature affected bacterial viability.
3. It is recommended to store immobilized *S. griseoviridis* products in the peat at room temperature (20 °C)
4. It is recommended to store *Azotobacter* sp. products in the peat at -18 °C.
5. It is recommended to store *R. leguminosarum* products in the peat at -18 °C or 4 °C.
6. In the case of bacterial immobilization in the ceramic granules, it is recommended to store microbial preparations at a low temperature, best of all at -18 °C.
7. Bacterial suspensions in sterile water can be stored at 4 °C for at least 10 months.

LATVIJAS UNIVERSITATE
ANNO 1919

ACKNOWLEDGEMENT

Authors are grateful to R. Švinka and V. Švinka for providing and characterization of ceramic granules. This study was supported by the National Research Programme of Latvia “Sustainable Use of Forests and Mineral Deposits — New Products and Technologies (ResProd)”, as well as EU FP7 Research Project EuroLegume, No. 613781.