IOCBE 2024 Conference
The 1st International Online Conference on Bioengineering
16–18 October 2024 | Online
MDPI

The impact of different chitosan viscosities on the proliferation and production of naphthoquinones in *Rindera graeca* hairy roots cultured on hybrid PLA–chitosan scaffolds

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INTRODUCTION & AIM

Plants are well known for centuries for their medical applications. The first detailed description of their use as a medicament was written in ancient Greece and China [1]. Nowadays they are considered as the greatest source of anticancer agents all over the world. They provide various chemical compounds characterized by the therapeutic effect. They have a wide range of activity and show less toxicity than chemically synthesized drug agents. The application of *in vitro* plant tissue cultures is a convenient alternative to the classical approach to plant breeding. Different *in vitro* techniques are designed to maximize biomass proliferation and secretion of the desired metabolites [2]. Biotechnological and bioengineering methods need to be applied to maximize the quantity of desired products occurring in the medium after culturing. Biomass immobilization is a very effective way to significantly increase plant tissue proliferation and the volume of the produced secondary metabolites. The polymeric-based scaffolds are useful and cheap bioengineering tool in *in vitro* culturing techniques [3].
The aim of the study was to evaluate influence of chitosan viscosity on *Rindera graeca* hairy root proliferation and naphthoquinone derivatives secretion.

MATERIALS & METHOD

The scaffolds made of PLA coated with different viscosity chitosan (**Figure 1**.) were separately placed in 250 cm^3 Erlenmeyer flasks filled by 50 cm^3 of hormone-free DCR medium. The next step was to put 1 g of 28-day *R. graeca* transgenic roots on the upper surface of the polymeric materials. All culture systems were incubated at 24°C in dark conditions for 28 days on the oscillatory shaker at 105 rpm. After that, each culture system were collected and analysed to determine the quantitative parameters: the increases in fresh (FB) and dry biomass (DB), values of specific growth rate (μ), and the concentration of naphthoquinones per culture system (m_p). The concentration of naphthoquinones per culture system was determined for all samples, included the samples of culture medium, dry hairy roots, and dried PLA constructs, which were separately extracted with HPLC grade methanol. All extraction procedures were supported with sonication until the solvent color faded. The obtained extracts were analysed by a chromatographic method.
The value of FB was calculated from the following equation:

$$FB_{28} = m_{28}^{FB} \text{ x } m_0^{FB^{-1}} \text{ [−]}$$

m_{28}^{FB} – fresh biomass weight for the sample harvested at 28-day of culture [g],
m_0^{FB} – fresh biomass weight of inoculum [g].

The value of DB was determined based on the equation:

$$DB_{28} = m_{28}^{DB} \text{ x } m_0^{DB^{-1}} \text{ [−]}$$

m_{28}^{DB} – dry biomass weight for the sample harvested at 28-day of culture [g],
m_0^{DB} – dry biomass weight of inoculum [g].

The value of μ was determined based on the equation:

$$\mu = (\ln m_{28}^{FB} - \ln m_0^{FB}) \text{ x } \Delta t^{-1} [h^{-1}]$$

Δt – time of culture [h].

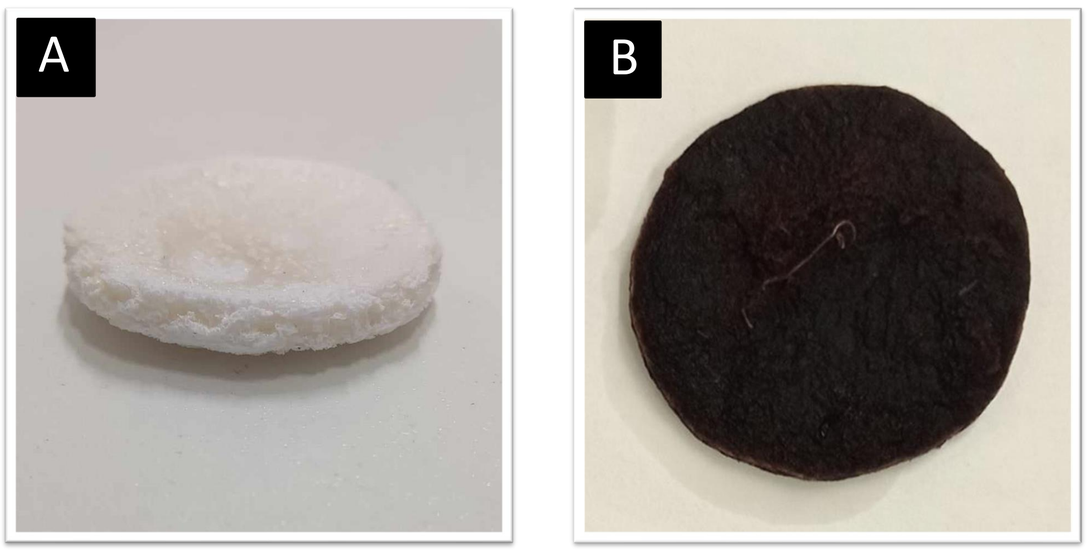

Figure 1. Polylactide (PLA) porous scaffold coated with chitosan before culturing (A) and after (B).

RESULTS

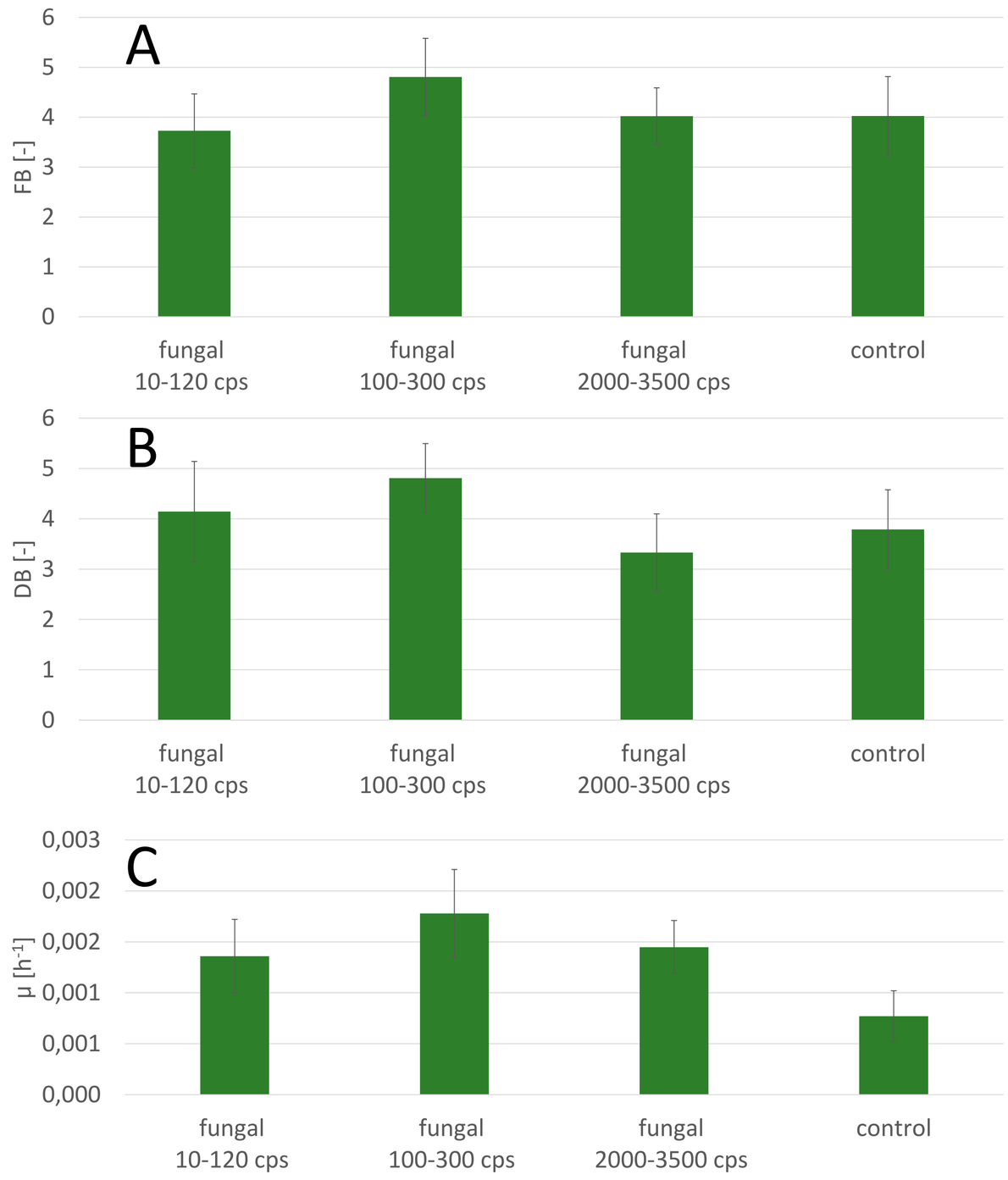

Figure 2. Values of FB (**A**), DB (**B**) and μ (**C**) obtained for *R. graeca* transgenic roots in the culture systems with polymeric scaffolds coated with with different viscosity and in the reference system with PLA scaffold without coating.

CONCLUSION

- *Rindera graeca* hairy roots immobilization on PLA scaffold coated with 100 – 300 cps chitosan slightly increased FB and DB, while immobilization on polymeric material coated with other viscosities chitosan doesn't show noticeably improvements in FB and DB in comparison to reference system.
- Immobilization on polymeric scaffolds coated with chitozan noticeable improve μ in comparison to reference system
- Secondary metabolites did not reach the detection threshold in all systems

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This research was funded by the National Science Centre (NCN), Poland, grant no. 2021/41/N/ST8/00958.