Novel Autochthonous Fungi for the Treatment of Lignocellulosic Biomass

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Introduction

Lignocellulosic biomass has become one of the most explored renewable substrates for the production of many valuable chemicals, biofuels, and food or feed ingredients or products. However, a complex structure blocs its direct utilization and directs to utilization of particular pretreatments in order to remove lignin and facilitate the biomass hydrolysis prior to its further processing. Biological methods involving the use of fungi or their enzymes in the pretreatment and hydrolysis of the lignocellulosic substrate are ecologically friendly, rather time consuming, but with no formation of harmless components that can inhibit the production microorganism. The limitations, such as process longevity and loss of valuable sugars, can be alleviated or overcome by utilization of the novel and efficient microorganisms, e.g. fungi which are selective biomass decomposers, as well as by optimizing conditions of the pretreatment.

This research aimed to isolate, select, and identify Serbian autochthonous fungi with a pronounced lignocellulolytic activity, and to define the conditions for their use in the pretreatment of lignocellulosic biomass. Potential candidates were selected based on their ligninolytic and hydrolytic activity. The best candidate for the pretreatment was chosen based on the selectivity in lignocellulose degradation. The research also included the effect of addition of sugar beet molasses stillage (MLS) in the biological pretreatment by the selected fungi.

Materials & Methods

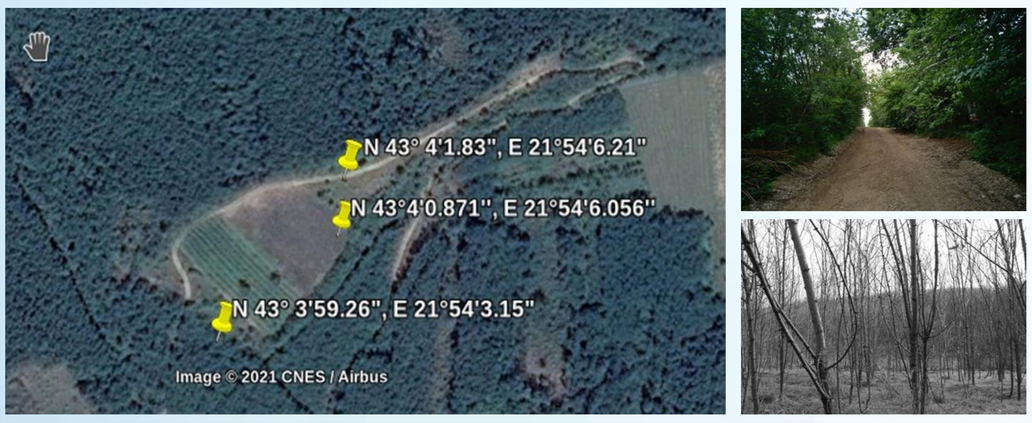

Figure 1. Images of the location where fungal isolates were collected. Location coordinates (left) and locality characteristics (right) – in spring (up) and winter (down). It is Southern Serbia near the city of Leskovac.

- 12 fungi were isolated from nature (stumps, fallen leaves, trees and branches) and tested.
- The three novel fungal isolates with pronounced lignocellulolytic activities were identified using ITS sequences, and the sequences were deposited in the NCBI GenBank database. Their accession numbers are KY264754.1 (***Trametes hirsuta* F13**), KY264753.1 (***Stereum gausapatum* F28**), and MF521930.1 (***Myrmaecium fulvopruinatum* F14**)
- Sugar beet molasses stilage (MLS) was obtained from a local alcohol industry.
- Beechwood sawdust (lignocellulosic biomass) was obtained from a local sawmill.
- Fungal enzymes were extracted with 50 ml of distilled water. Enzyme activities were determined using spectrophotometric assays.
- The dry substrate mass was determined according to the NREL/TP 510-42621 protocol.
- The share of acid soluble and acid insoluble lignin was determined according to the LAP-003 and LAP-004 protocols.

Results & Discussion

1. Selection of Fungal Isolates Based on the Enzyme Activity

Table 1. Hydrolytic Activity

Fungal Isolate	Enzyme activity (U/L)	
	Cellulase	Xylanase
***T. hirsuta* F13**	**1069±114**	**1054±121**
***M. fulvopruinatum* F14**	**5682±327**	**7721±293**
***S. gausapatum* F28**	**870±94**	**947±129**

*The best hydro*lytic enzyme producer was ***M. fulvopruinatum* F14.**

Table 2. Ligninolytic Activity

Fungal Isolate	Enzyme activity (U/L)		
	Laccase	MnP	VP
***T. hirsuta* F13**	**110.3±0.7**	**1.5±0.1**	**1±0.1**
***S. gausapatum* F28**	**19.2±0.1**	**5.1±0.2**	

***Trametes hirsuta* F13** and ***Stereum gausapatum* F28** were selected as best ligninolytic enzyme producers for potential use in pretreatment of waste lignocellulosic biomass .

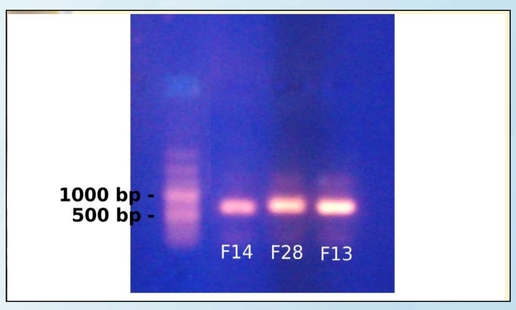

Figure 2. Identification of the selected fungi by PCR using ITS sequences

2. Selection of Fungal Isolates Based on Biomass Decomposition

Monitored parameters	*T. hirsuta* F 13	*S. gausapatum* F 28
Biomass reduction (%)	**19**	**24**
Total Lignin reduction (%)	**33.8**	**28**
Selectivity coefficient 1	**1.7**	**1.1**
Klason`s lignin reduction (%)	**28**	**19**
Selectivity coefficient 2	**1.47**	**0.80**

Table 3. Biomass and lignin reduction and selectivity coefficient relative to the lignin reduction (selectivity coefficient 1), and relative to the Klason`s lignin reduction (selectivity coefficient 2) after 35 days of incubation under non-optimized cultivation conditions and without addition supplements for stimulation of enzyme activity (such as sugar beet molasses stillage).

3. Molasses Stilage as a Supplement for Enzyme Production and Pretreatment

Addition of molasses stillage increased the enzyme production of lacasse and MnP in *T. hirsuta* F13. Under optimal conditions biomass reduction is improved.

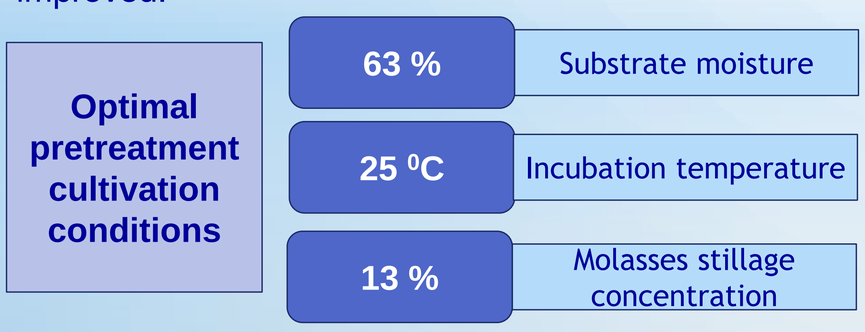

Figure 3. Optimal conditions for the fungal pretreatment with *T. hirsuta* F13.

Figure 4. Pretreatment with *T. hirsuta* F13.

Table 4. Biomass and lignin reduction in the presence of MLS

Monitored Parameters	Pretreatment under optimal cultivation conditions			
	18 days		35 days	
	MLS	dH_2O	MLS	dH_2O
Biomass Reduction (%)	**15**	**13,9**	**22**	**19,6**
Klason's Lignin Reduction (%)	**29,2**	**23,5**	**32,7**	**29,1**
Selectivity Coefficient	**1,9**	**1,7**	**1,49**	**1.48**

Conclusions

Serbian autochthonous fungi are due to their enzyme production great unexplored potential for application in various industries (from the pulp and paper industry, to the textile industry and biofuel production). Initially from twelve isolates, the three were selected in this research, ***T. hirsuta* F13**, ***M. fulvopruinatum* F14**, and ***S. gausapatum* F28**, and identified as producers of industrially important lignocellulolytic enzymes and can be used for their production or in biomass pretreatment. The best candidate for the pretreatment was ***T. hirsuta* F13,** which showed a high selectivity of lignocellulosic biomass degradation. The research has also shown that the use of sugar beet MLS as a supplement improved the pretreatment: the pretreatment duration could be shortened from 35 to 18 days, and the selectivity of biomass degradation was improved. This effect is due to effect on fungal enzyme activity.

Acknowledgements

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