**Development of a novel biological plant-based protective agent**

 **for wood-based materials**

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**Highlights**

* Plant-based protective agent for wood-based materials
* Biotechnological process with *Salvia* cell suspension cultures
* Combination of sucrose fed-batch and elicitor increase the volumetric triterpene yield

**1. Introduction**

Wood is one of the most important renewable raw materials. The use of wood preservatives contributes to the protection and increases the resistance of the material against harmful organisms such as mold fungi. Most currently available protective agents for the treatment of wood-based materials are almost completely chemicals that contain substances that are harmful to the environment and hazardous to health. In order to minimize these harmful effects on the environment and humans, manufacturers are searching new bio-active substances as alternative to conventional fungicides for products made of renewable raw materials.

Plant secondary metabolites occur in a wide variety and high structural diversity in all higher plants. They are involved in the defense mechanisms of plants and accumulate in cells when abiotic or biotic stress occurs. Therefore, many secondary metabolites have an antimicrobial, hydrophobic and antifungal effect. *Salvia* species contain a large number of bioactive pharmaceutical ingredients, like flavonoids, terpenes or phenols that exhibit antibacterial, antiviral and antifungal activities. Two of these compounds are oleanolic acid (OA) and ursolic acid (UA). Both are constitutional isomers and belong to the group of pentacyclic triterpenic acids.

Extracts or ingredients of plants are traditional produced from agricultural material. Depending on several influencing factors such as the location and period of production, the obtained extractives differ in quality and quantity. For a continuous, sustainable production of plant ingredients, the use of biotechnological processes based on plant *in vitro* cultures is required. The cultivation of plant cells in a closed bioreactor system ensures the sustainable production of plant secondary metabolites with consistent quality and quantity [1]. To reach reasonable productivities with plant cell suspension cultures, elicitation is a widely used strategy. The systematic use of so-called elicitors in plant cell cultures stimulates the plant defense mechanisms, which can be used to increase the yield of the active substances [2].

The aim of the presented work is to develop a sustainably produced bio-based wood protective agent with plant *in vitro* cultures in a closed bioreactor system. To increase the productivity of the process, an elicitation strategy combining treatment with fungal medium filtrate and sucrose feeding will be applied.

**2. Methods**

The development process of the production for a protective agent for plant-based wood materials is illustrated in Figure 1.



**Figure 1.** Planned process flow for the production of a bio based protective agent for wood-based materials.

For the fungal medium filtrates, the mold fungi *Aspergillus* *niger* (DSM 1957) and *Trichoderma* *virens* (DSM 1963) were each cultivated in 1 l shaking flasks with 500 ml of 1.5 % malt extract medium for 14 days. The mycelium pellets were separated from medium by a three-step filtration [3]. For the plant cell culture, *Salvia fruticosa* was cultivated in Linsmaier and Skoog medium with 30 g l-1 sucrose and 0.2 mg l-1 2.4-dichlorophenoxyacetic acid at 26 °C, 110 rpm in darkness and sub-cultivated every 10 days with 20 % inoculum volume. The fed-batch cultivation and the elicitation procedure were performed as described in [3]. The extraction of intracellular triterpenes was carried out with a mortar and ethanol as extraction agent. The analysis of the extracts for OS and US was performed by HPLC [4].

**3. Results and discussion**

To further increase triterpene productivity of *S. fruticosa* cell suspension, preliminary experiments of nutrient feeding with the application of 30 g l-1 sucrose at the end of the cell growth on day 10 successfully led to additional growth and higher levels of triterpene contents, volumetric yield and productivity. With the combination of sucrose fed-batch and elicitation with fungal medium filtrate, this value was further exceeded. Expressed in analytical values, a content of 16.3 mg l-1 OA was determined for the pure *S. fruticosa* cell suspension. The yield was increased to 32.6 mg l-1 by the addition of *T. virens* fungal medium filtrate. Through the combination of elicitor and sucrose fed-batch the yield could be increased to 112.9 mg l-1, which represents an increase of 500 % in the volumetric triterpene yield. The results with the fungal filtrate of *A. niger* as elicitor showed similar results. For the elicitation, a concentration of 3 % *(v/v)* of each fungal medium filtrate was selected because higher concentrations of 12 % *(v/v)* demonstrated strong growth inhibition of the plant cells. The detailed results of the effects of fungal medium filtrates of *A.* *niger* and *T. virens* as well as of the sucrose fed-batches on growth and productivity are described in [3].

**4. Conclusions**

A biotechnological process for the production of triterpenic acids with *S. fruticosa* cell suspension cultures has been developed on a laboratory scale. By an elicitation strategy combining treatment of the cell cultures with fungal medium filtrate and sucrose feeding, the yields of the secondary metabolites could be significantly increased. It was demonstrated that the eliciting effect of the fungal extracts depends on the concentration, the time of addition and the age of the culture. In further studies, the eliciting substances in the plant extracts have to be determined. In addition, the protecting effects of the extracted triterpenic acids for wood-based materials have to be intensively evaluated. Subsequently, the process has to be scaled up into the industrial scale. This work has been financially supported by the Central Innovation Program for SMEs of the Federal Ministry of Economics and Technology (BMWi, grant number KF2049810SA2), German Research Foundation (DFG, Project ID BL345/10-2) and Federal Ministry of Education and Research (BMBF), the project ID is: 031B0691.

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